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DARWIN REVIEW

Hormonal regulation of stem cell maintenance in roots

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Abstract

During plant embryogenesis, the apical–basal axis is established and both the shoot apical meristem (SAM) and the root apical meristem (RAM) are formed. In both meristems, there are slowly dividing cells which control the differentiation of their surrounding cells called the organizing centre (OC) and the quiescent centre (QC) in the shoot and root, respectively. These centres with their surrounding initial cells form a 'stem cell niche'. The initial cells eventually differentiate into various plant tissues, giving rise to plant organs such as lateral shoots, flowers, leaves, and lateral roots. Plant hormones are important factors involved in the balance between cell division and differentiation such that plant growth and development are tightly controlled in space and time. No single hormone acts by itself in regulating the meristematic activity in the root meristem. Division and differentiation are controlled by interactions between several hormones. Intensive research on plant stem cells has focused on how cell division is regulated to form specific plant organs and tissues, how differentiation is controlled, and how stem cell fate is coordinated. In this review, recent knowledge pertaining to the role of plant hormones in maintaining root stem cells including the QC is summarized and discussed. Furthermore, we suggest diverse approaches to answering the main question of how root stem cells are regulated and maintained by plant hormones.

Key words: Hormone cross-talk, plant hormone, quiescent centre, root apical meristem, root development, stem cell niche.

Introduction

A root is formed from a reservoir of undifferentiated cells, called root stem cells, in the root apical meristem (RAM). Plant hormones control root growth and development by balancing between cell division and differentiation, and their interactions are crucial for the temporal and spatial coordination of root development. Six classical plant hormones, auxin, abscisic acid (ABA), brassinosteroids (BRs), cytokinin (CK), ethylene, and gibberellins (GAs), are all involved in post-embryogenetic root organogenesis and regulate the formation and maintenance of the RAM. The effects of plant hormones and the regulating proteins are discussed in several reviews largely based on genetic and molecular studies (Benkova and Hejatko, 2009; Depuydt and Hardtke, 2011). In this review, we discuss the effect of individual hormones on the formation and maintenance of the RAM, especially the quiescent centre (QC) and root stem cells, and discuss recent findings on the effect of hormonal interactions. The possible involvement of non-hormonal factors that might interact with plant hormones through cell–cell communication is also discussed. Furthermore, we suggest diverse approaches to answering the main question of how root stem cells are regulated and maintained by plant hormones.

Structure and organization of the root cell

The *Arabidopsis* root has a simple, concentric structure (Fig. 1; Dolan *et al.*, 1993). The radial pattern of the root is composed of the outermost lateral root cap, epidermis, ground tissue (cortex and endodermis), pericycle, and the innermost stele. Along the longitudinal axis, the root is composed of a lateral root cap, columella, QC and initials/stem

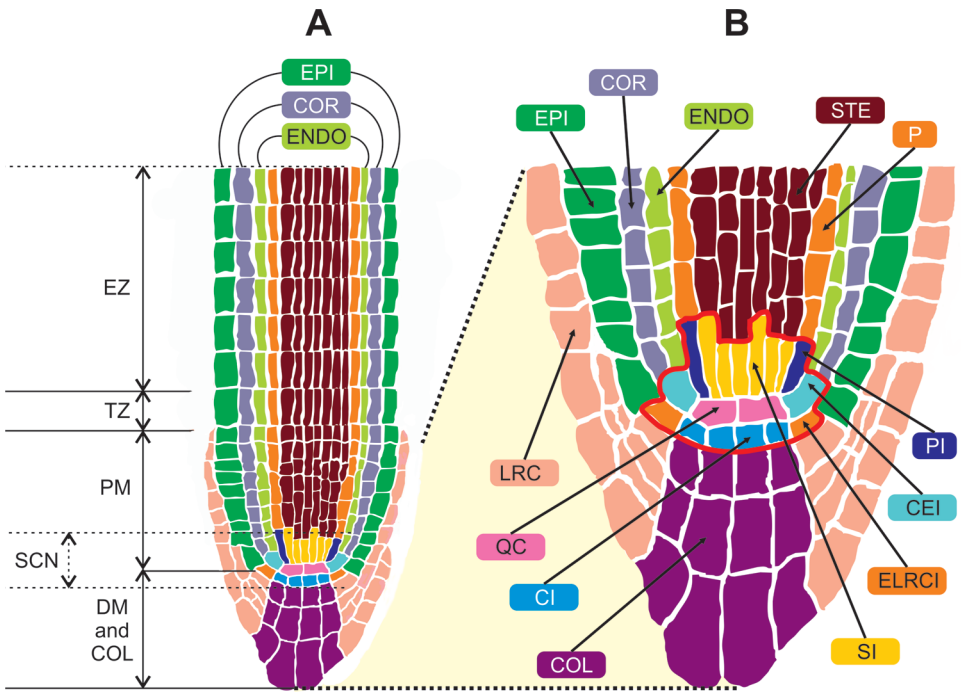

Fig. 1. Structure of the *Arabidopsis* root. (A) Schematic longitudinal section of the *Arabidopsis* root. There are three distinct developmental zones: the meristematic zone (MZ), the transition zone (TZ), and the elongation zone (EZ). The meristematic zone can be divided into the distal meristem (DM) and the proximal meristem (PM). In the meristematic zone, there is a 'stem cell niche' (SCN) that consists of the QC and initials (stem cells). (B) Schematic longitudinal section of the *Arabidopsis* root tip. The area enclosed with the red line shows the SCN. Around the QC, there are four initials (root stem cells). QC, quiescent centre (purple); CEI, cortex/endodermis initials (light green); ELRCI, epidermis/lateral root cap initials (light brown); CI, columella initials (sky blue); SI, stele initials (light ochre); LRC, lateral root cap (pink); EPI, epidermis (green); COR, cortex (light sky blue); ENDO, endodermis (dark ochre); STE, stele (dark brown. The same colours are used to represent the same tissues (or cells) in Fig. 2.

cells, proximal meristem, transition zone, elongation zone, and differentiation zone.

