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REVIEW PAPER

Hormonal control of cell division and elongation along differentiation trajectories in roots

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Abstract

The continuous development of roots is supported by a sustainable system for cell production and growth at the root tip. In the stem cell niche that consists of a quiescent centre and surrounding stem cells, an undifferentiated state and low mitotic activity are preserved by the action of auxin and abscisic acid. Stem cell daughters divide several times in the proximal meristem, where auxin and gibberellin mainly promote cell proliferation. Cells then elongate with the help of gibberellin, and become finally differentiated as a constituent of a cell file in the elongation/differentiation zone. In the model plant *Arabidopsis thaliana*, the transition zone is located between the proximal meristem and the elongation/differentiation zone, and plays an important role in switching from mitosis to the endoreplication that causes DNA polyploidization. Recent studies have shown that cytokinins are essentially required for this transition by antagonizing auxin signalling and promoting degradation of mitotic regulators. In each root zone, different phytohormones interact with one another and coordinately control cell proliferation, cell elongation, cell differentiation, and endoreplication. Such hormonal networks maintain the elaborate structure of the root tip under various environmental conditions. In this review, we summarize and discuss key issues related to hormonal regulation of root growth, and describe how phytohormones are associated with the control of cell cycle machinery.

Key words: Cell cycle, cell division, cell elongation, endoreplication, phytohormone, root.

Introduction

In higher plants, the root has four essential functions: (i) absorbance of water and nutrients; (ii) anchorage of the plant body to the ground and supporting the plant body; (iii) storage of nutrients; and (iv) vegetative reproduction. Generally, no genetic programme restricts root size, meaning that roots exhibit so-called indeterminate growth. For example, roots of woody plants sometimes continue growing for several hundred years, forming very long roots. The maximum rooting depth of *Juniperus monosperm* is >60 m (Stone and Kalisz, 1991). However, roots do not grow constantly. Root growth is greatly affected by external factors in the soil and by above-ground conditions (Schenk and Jackson, 2002; Chapman *et al.*, 2012).

Root cells are produced in the proximal meristem (PM), where most cells are dividing, and daughter cells accumulate in the longitudinal direction (Fig. 1). Most PM cells are small and rich in cytoplasm, but, after undergoing several mitotic divisions, they begin irreversible post-mitotic growth. The elongation/differentiation zone (EDZ) is a region of fast cell elongation without growth in the transverse direction (Fig. 1). In EDZ cells, nuclei are pushed to the side of cell walls by large vacuoles. In the model plant *Arabidopsis thaliana*, a characteristic zone, referred to as the transition zone (TZ), resides between the PM and the EDZ (Fig. 1). Cells in the TZ grow slowly in both length and breadth. This zone is assumed to function as a buffer for the transition from cell division to cell elongation, but its precise role remains speculative (for a review, see Verbelen *et al.*, 2006).