The root structure of plants is formed by a balance between cell division and differentiation. There are groups of undifferentiated meristematic cells which have the potential to divide, and they are called initials or stem cells. The mitotically less active QC is surrounded by the initial cells, which forms a 'stem cell niche' (SCN), and an unknown factor from the QC inhibits the differentiation of the abutting stem cells (van den Berg *et al.*, 1997). Stem cells divide infrequently, and their descendants do not directly differentiate but instead constitute an intermediate cell population of more rapidly dividing progenitors. Upon developmental cues, these progenitor cells give rise to certain types of cells in each layer by asymmetric divisions that produce clonally related cells. These root cells, which reside in the root tip, undergo asymmetric divisions to form transit amplifying cells at the boundary of the proximal meristem, and they exit the cell cycle at the transition zone to start differentiating into specific tissues, including epidermis, cortex/endodermis, and vascular tissues in the elongation/differentiation zone (Perilli *et al.*, 2012). At the distal part, there are columella initials beneath the QC, which differentiate into the columella root cap. The columella and lateral root cap form a root cap that surrounds the RAM. The root cap protects the SCN (root meristematic cells) and is also able to detect changes in the gravity vector and in the state of the rhizosphere (Arnaud *et al.*, 2010).

QC cells are initially formed by the transverse division of a hypophyseal cell during the heart stage of embryogenesis (Scheres *et al.*, 1994). The QC is the source of stem cell initials. QC cells are pluripotent and are maintained at the G_1/S checkpoint in the cell cycle; thus, they divide infrequently (Jiang and Feldman, 2005). QC cells act as integrators for many processes and events requisite for root meristem establishment and maintenance. It was proposed that QC cells may send short-range non-cell-autonomous signals which help the initials to remain in an undifferentiated state (van den Berg et al., 1997; Scheres, 2007). When an asymmetric division of a stem cell initial occurs, generally a daughter cell which has contact with the QC remains as an initial cell while another daughter cell that is separated from the QC divides (Scheres, 2007).

Role of plant hormones on stem cell maintenance in roots

Auxin

Auxin is the first hormone whose signal transduction pathway was well characterized. Auxin binds to the auxin receptor

known as TRANSPORT INHIBITOR RESPONSE 1/ AUXIN-SIGNALING F-BOX PROTEINs (TIR1/AFBs), which is a component of the E3 ubiquitin ligase complex. Auxin-bound TIR1/AFBs targets the transcriptional repressor of the auxin/indole-3-acetic acid (Aux/IAA) family for proteosome-mediated degradation. Without auxin, the Aux/IAA repressor is bound to auxin response factors (ARFs). After the proteolysis of the Aux/IAA repressor, ARFs activate the transcription of auxin-responsive genes (Dharmasiri *et al*., 2005; Kepinski and Leyser, 2005; reviewed in Berleth *et al*., 2004).

Auxin is synthesized at the shoot tip and is transported down to the QC and to the columella initials in the root tip in which the auxin maximum is formed (Friml *et al*., 2003; Blilou *et al*., 2005). In addition, local biosynthesis of auxin in the root substantially contributes to auxin homeostasis in the root tip (Petersson *et al*., 2009). This was recently confirmed by a study demonstrating that auxin accumulates in the QC, root initials, and the lateral root cap, shown by an auxin sensor DII-VENUS (Brunoud *et al*., 2012). The auxin maximum specifies the hypophysis and the QC, regulates root meristem formation, and is a positional cue for the SCN (Sabatini *et al*., 1999). This feature arises due to the spatially distinct acropetal and basipetal auxin transport system in the root tip. The presence of the auxin maximum and gradient along the root is due to the collective activities and topology of the PIN-formed (PIN) proteins, the AUX1/LAX family proteins (Blilou *et al*., 2005; Grieneisen *et al*., 2007; Ugartechea-Chirino *et al*., 2010), and the multidrug-resistant/P-glycoprotein (MDR/PGP) family proteins (BlakesLee *et al*., 2007). In particular, AtPIN4 mediates the sink-driven auxin gradients and the resulting auxin maximum in the QC and the columella initial, and it signals the QC to regulate auxin-driven root patterning (Friml *et al*., 2002). PIN proteins are expressed in specific but overlapping regions in the RAM (Blilou *et al*., 2005). AUX1/LAX proteins are also involved in the formation of the RAM during the early stage of embryogenesis, especially formation of the root cap (Ugartechea-Chirino *et al*., 2010). However, AUX1/LAX proteins do not seem to be directly involved in the QC and stem cell maintenance or activity.

The topological patterning of PIN proteins in the root is regulated by the PINOID (PID) protein kinase (Benjamins *et al*., 2001). PID phosphorylates PIN proteins to drive them to the basal side (rootward) of the plasma membrane (PM). Apical generation of PIN polarity is caused by non-polar PIN secretion and clathrin-mediated PIN endocytosis, as well as ARF-GNOM-dependent basal PIN endocytotic recycling (Geldner *et al*., 2003; Dhonukshe *et al*., 2007, 2008). Another way to change the topology of PIN proteins is by controlling endocytosis through clathrin (Dhonukshe *et al*., 2007; Robert *et al*., 2010; Kitakura *et al*., 2011). Clathrin-mediated endocytosis is mediated by ABP1 and ROP6 GTPase (Robert *et al*., 2010; Chen *et al*., 2012). It might be interesting to determine if the phosphorylation of PIN proteins and clathrin-mediated endocytosis are directly involved in the maintenance or activation of the QC and the activity of the root initials.