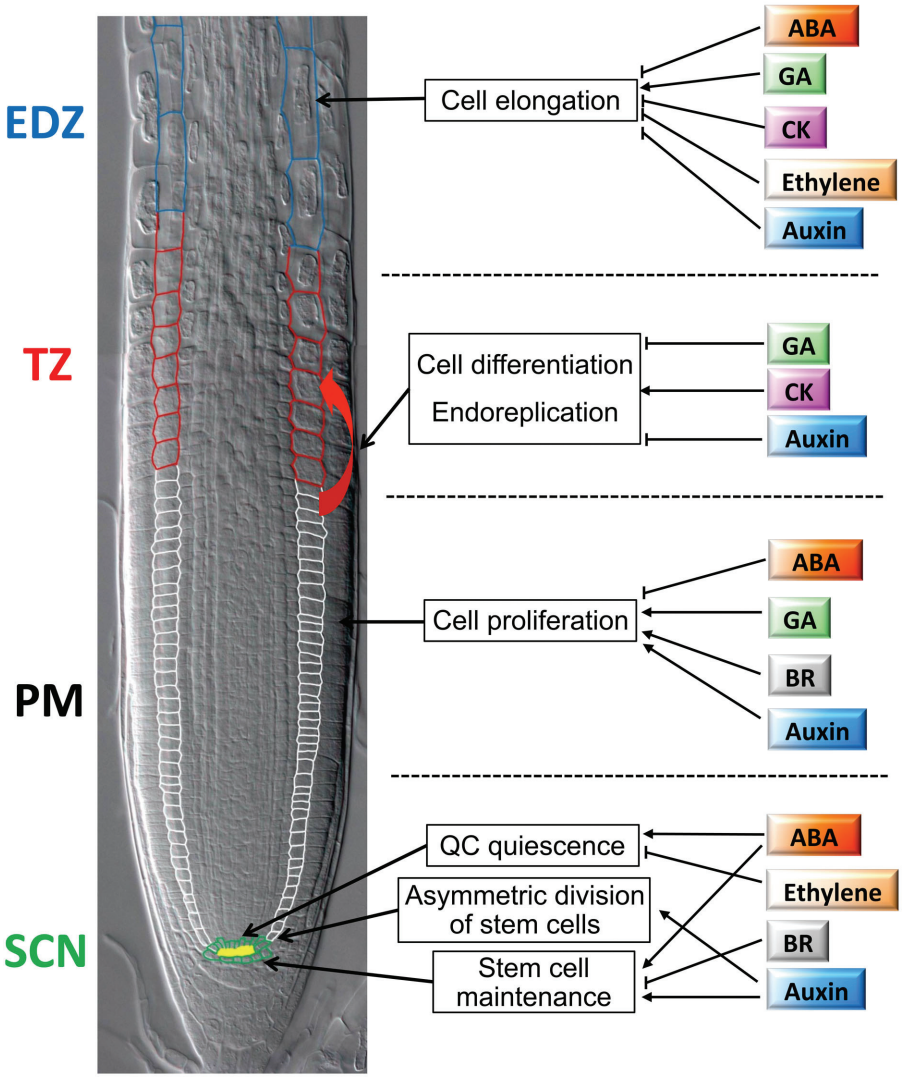

Fig. 1. Role of phytohormones in distinct root zones of *Arabidopsis*. The stem cell niche (SCN) contains the quiescent centre (yellow) and surrounding stem cells (green). The cortex cell file is divided into three zones: the proximal meristem (PM), the transition zone (TZ), and the elongation/differentiation zone (EDZ). The involvement of phytohormones in each zone is shown on the right-hand side of the figure.

Recent studies have revealed that phytohormones play an important role in controlling cell division, cell growth, and cell differentiation in distinct zones of roots. Their involvement in root growth has been highlighted in terms of cell cycle regulation and DNA polyploidization. Here, we review the functional role of various phytohormones in the establishment and maintenance of the three root zones, and discuss the cross-talk of phytohormones during continuous root development. Although secreted peptides and their receptors have recently been shown to play roles in root development (for reviews, see Delay *et al.*, 2013; Yamada and Sawa, 2013), we have chosen to focus only on the roles of phytohormones in the development and maintenance of root zones.

Stem cell specification and cell division in the proximal meristem

At the apical end of the root meristem, multipotent stem cells surround the quiescent centre (QC), which maintains the undifferentiated state of stem cells by sending short-range non-autonomous signals (Perilli *et al.*, 2012). Thus, the QC and surrounding stem cells constitute the stem cell niche (SCN; Fig. 1). Stem cells undergo asymmetric cell division, giving rise to daughter cells that divide several times to generate a transit amplifying cell population in the PM. The activity of the root meristem is determined by stem cell specification and cell division in the PM.

Auxin

Auxin controls expression of core cell cycle regulators

Auxin is an important long- and short-distance signal and controls multiple developmental processes, including root patterning (Sabatini *et al.*, 1999; Friml *et al.*, 2002; Petersson *et al.*, 2009), cell division, and cell elongation in roots (Ding and Friml, 2010). Polar auxin transport, which is mediated by PIN-FORMED (PIN) efflux carriers, is essential for creating auxin gradients and for proper development of organs

(Tanaka *et al.*, 2006). Auxin signalling involves transport inhibitor response 1 (TIR1), auxin response factors (ARFs), and auxin/indole acetic acid (Aux/IAA) transcriptional repressors. Aux/IAA proteins bind ARFs and prevent them from transcribing auxin-responsive genes. However, in the presence of auxin, the F-box protein TIR1 is activated and promotes degradation of Aux/IAA proteins, thereby inducing ARF-dependent expression of auxin-responsive genes (Ljung, 2013).

Classical experiments revealed that exogenously applied auxin stimulates cell division in plant tissues and cultured cells (Davies, 1995). Accumulating evidence indicates that auxin acts on multiple targets which control cell proliferation. Plants contain eight types of cyclin-dependent kinases (CDKs), CDKA–CDKG and the CDK-like kinase (CKL). Of these, CDKA plays a critical role in both G_1 to S and G_2 to M progressions (Inagaki and Umeda, 2011). Auxin has been shown to induce the expression of *CDKA;1* in *Arabidopsis* seedlings (Hemerly *et al.*, 1993; Ferreira *et al.*, 1994; Doerner and Celenza, 2000). Global transcript profiling analysis revealed that various cyclin genes, such as *CYCB1* and *CYCA2*, are also potentially regulated by auxin (Roudier *et al.*, 2003; Hartig *et al.*, 2005). Indeed, auxin-responsive elements (AuxREs) were found in the promoter regions of these cyclins (Hu *et al.*, 2003; Roudier *et al.*, 2003); however, the functional relevance of such AuxREs has not yet been investigated. Himanen *et al.* (2002) showed that the transcript levels of the CDK inhibitors, *KIP-RELATED PROTEIN 1* (*KRP1*) and *KIP-RELATED PROTEIN 2* (*KRP2*), were reduced by treatment with 1-naphthaleneacetic acid (NAA), which induces simultaneous formation of lateral roots. In other words, KRP1 and KRP2 prevent pericycle cells from proliferating under normal conditions; this idea is supported by the fact that the *krp2* mutant produces lateral roots at a higher density than wild-type plants (Sanz *et al.*, 2011). This suggests that auxin is involved in the activation of cell division by down-regulating CDK inhibitors. In addition to transcriptional control, auxin is also involved in the stabilization of cell cycle regulators. In the *Arabidopsis* genome, there are six types of E2F transcription factor. E2F transcription factors are crucial for the control of the G_1 to S progression. One of the *Arabidopsis* E2F transcription factors, E2FB, is stabilized by auxin in cultured cells, suggesting that auxin promotes G_1 to S progression by regulating the protein stability of E2FB (Magyar *et al.*, 2005).

Auxin controls stem cell specification and cell division in the meristem

Arabidopsis plants harbouring *DR5::GUS*, an auxin-responsive reporter, display maximum β-glucuronidase (GUS) activity in the columella initial cells and relatively low activity in the QC and mature columella cells. The newly developed auxin sensor *DII-VENUS* shows a complementary expression pattern to *DR5::GUS*. Similar maps of cell type-specific auxin responses were obtained using both reporters (Brunoud *et al.*, 2012), indicating that these auxin reporters are valid. Inhibition of polar auxin transport by *N*-1-naphthylphthalamic acid (NPA) shifts the DR5 maximum to more proximal cortical and epidermis cells. The newly formed lateral auxin response maximum leads to ectopic QC and columella specification, indicating that auxin plays a crucial role in stem cell specification in roots (Fig. 1; Sabatini *et al.*, 1999). RETINOBLASTOMA-RELATED protein (RBR) is the plant orthologue of mammalian retinoblastoma protein, and inhibits G_1 to S transition by repressing E2F transcription factors (Xie *et al.*, 1996; Nakagami *et al.*, 1999). Reduction in *RBR* expression in *Arabidopsis* roots increases the number of stem cells without changing the duration of cell cycles in the meristem (Wildwater *et al.*, 2005). Conversely, overexpression of *RBR* results in a loss of stem cells, indicating that an appropriate level of RBR is essential for maintaining the appropriate number of stem cells (Wildwater *et al.*, 2005). RBR is phosphorylated and inactivated by CDKs, which are up-regulated by auxin as described above. Thus, one of the mechanisms maintaining the stem cell pool may be CDK-mediated RBR phosphorylation that is enhanced by higher auxin levels at the root tip.