Auxin regulates the maintenance of the QC and the activity of the root meristem through PLETHORA (PLTs) (Galinha *et al*., 2007), ARFs (Aida *et al*., 2004), and PID (Friml *et al*., 2004). Auxin determines the position of the SCN in the developing root given that the location of the auxin maximum matches the SCN in the root (Jiang and Feldman, 2010). PLT1, PLT2, PLT3, and BABYBOOM (BBM) are involved in embryonic root development and stem cell maintenance (Aida *et al*., 2004; Galinha *et al*., 2007). Expression of PLTs and BBM is induced by the action of PIN protein-driven auxin gradients, and they are positively regulated by MONOPTEROS/ARF5 (MP/ARF5) and NONPHOTOTROPIC HYPOCOTYL4/ AUXIN RESPONSE FACTOR 7 (NPH4/ARF7) to direct embryonic root patterning (Aida *et al*., 2004; Galinha *et al*, 2007). Conversely, PIN transcription is maintained by PLT proteins to stabilize the position of the SCN (Blilou *et al*., 2005; Grieneisen *et al*., 2007). PLTs are dose-dependent master regulators of root development (Galinha *et al*., 2007). PLT at a high concentration maintains the QC and stem cell activity and, at a low concentration, regulates the division and differentiation of the transit amplifying cells.

The expression of auxin-regulated transcription factors such as *PLT* genes overlaps with the expression of QC-specifying genes such as *SHORT-ROOT* (*SHR*) (Benfey *et al*., 1993), *SCARECRAW* (*SCR*) (Sabatini *et al*., 2003), and *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*) around the SCN (Haecker *et al*., 2004; Sarkar *et al*., 2007). Auxin acts as a signal for all SCR- and SHR-expressing cells indirectly through PINs and PLTs to acquire a QC identity (Sabatini *et al*., 2003). SHR and SCR proteins belong to the GRAS family of transcription factors and they direct the QC to maintain the proper functioning of the QC and SCN (Helariutta *et al*., 2000; Sabatini *et al*., 2003). The asymmetric division of the endodermis/cortex initial separates daughter cells into two ground tissue layers, the endodermis and cortex layers, due to the actions of SCR and SHR (Hirsch and Oldroyd, 2009). Interestingly, the defective *shr* primary root was not due to the reduced level of auxin or its synthesis but was instead associated with the loss of PIN auxin carrier accumulation (Lucas *et al*., 2011). Since *PIN* genes are not direct targets of SHR (Levesque *et al*., 2006), PIN protein abundance must be regulated by SHR at the post-transcriptional level (Lucas *et al*., 2011).

It has been demonstrated that the auxin-regulated redox status of the QC might be one of the factors involved in maintaining the slowly dividing cells. Glutathione/glutathione disulphide (GSH/GSSH) and ascorbic acid/ dehydroascorbic acid (AA/DHA) are important redox-regulating couples for maintaining the QC at the root tip (Jiang *et al*., 2003). Treatment with ascorbic acid (AA) abolished the normal establishment of the auxin maximum (Lee *et al*., 2007), whereas treatment with exogenous auxin and DHA (the oxidized form of AA) had the opposite effect (Potters *et al*., 2004; Lee *et al*., 2007). The QC is in a more oxidized state than the fast-dividing neighbouring cells because the QC has lower levels of GSH and AA than its surrounding cells (Jiang *et al*., 2003). Considering that the expression of ascorbate oxidase (AAO) can be transcriptionally activated by auxin and can degrade IAA in turn, it has been proposed that the activity of the cell division at the QC could be established

by mutual control between auxin and the AAO level (Jiang *et al*., 2003; Jiang and Feldman, 2005).

In addition to the redox status and/or reactive oxygen species (ROS) in the RAM, reactive nitrogen species (RNS) such as nitrogen oxide (NO) also regulates primary root growth and auxin transport (Fernandez-Marcos *et al*., 2011). An increase in the endogenous level of NO stimulated cell differentiation. Genetic studies using mutants with an elevated level of NO exhibited reduced expression of *DR5pro::GUS/GFP*, auxin transport, and the PIN1 level in the primary root (Fernandez-Marcos *et al*., 2011). As a consequence, NO-overexpressing mutants had a similar root phenotype to that of the *pin1* mutant, which has a disrupted organization of the QC and the surrounding stem cells including the columella stem cells. Recently, it was shown that an increase in the NO level in the cell stimulates auxin-induced gene expression, and depletion of NO inhibits AUX/IAA degradation (Terrile *et al*., 2012). Surprisingly, NO enhances the interaction between TIR1/AFB(s) and AUX/IAA and stimulates S-nitrosylation on the TIR1/AFB(s) protein (Terrile *et al*., 2012). This report provides evidence that TIR1 is post-translationally modified by NO and that there is another redox control through oxidative molecules. It has yet to be determined if this mechanism controls the QC and/or root stem cells.

WOX5 and quiescent-centre-specific homeobox (QHB) are found in the root of *Arabidopsis* and rice, respectively (Kamiya *et al*., 2003; Haecker *et al*., 2004). Both are specifically expressed in the QC cells and are thought to be important in QC specification. Interestingly, QC-specific *WOX5* expression is also required to maintain the distal root stem cells, such as columella initials (Sarkar *et al*., 2007). Recently, auxin was found to act upstream of *WOX5* and *PLT1* and regulate distal stem cell (DSC) differentiation (Ding and Friml, 2010). AUXIN RESISTANT 3/IAA17 (AXR3/IAA17) promoted DSC differentiation, while auxin-responsive ARF10 and ARF16 maintained the state of DSCs by inhibiting *WOX5* and *PLT1* gene expression.

Auxin regulates the formation of the root cap through the action of auxin-responsive ARFs. ARFs interact with AP3/ERF and NAC transcription factors, and they control root cap differentiation and root cap cell division (Arnaud *et al*., 2010). Auxin-responsive ARF5 and ARF7 stimulate expression of PLT genes, and PLTs and BBM also stimulate root cap differentiation (Aida *et al*., 2004; Galinha *et al*., 2007). ARF10, ARF16, and ARF17 are highly homologous ARFs and are regulated post-transcriptionally by the microRNA (miRNA), *MIR160* (Wang *et al*., 2005). The overexpression of *MIR160* and the *arf10arf16* T-DNA knock-out double mutant have the same mutant phenotypes—a retarded root, insensitivity to gravity, no starch granules in the columella, ectopic columella cell division, and enlarged columella initial cells which are unable to differentiate (Wang *et al*., 2005). In addition, *miR160* inhibits ARF10 and ARF16 at the distal meristem so that it depresses *WOX5* expression (Ding and Friml, 2010). To sum up, auxin regulates the SCN in two ways; it inhibits the activation of the QC (Jiang *et al*., 2003) and promotes differentiation in the distal meristem (Wang *et al*., 2005; Ding and Friml, 2010).