Růžička *et al.* (2009) reported that mitotic activity in the PM is dramatically elevated by NAA, supporting the function of auxin in promoting cell proliferation (Fig. 1). The PIN auxin efflux carriers determine auxin distribution in roots. The five *Arabidopsis PIN* genes *PIN1*, *PIN2*, *PIN3*, *PIN4*, and *PIN7* are expressed at the root tip in a slightly overlapping, but distinct, manner (Křeček *et al.*, 2009). Blilou *et al.* (2005) found that all *pin* mutants displayed decreased PM activity, demonstrating that auxin homeostasis, as controlled by multiple *PIN* genes, has a crucial role in sustaining cell proliferation in the PM.

PLETHORA 1 (PLT1) and PLETHORA 2 (PLT2) belong to the AP2-domain transcription factor family, and are essential for determining the root SCN (Aida *et al.*, 2004). The expression patterns of *PLT1* and *PLT2* strongly correlate with the auxin maximum in the root meristem, and the *plt1 plt2* double mutant lacks stem cells. However, *plt1 plt2* also exhibits a defect in cell elongation in the EDZ, indicating a pivotal function of these PLTs in root development. Galinha *et al.* (2007) reported that the *PLT* genes function in a dose-dependent manner in terms of their expression gradients. High levels of *PLT* genes promote stem cell identity and maintenance, lower levels enhance the mitotic activity of stem cell daughters, and further reduction in expression levels is prerequisite for cell differentiation. All PLT proteins also show obvious gradients that extend to the TZ, while PLT2 and PLT3 extend to the EDZ (Galinha *et al.*, 2007). Altering the PLT2 gradient affects root meristem size, supporting the idea that PLTs maintain not only stem cell identity but also cell proliferation in the PM as a graded outputter of auxin distribution. Expression of *PLT1* and *PLT2* is regulated at the transcriptional level by auxin and is dependent on ARFs (Aida *et al.*, 2004). However, it is likely that auxin does not directly regulate *PLT* expression because *PLT* genes do not immediately respond to exogenously applied auxin (Fig. 2; Aida *et al.*, 2004). Nevertheless, auxin gradients probably underlie the observed *PLT* expression patterns, as the dynamic auxin distribution established by PIN-mediated polar transport overlaps well with