Ethylene

Ethylene is a gas hormone produced by plants. This hormone inhibits cell elongation, and stimulates fruit ripening and senescence (Abeles *et al*., 1992). Regarding root development, ethylene up-regulates auxin biosynthesis and modulates transport-dependent auxin distribution, thereby inhibiting the expansion of cells leaving the root meristem and resulting in root cell inhibition (Swarup *et al*., 2007). *ETHYLENE OVEREXPRESSOR 1* (*ETO1*) encodes a ubiquitin E3 ligase that modulates the level of ACC SYNTHASE 5 (ACS5), and the *eto1* mutant overproduces ethylene. Interestingly, the *eto1* mutant showed supernumerary cell division of the QC, which provides evidence that ethylene promotes cell division in the QC (Ortega-Martinez *et al*., 2007). By the same reasoning, the mutant of CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), a negative regulator of ethylene action, phenocopied the *eto1* mutant. Because extra division was also seen in the naphthylphthalamic acid (NPA)-treated root, it was proposed that the effect of auxin on the QC depends on auxin-dependent ethylene biosynthesis for the QC (Ortega-Martinez *et al*., 2007). In maize, NPA enhanced the mitotic activity within the QC (Kerk and Feldman, 1995), and the NPA effect was reversed by ethylene (Ponce *et al*., 2005). The effect of ethylene on the QC activity in maize was the opposite of that reported by Ortega-Martinez *et al*. (2007) in which ethylene inhibited the QC cell division (Ponce *et al*., 2005). Ethylene also regulates statolith formation, which is a unique characteristic of the gravity-sensing columella cell in maize. In this regard, it was proposed that the communication between the root cap and QC and the resulting alteration of auxin distribution are the main controlling factors for the regulation of root cap size (Ponce *et al*., 2005).

Abscisic acid

ABA is a growth-inhibiting hormone that is involved in the induction of desiccation tolerance, dormancy, stress tolerance, and stomatal closure (Finkelstein *et al*., 2002). In addition, ABA inhibits root growth at micromolar concentration, but stimulates it at a lower concentration. Recently, it has been demonstrated that ABA promotes QC maintenance and suppresses stem cell differentiation (Zhang *et al*., 2010). ABA also inhibits cell division in the root meristem and reduces the cell differentiation rate, resulting in an increase in the number of cells in the division zone and in the transition zone, through the actions of MP and WOX5, but without involvement of ethylene biosynthesis and its action (Zhang *et al*., 2010).

There are several ABA-related genes whose mutations cause defects in the RAM structure. A loss-of-function mutant for *TETRATRICOPEPTIDE-REPEAT THIOREDOXIN-LIKE 1* (*TTL1*) reduced tolerance to NaCl and osmotic stress and had a disorganized RAM (Rosado *et al*., 2006). The *lateral root organ-defective* (*latd*) mutant of *Medicago truncatula* has an arrested primary root and shows a disorganized root tip, and there are no root meristem cells or root cap columella cells (Liang *et al*., 2007). The semi-dominant *no-hydrotropic response 1* (*nhr1*) mutant has an abnormal RAM, root cap,

and QC cells, and its seedlings showed reduced root sensitivity to ABA (Eapen *et al*., 2003). A mutation in *ABSCISIC ACID INSENSITIVE 8* (*ABI8*) caused a loss of RAM and produced a short root, and this root phenotype was due to the cessation of cell division at the root tip, leading to terminal differentiation of the RAM (Brocard-Gifford *et al*., 2004). Recently, it was demonstrated that ABA can act through ARF2 to inhibit HB33 and control RAM (Wang *et al*., 2011).

Brassinosteroids

BRs are polyhydroxylated steroid hormones that have pivotal roles in a wide range of plant growth and developmental processes, such as elongation/expansion in stems and roots, tolerance to various environmental stresses, and xylem differentiation during vascular development (reviewed in Yang *et al*., 2011). Upon the binding of BRs to BRASSINOSTEROID INSENSITIVE 1 (BRI1), a membrane-localized LRR-RLK (leucine-rich repeat receptor-like kinase) protein, BRI1 forms a heterodimeric complex with bri1-associated receptor kinase1/SOMATIC EMBRYOGENESIS RECEPTOR KINASE3 (BAK1/SERK3), and the fully activated BRI1/BAK1 complex then starts a signalling cascade activating the positively regulated transcription factors, brassinazole resistant1/bri1 EMS suppressor2 (BZR1/BES2) and BZR2/BES1 to initiate a wide range of gene expression and the subsequent plant growth and development (Sun *et al*., 2010; Yu *et al*., 2011).

BRs regulate root growth in a concentration-dependent manner. They promote root growth at low concentrations and inhibit it at high concentrations (Kim *et al*., 2007). Many BR-deficient or -insensitive mutants are defective in root growth and show a short-rooted phenotype (Chory *et al*., 1991; Mouchel *et al*., 2004). Whole-genome microarray analysis revealed that the BR biosynthetic enzyme, BR6ox2/Cyp85A2 cytochrome P450, is a direct endogenous target of SHR, and its expression domain in roots overlapped with that of SHR, which implies that BRs together with SHR are important regulators of vascular development in roots (Levesque *et al*., 2006). Interestingly, transcriptional profiling of the *Arabidopsis* root QC cells revealed that the BRL1 (BRI1-Like 1) transcript is significantly enriched in the QC where *PLT1* and *SCR* are highly expressed, playing a key role in QC establishment and maintenance.

Recently, it was demonstrated that BRs have a regulatory role in the control of cell cycle progression and differentiation in *Arabidopsis* root meristem. Mutants with enhanced BR signalling, such as *bes1-D*, or plants treated with BR showed a premature cell cycle exit that resulted in the early differentiation of meristematic cells, thus negatively influencing the meristem size and overall root growth (Gonzalez-Garcia *et al*., 2011). In addition, BRs promoted QC division and differentiation of distal stem cells. It is interesting to note that only brassinosteroids seem to alter the expression of the regulators of the SCN, such as *SCR* and *WOX5*, for the maintenance of stem cell identity and organization (Hacham *et al*., 2011). Despite these recent reports, the molecular links between BR signalling and control of root stem cell maintenance are not well established as yet.

Cytokinin

CK signal transduction involves the histidine to aspartate phosphorelay system. It is well known that three *Arabidopsis* histidine kinases (AHKs), six histidine phosphotransfer factors (AHPs), and 23 response regulators (ARRs) are involved in the *Arabidopsis* CK signal transduction (To and Kieber, 2008).

Many reports demonstrated that CK stimulates the differentiation in the root proximal meristem which leads to a decrease in the meristem size and growth (Dello Ioio *et al*., 2007, 2008; Moubayidin *et al*., 2010); thus, it acts as an antagonistic counterpart of the non-cell-autonomous cell division signal, auxin. CK negatively regulated *PIN1* and *PIN4* gene expression and up-regulated *PIN7* expression, and thus modulated auxin distribution which is important for the regulation of the activity and size of the root meristem (Ruzicka *et al*., 2009). Recently, SHORT HYPOCOTYL 2 (SHY2/IAA3), an auxin signalling repressor, has emerged as the common target for auxin/CK regulation of meristem propagation and differentiation. Up-regulation of *SHY2* induced by both CK-activated ARABIDOPSIS RESPONSE REGULATOR1 (ARR1) and ARR2 increased cell differentiation at the division–elongation transition zone of the proximal meristem. In contrast, suppression of SHY2 and enhancement of GA biosynthesis driven by auxin resulted in the suppression of ARR1, and thus it ensured a prevalence of cell division over cell differentiation at the proximal meristem area (Moubayidin *et al*., 2010). However, CK seems to have no role in the specification of the QC and the maintenance of the root stem cells (Dello Ioio *et al*., 2007).

Gibberellin

GA is a regulator of shoot and root growth, germination, flowering time, and elongation. GA signal transduction starts when GA binds to its receptor GA INSENSITIVE DWARF1 (GID1). This binding enhances the GID1–DELLA interaction and results in the rapid degradation of DELLA by the ubiquitin–proteosome pathway and allows for the transcriptional regulation of GA-responsive genes (reviewed in Schwechheimer, 2008).

GA controls cell proliferation in the root meristem. GA promoted the cell proliferation rate in the root meristem by removing DELLA proteins which enhanced the levels of the cell cycle inhibitors *Kip-relate protein 2* (*KRP2*) and *SIAMESE* (*SIM*) (Achard *et al*., 2009). In particular, GA signalling in the endodermis controlled the equivalent DELLA pathway-dependent cell division in the proximal meristem (Ubeda-Tomás *et al*., 2009). GA biosynthesis genes such as *ent*-kaurene oxidases (*GA3*, *GA4*) are up-regulated in the QC (Nawy *et al*., 2005), and the GA biosynthesis mutant has a smaller meristem than the wild type (Achard *et al*., 2009; Ubeda-Tomás *et al*., 2009). Nonetheless, there was no change in the expression of cell type-specific markers such as *pSCR::SCR-GFP*, *pSHR::SHR-GFP*, and *QC46*. Therefore, GA signalling does not seem to be involved in the control of SCN specification (Achard *et al*., 2009).

Hormonal cross-talk

The size of the root meristem is determined by the balance between the rate of cell division of stem cells and transit amplifying cells in the meristem and the rate of cell elongation followed by the differentiation of cells in the differentiation zone. No single hormone can function to strike a balance between cell division and cell differentiation. Plant hormones act synergistically or antagonistically in developmental processes. There are several known incidences of cross-talk between the different hormones regulating cell division and differentiation in roots, but not many studies have been reported on the establishment and maintenance of the root stem cells including the QC.

Auxin and CK

Auxin and CK have an antagonistic functional relationship in controlling root growth during post-embryonic development, which regulates the size of the meristem. On the one hand, CK stimulates cell differentiation at the cell division and elongation zone by suppressing auxin signalling and transport. On the other hand, auxin promotes cell division by inactivating CK signalling (Dello Ioio *et al.*, 2007, 2008; Ruzicka *et al.*, 2009). CK induces ARR1 and ARR12, and these proteins up-regulate *SHY2*/*IAA3* in the transition zone of the protophloem (Moubayidin *et al.*, 2010). SHY2/IAA3 negatively regulates ARFs through protein–protein interaction and then down-regulates the *PIN* genes—*PIN1*, *PIN3*, and *PIN7* (Dello Ioio *et al.*, 2008). Recently, it was reported that polar auxin transport is regulated by phloem-transported, symplastic CK transport and that it maintains the vascular pattern of the root meristem (Bishop *et al.*, 2011). More surprisingly, PIN1 abundance can be rapidly modulated by CK through endocytosis during plant organogenesis (Marhavy *et al.*, 2011). Auxin may influence the CK level because SHY2 down-regulates *Arabidopsis ISOPENTENYLTRANSFERASE* (*AtIPT*), which is the rate-limiting enzyme in CK biosynthesis (Dello Ioio *et al.*, 2008). The level of PIN proteins in the RAM can also be changed at the post-transcriptional level (Zhang *et al.*, 2011). In addition, CK modulates the endocytotic trafficking of PIN1, which is independent of transcriptional control by CK (Marhavy *et al.*, 2011). Interestingly, in the *arr7* and *arr15* mutants, there was a misexpression of the *SCR*, *PLT1*, and *WOX5* genes. However, there are few reported studies on the effect of auxin–CK cross-talk on the maintenance of root stem cells. It would be interesting to know whether similar mechanisms related to the auxin–CK interaction in the proximal root meristem and transition zone can be found in the maintenance of stem cells.