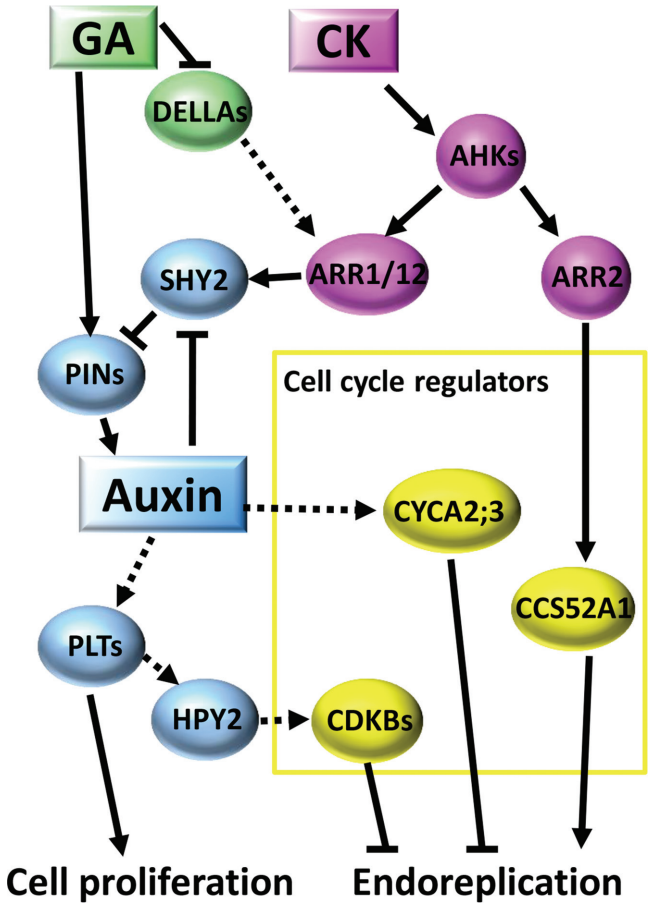

Fig. 2. Hormonal interactions controlling cell proliferation and endoreplication in the roots of *Arabidopsis*. Auxin and gibberellins (GAs) synergistically promote cell proliferation in the proximal meristem (PM), and auxin delays the onset of endoreplication by up-regulating the expression of CYCA2;3 and CDKB1. Cytokinins (CKs) promote the transition from cell proliferation to endoreplication by inhibiting auxin signalling (via SHY2) and by inducing endoreplication (via CCS52A1). GA inhibits CK signalling by suppressing the expression of *ARR1* (but not *ARR12*). Solid and dotted lines represent direct and indirect regulation, respectively.

the *PLT* gradient in roots (Grieneisen *et al.*, 2007). It has been reported that PLTs control PIN polarization, suggesting that a feedback loop between the auxin level and *PLT* expression maintains the PM (Blilou *et al.*, 2005).

PLTs play an important role in controlling stem cell identity, cell division, and cell differentiation. Thus, they act as a hub for the regulation of root development. Indeed, recent studies have demonstrated that *PLT* expression is finely controlled by several factors in *Arabidopsis*. Secreted, tyrosine-sulphated peptides, named ROOT MERISTEM GROWTH FACTORs (RGFs), are required for maintenance of the root SCN and for cell proliferation in the PM. Although *RGF* expression is not affected by auxin, RGF1 up-regulates *PLT* expression mainly at the post-transcriptional level (Matsuzaki *et al.*, 2010). In the QC, the RAC/ROP GTPase activator RopGEF7 is expressed in an auxin-dependent manner, and is required for *PLT* expression, suggesting that RopGEF7 transmits an auxin signal to PLTs in the QC (Chen *et al.*, 2011*a*). In contrast, jasmonate (JA) reduces *PLT* expression, resulting in aberrant differentiation of initial cells. Gel-shift and chromatin immunoprecipitation (ChIP) experiments revealed that MYC2/JASMONATE INSENSITIVE 1, a basic helix–loop–helix transcription factor controlling JA-related gene expression, directly binds to promoters of *PLT1* and *PLT2* and represses their expression (Chen *et al.*, 2011*b*).

Auxin promotes asymmetric division of CEI daughter cells

SHORT-ROOT (*SHR*) and *SCARECROW* (*SCR*) transcription factors regulate ground tissue patterning by controlling asymmetric division in the immediate progeny of ground tissue stem cells, known as cortex/endodermis initial (CEI) cells (Benfey *et al.*, 1993; Pysh *et al.*, 1999). Sozzani *et al.* (2010) revealed that SHR and SCR control this asymmetric division by directly inducing the expression of *CYCD6;1*, a D-type cyclin that is specifically expressed in CEI and CEI daughter cells. Ectopic expression of *CYCD6;1* in the endodermis results in asymmetric division of endodermal cells (Sozzani *et al.*, 2010). When auxin accumulation is enhanced at the root tip by simultaneous application of auxin and NPA, *CYCD6;1* was strongly expressed in the basal region of roots and successive asymmetric division was observed in the endodermis. The promoter of *CYCD6;1*, but not *SHR* or *SCR*, contains the auxin-responsive element (AuxRE). Nevertheless, in the *shr* mutant, auxin and NPA treatment did not induce *CYCD6;1* expression (Cruz-Ramírez *et al.*, 2012). These results suggest that auxin up-regulates *CYCD6;1* expression and promotes the asymmetric division of CEI daughter cells and this regulation requires SHR/SCR (Fig. 1).