Auxin and BR

The root-specific, BR deficiency mutant *brevis radix* (*brx*) has a reduced meristem size (Mouchel *et al.*, 2004). BRX is a PM-associated protein, and it is translocated to the nucleus upon auxin treatment. Interestingly, the application of an auxin transport inhibitor retained BRX in the PM and phenocopied the *brx* root meristem phenotype, which implies that the nuclear localization and the following action of BRX is under control of the cellular auxin concentration or auxin flux (Scacchi *et al.*, 2009). Recently, it was found that nuclear translocation of BRX from the PM is related to the auxin gradient and endocytosis in the division and transition zones in the root (Santuari *et al.*, 2011). The BRX-mediated BR signalling event may have cross-talk with auxin signalling, as *PIN3* is down-regulated in the *brx* mutant, which can be restored by treatment with brassinolide (Mouchel *et al.*, 2006). Furthermore, auxin-responsive gene expression was globally impaired in the *brx* mutant, demonstrating that the BR levels are rate limiting for auxin-responsive transcription. Interestingly, BRX seems to be a common target of both negative and positive auxin signalling pathways. SHY2/IAA3 (an auxin signalling repressor) in the root transition zone down-regulates *BRX* expression. Actually, the phenotype of *brx* resembles that of *shy2-D* carrying a gain-of-function mutation in the *SHY2* gene. In reverse, *BRX* is a target gene of MP/ARF5 (Scacchi *et al.*, 2010). So far, there is no direct evidence to prove that the auxin–BR interaction directly controls maintenance of the QC and the stem cell activity.

Auxin, GA, and ethylene

Auxin stimulates root growth by modulating the GA response (Fu and Harberd, 2003). Several reports have shown that GA-promoted root growth depends on auxin. For example, defects in auxin transport and signalling delayed GA-mediated root growth by decreasing the degradation of the DELLA protein (RGA, REPRESSOR-OF-*ga1-3*) in the nucleus (Fu and Harberd, 2003). Recently, it was reported that GA increased the abundance of PIN, and a decrease in PIN proteins was not at the transcriptional level but instead occurred post-translationally during vacuolar degradation (Willige *et al.*, 2011).

There have been numerous reports that mutants defective in auxin transport and signalling show ethylene-insensitive root growth (reviewed in Benková and Hejátko, 2009). The ethylene-overexpressing *acc-related long hypocotyl 1* (*ahl1*) mutant shows disorganized columella cells or an additional columella column, and has altered sensitivity to auxin (Vandenbussche *et al.*, 2003). Recently, it was found that the inhibitory action of ethylene on root growth requires auxin biosynthesis, transport, and responses. *TRYPTOPHAN AMINOTRANSFERASE* (*TAA1*)-mediated local activation of auxin biosynthesis modulates tissue-specific ethylene responses; thus, mutations in *TAA1* resulted in root-specific ethylene insensitivity for root growth inhibition. Additionally, *taa1* and *taa2* show a reduction in the meristem size and collapse of the root meristem (Stepanova *et al.*, 2008).

An antagonistic auxin–ethylene interaction has an important role in DELLA protein-mediated root patterning. DELLA stimulates cell cycle inhibitors to lower the cell proliferation rate without affecting the SCN (Achard *et al.*, 2003). Ethylene inhibits the degradation of DELLA, whereas auxin stimulates its degradation (Achard *et al.*, 2003; Fu and Harberd, 2003). Recently, CULLIN3 (CUL3)-based

E3 ligase was shown to modulate ethylene production and influence root growth. CUL3 directly bound to ETO1 and regulated the ACC SYNTHASE 5 (ACS5) turnover rate. The *cul3* knock-down mutant inhibited primary root growth by reducing the size of the RAM and disrupting the distal root patterning process in an ethylene-dependent manner, which implies that CUL3 is required for the division and organization of the root SCN and the columella root cap (Thomann *et al*., 2009).

BR, JA, and auxin

Jasmonic acid (JA) has been known to inhibit primary root growth, and a *coi1* mutant defective in the JA receptor CORONATINE INSENSITIVE 1 (COI1) was fully insensitive to JA in root growth inhibition (Feys *et al*., 1994). BR treatment or a genetic transformation of *DWF4* into a JA-sensitive double mutant of *coi1* and *partially suppressing coi1* (*psc1coi1*) attenuated this jasmonate inhibition of root growth. In return, JA suppressed *DWF4* gene expression through the COI1-mediated JA signalling pathway (Ren *et al*., 2009; Huang *et al*., 2010). Recently, it was reported that JA-induced root inhibition was involved in the reduction of root meristem activity; the JA-activated MYC2 transcription factor, directly bound to the promoter of the *PLT1* and *PLT2* gene, reduced their gene expression and resulted in the promotion of QC division and columella stem cell differentiation (Chen *et al*., 2011).

Concluding remarks and perspectives

Many plant hormones and protein factors have been reported to control establishment, maintenance, and mitotic activation of the QC (summarized in Table 1). Moreover, interactive coordination of plant hormones regulates cell division and differentiation in the root meristems (summarized in Fig. 2). Among them, auxin is the core hormone for the regulation of root growth and differentiation. Auxin appears to be a central factor that allows molecular communication between different tissue layers. In addition, auxin acts as an integrating factor of the activities of other hormones (Jaillais and Chory, 2010). Recently, endocytosis has emerged as one of the main mechanisms for the regulation of root meristem growth. Localization of PIN proteins is regulated by endocytosis (Kitakura *et al*., 2011). Interestingly, endocytosis appears to be very active in the QC, where the auxin level is the highest (Grieneisen *et al*., 2007; Petersson *et al*., 2009). The expression of *DR5::GUS* around the QC is inhibited by tyrphostin A23, an endocytosis inhibitor (Santuari *et al*., 2011). Therefore, it might be interesting to see if endocytosis influences the maintenance of root stem cells, including the QC. It was previously postulated that endocytotic activity in the RAM might provide positional information to interpret the auxin gradient and the localized biosynthesis and/or action site of plant hormones (Ubeda-Tomás *et al*., 2012). Endocytosis-driven PIN localization is mediated by auxin, BR, cytokinin, and JA, and is mediated by PIN proteins. Therefore, endocytosis might have a role in plant hormonal cross-talk.

Recently, two studies from Wang and his colleagues paved the way to interpret the interaction among hormones such as BR, phytochrome, and GA and their downstream regulators BZR1, PIF4, and DELLA, respectively, during photomorphogenesis and hypocotyl elongation (Bai *et al*., 2012; Oh *et al*., 2012). ChIP-seq combined with sequential immunoprecipitation of chromatin in doubly transformed *Arabidopsis* by different myc- or yellow fluorescent protein (YFP)-tagged transcription factors (i.e. BZR-myc and PIF4–YFP) led to the finding of common binding sites on the chromatin and their regulatory genes. This elegant strategy can be applied to the field of roots to dissect molecularly the effect of hormonal cross-talk on the regulation of root stem cell activity at the transcriptional level since the fluorescence-activated cell sorting method is now widely available for the analysis of cell type-specific responses in plants (Nawy *et al*., 2005; Evrard *et al*., 2012). Hopefully, a similar large-scale approach can also be applied to protein interactome and

Table 1. Effect of plant hormones on the root apical meristem activity.

Hormone	QC activation	DM differentiation	PM division	PM differentiation	Proteins involved
Auxin	–	+	+	–	PINs (Blilou *et al*., 2005; Griffiths *et al*., 2006); PLTs (Blilou *et al*., 2005); AXR3/IAA17 (Sabatini *et al*., 1999); TMO5 and TMO7 (Schlereth et al., 2010); ARF10 and ARF16 (Wang *et al*., 2005); TAA1 and TAR2 (Stepanova *et al*., 2008); SHY2 (Moubayidin *et al*., 2010)
Ethylene	+	ND	ND	ND	ETO1 (Ortega-Martinez *et al*., 2007);
ABA	–	–	ND	ND	TTL1 (Rosado *et al*., 2006); ABI8 (Brocard-Gifford *et al*., 2004); NHR1 (Eapen *et al*., 2003)
BR	+	+	–	+.	BRX (Santuari *et al*., 2011); BRI1and BES1 (Gonzalez-Garcia *et al*., 2011; Hacham *et al*., 2011; Santuari *et al*., 2011)
CK	NE	ND	–	+	ARR1 and ARR12 (Moubayidin *et al*., 2010); ARR7 and ARR15 (Müller and Sheen, 2008)
GA	NE	ND	+	–	SCR (Sarkar *et al*., 2007); SHR (Lucas *et al*., 2011)
JA	+	+	ND	ND	MYC2, JAZ and COI1 (Chen *et al*., 2011; Sun *et al*., 2011)

–, inhibition; +, stimulation; NE, no effect; ND, not determined; DM, distal meristem; PM, proximal meristem; QC, quiescent centre.