Cytokinin

Cytokinins (CKs) are mainly classified into two types, *trans*-zeatin and N^6-(Δ2- isopentenyl)adenine (Hirose *et al.*, 2008). Each CK species has differential distribution patterns in plant tissues, but whether they have distinct functions in root development is not yet known (Hirose *et al.*, 2008). CK signalling is mediated by a two-component system, in which three ARABIDOPSIS HISTIDINE KINASEs (AHKs), AHK2, AHK3, and AHK4/WOL1/CRE1, act as transmembrane CK receptors (Hwang and Sheen, 2001; Inoue *et al.*, 2001; To and Kieber, 2008). These receptors transmit signals to the nucleus via the phosphorelay pathway, leading to phosphorylation and activation of transcription factors known as B-type ARABIDOPSIS RESPONSE REGULATORs (ARRs) (Sakai *et al.*, 2001; To *et al.*, 2004; Mason *et al.*, 2005; To and Kieber, 2008). B-type ARRs then induce the expression of CK primary response genes, including A-type ARRs, which attenuate CK signalling (To *et al.*, 2004; To and Kieber, 2008).

CKs promote cell proliferation in shoots and calli. *Arabidopsis* has three D3-type cyclins (CYCD3;1, CYCD3;2, and CYCD3;3). CYCD3;1 is a key target of CKs. *CYCD3;1* expression is up-regulated by CKs, and its overexpression induces callus formation on *Arabidopsis* leaf explants without exogenous cytokinin, suggesting that CKs promote cell proliferation by inducing *CYCD3;1* expression (Riou-Khamlichi

et al., 1999). On the other hand, Beemster and Baskin (2000) demonstrated that CKs drastically reduce the cell production rate in the PM of *Arabidopsis* roots. The primary role of CKs in roots is the promotion of cell differentiation in the TZ, not the inhibition of cell proliferation in the PM (Dello Ioio *et al.*, 2007). Therefore, reduced cell division in the CK-treated PM may be an indirect consequence of the early onset of cell differentiation (Fig. 1). Nevertheless, it is also likely that CKs actively down-regulate cell division in the PM, where distinct sets of AHKs and B-type ARRs control CK signalling in a manner different from that in shoots. Further studies will reveal how the phosphorelay pathway is associated with cell division in the PM.

Zhang *et al.* (2013) recently showed that CKs are involved in the control of cell division in the QC; CKs induce QC divisions in part by down-regulating the expression of an auxin influx carrier, *LAX2*. This indicates that CKs negatively control auxin maxima and QC specifications, which are associated with enhanced cell division in the QC.

Gibberellin

Since gibberellin (GA) was first discovered, >130 GAs have been identified (Yamaguchi, 2008; Shani *et al.*, 2013). Among these, a few (e.g. GA1, GA3, and GA4) are bioactive, but their individual functions remain elusive. GAs regulate stem elongation, germination, dormancy, flowering, sex expression, and senescence of leaves and fruit (Davière and Achard, 2013). In these developmental processes, GAs primarily promote cell growth. Binding of GAs to the GA receptor GID1 enhances destruction of nuclear DELLAs, which are transcriptional regulators that repress GA signalling, via the ubiquitin–proteasome pathway (Davière and Achard, 2013). Thus, *Arabidopsis* mutants that accumulate large amounts of DELLAs, such as GA-deficient *ga1-3* and the F-box mutant *sly1-10*, are dwarf due to impairment of GA-induced cell growth (Dill *et al.*, 2001; Strader *et al.*, 2004). However, Achard *et al.* (2009) showed that GAs also control cell proliferation in the PM of *Arabidopsis* roots. The number of dividing cells was reduced in *ga1-3* and in wild-type plants treated with paclobutrazol, an inhibitor of GA biosynthesis, whereas the quadruple-DELLA mutation in *ga1-3* restored meristem size. DELLAs elevate the expression level of CDK inhibitors, such as *KRP2* and *SIAMESE* (*SIM*), by unknown mechanisms (Achard *et al.*, 2009). This suggests that GAs promote cell proliferation in the PM by degrading DELLAs and suppressing expression of CDK inhibitors (Fig. 1).