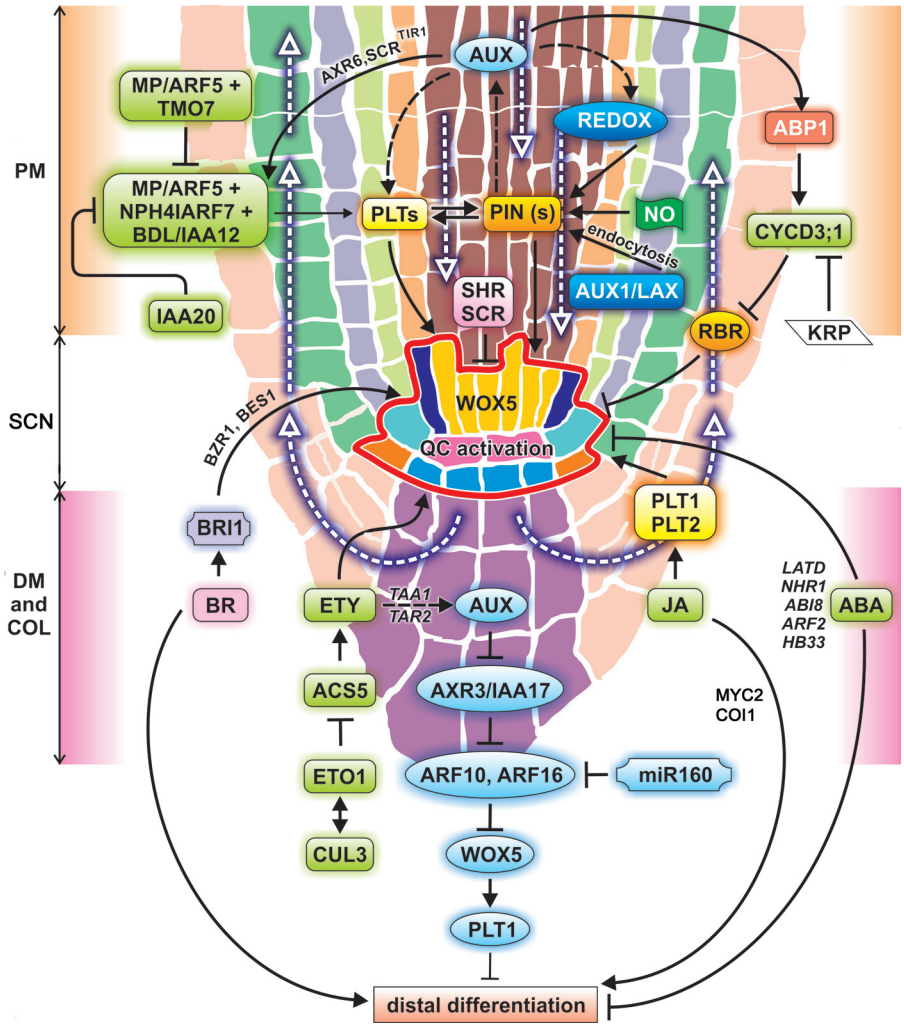

Fig. 2. Control of root stem cells by plant hormones and their cross-talks. Different colours and diagrams are used for each hormone and its components. Auxin might inhibit the activation of the QC in several ways. One is through the canonical AXR6, $SCF^{TIR1/AFB(s)}$, MP/ARF5, BDL/IAA12, PLT, and WOX5 pathways. The second way is through the ABP1, CYCD3;1, RBR, and WOX5 pathway. Domain II-less IAA20 also has an effect on the QC and root initials. Auxin can exert an effect on the QC and initials through PIN proteins, which are regulated by the redox status of the root tip and NO. Recently, it was found that AUX1/LAX increases endocytosis at the root apical meristem, which might change the location of the PIN protein. However, it is unknown whether this mechanism directly influences the QC. BR might stimulate QC activation by activating BRI1 kinase, BZR1, and BES1. ABA inhibits QC activation, which is supported by the results from studies on several ABA-related mutants such as *latd*, *nhr1*, *abi8*, and *arf2*. However, there is no direct evidence that they are involved in QC maintenance and/or activation. Ethylene stimulates QC cell division, and ETO1 and ACS5 are involved. JA activates the QC by transcriptionally repressing *PLT1* and *PLT2* through MYC2. It is interesting to note that auxin activates distal differentiation (i.e. differentiation of columella initials) by inhibiting *WOX5* expression through AXR3/IAA17, ARF10, and ARF16. This differs from the effect of auxin on the *WOX5* expression at the QC. This finding suggests that there may be different control mechanisms for hormones on the QC and columella initials. COL, columella; arrows, stimulation; lines ending in bar, inhibition; dotted lines with an arrowhead, activation of biosynthesis; antiparallel arrows, interaction; empty dotted lines with empty arrowhead, flow of AUX. Refer to the text for more detailed information.

phosphoproteome analyses to discover the point of cross-talk at the post-translational level.

In multicellular organisms, intercellular communication is one of the essential processes that coordinate collective growth, development, and responses to environmental stimuli. Since plant cells are surrounded by rigid cell walls, precise intercellular communication between cells has great importance in the shaping of the correct plant body plan. In the case of transcription factors in plants, >15% are mobile between cells in *Arabidopsis* (Lee *et al.*, 2006). Recently, mobile factors other than hormones have been reported to be involved in the regulation of the root meristem. Those mobile signals include post-translationally modified peptides, transcription factors, miRNAs, and redox-related protein(s) (Table 2). Considering that hormones are the initial signal that determines the direction of root development, mobile factors could be effectors

Table 2. Non-hormonal mobile factors that act in the root apical meristem.

Mobile factors	Function	Proposed movement	Reference
ACR4-CLE40	Receptor kinase–peptide	Columella cells to columella initials	De Smet *et al*. (2008); Stahl *et al*. (2009)
RGFs	Peptide	QC, columella, and its initials to cells in meristematic zone	Matsuzaki *et al*. (2010)
SHR	Transcription factor	Stele to endodermis	Gallagher and Benfey (2009)
TMO7	Transcription factor	Embryo proper to suspensor	Schlereth *et al*. (2010)
UBT1	Transcription factor	Lateral root cap to cells in elongation zone	Tsukagoshi *et al*. (2010)
MIR165/166	MicroRNA	Endodermis to stele	Carlsbecker *et al*. (2010)

ACR4, *ARABIDOPSIS* CRINKLY4; RGFs, root meristem growth factors; UBT, UPBEAT; MIR165/166, microRNA 165/166.

transmitting the hormonal commands among cells. Therefore, it is now necessary to understand the action of these mobile factors under the control of plant hormones beyond hormonal cross-talk. How do hormones regulate the activity and localization of non-hormonal mobile factors? How do the non-hormonal mobile factors cross-talk with hormones? How is the mobility of the mobile factor controlled in moving to a localized area so that development proceeds? In addition, filling in the gap between the site of synthesis and the site of action of hormones and non-hormonal molecules such as peptides, miRNA, and small interfering RNAs (siRNAs) in the root would be another puzzle to solve. This kind of non-autonomous control is accomplished through the plasmodesmatal regulation of intercellular transport. Taking advantage of using plasmodesmatal mutants defective in cell–cell transport (Ham *et al*., 2012) in combination with the real-time imaging of fluorescent proteins or molecules (reviewed in Beeckman and Friml, 2012) might be a choice for answering how hormones affect other mobile signals and discriminate between different sites for their synthesis and actions.

Morphogens are mobile signalling molecules that pattern developing cells and tissues in a dose-dependent manner (Rogers and Schier, 2011). For the past several years, auxin (Dubrovsky *et al*., 2011), SHR transcription factor (Koizumi *et al*., 2012), secreted peptides (Matsuzaki *et al*., 2010), and miRNAs (Miyashima *et al*., 2011) have been proposed to be plant morphogens. However, there is little direct connection between the concentration-dependent perception of the mobile molecules in a cell or tissue and the subsequent developmental patterning occurring accordingly. For example, it has been possible to examine the auxin gradient by using an auxin-responsive marker, such as *DR5::GFP* in roots. However, the gradient of auxin alone is not enough to demonstrate auxin as a morphogen. It is necessary to prove that there is a direct connection among the number of bound hormone receptors or mobile signalling molecules in a cell, the number of transcripts changed in the cell or specific tissue, and the concomitant change in developmental patterning. To prove and link gradient-dependent signals with the resulting developmental outcomes, recent biophysical approaches such as using nanosensors (Cullum and Vo-Dinh, 2000) and aptamers (Song *et al*., 2012) could be adopted to track the quantitative change in the putative morphogens and in the transcripts in a cell or tissue. By taking these approaches, the concept of morphogen-regulated development can be applied to the plant field, and it will be feasible to find the cross-talking points between two plant morphogens. Is it the concentration gradient of the mobile factors including plant hormones that is critical in determining cell identity or is it the intrinsic tissue sensitivity that determines cell fate? That is the next challenging question we need to answer in the near future.

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