In the PM, the promotion of cell elongation by GAs is required to enhance division of adjacent cells. Ectopic expression of a non-GA-degradable form of GAI, which is one of the five *Arabidopsis* DELLAs, in dividing endodermal cells was sufficient to inhibit cell division in the root meristem (Ubeda-Tomás *et al.*, 2009). Endodermal cells must double in size due to GA signalling before undergoing mitosis, which then enables adjacent cells to elongate and divide, leading to enlargement of the root meristem (Ubeda-Tomás *et al.*, 2009).

Brassinosteroid

Brassinosteroids (BRs) are a group of polyhydroxylated steroidal hormones found in almost all plant species. To date, >70 BR-related phytosteroids have been identified from plants (Zhao and Li, 2012). BRs control numerous developmental processes (i.e. promotion of cell expansion, cell elongation and vascular differentiation, pollen elongation, and acceleration of senescence; Clouse, 2002). BRs up-regulate *CYCD3;1* expression and promote cell proliferation, in a manner similar to CKs (Hu *et al.*, 2000). However, the mode of BR action is rather complicated at the root apex. González-García *et al.* (2011) reported that both loss- and gain-of-function BR-related mutants of *Arabidopsis* displayed a reduced meristem size. In the BR-insensitive mutant *bri1-116*, expression of cell division-related genes, such as *CYCB1;1*, *KRP2*, and *KNOLLE*, was dramatically reduced, while BR-treated plants or mutants with enhanced BR signalling (*bes1-D*) exhibited a premature cell cycle exit that resulted in early differentiation of meristematic cells (González-García *et al.*, 2011). These observations suggest that balanced BR signalling is required for the maintenance of the root meristem (Fig. 1). The reduction of root meristem size in *bri1-116* was suppressed by overexpression of a cyclin gene, *CYCD3;1* (González-García *et al.*, 2011), indicating that BR signalling controls a set of regulators which fine-tune CDK activity to maintain root meristem size.

In the SCN, expression of QC markers is differentially altered by BR treatment. For example, the expression of *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*), *AGAMOUS-LIKE 42* (*AGL42*), *SCR*, *QC25*, and *QC142* is elevated by the application of BRs, whereas that of *QC46* and *QC184* is reduced by BR application (González-García *et al.*, 2011). Moreover, in the columella of *bes1-D* or BR-treated wild-type plants, starch granules accumulate not only in mature differentiated cells but also in columella initials, suggesting that BRs negatively control the undifferentiated state of distal stem cells (Fig. 1; González-García *et al.*, 2011). It has been suggested that BRs function upstream of known regulators of stem cell dynamics, such as *WOX5* (González-García *et al.*, 2011). BRs positively control the expression of *ETHYLENE RESPONSE FACTOR 115* (*ERF115*), which encodes a rate-limiting factor of QC divisions. This indicates that BRs function in promoting QC divisions (Heyman *et al.*, 2013). Further studies will reveal how BRs are involved in the control of cell division and differentiation in the SCN.

Ethylene

Ethylene is a gaseous messenger which transmits environmental signals caused by flooding, drought, chilling, wounding, or pathogen attack. Enhanced ethylene signalling brings about pleiotropic effects on plant development, such as fruit ripening, senescence of leaves and flowers, and seedling triple response (Lin *et al.*, 2009). Ortega-Martínez *et al.* (2007) showed that ethylene promotes cell division in the QC of *Arabidopsis* roots. QC cells of the *ethylene overproducer1* (*eto1*) mutant, which produces excessive amounts of ethylene,

undergo supernumerary cell divisions. The loss-of-function mutant, *CONSTITUTIVE TRIPLE RESPONSE 1* (*CTR1*), in which ethylene signalling is constitutively activated, also exhibits the QC division phenotype. These observations suggest that ethylene is a part of signalling pathways which modulate mitotic activity of the QC during post-embryonic root development (Fig. 1). Interestingly, ethylene-induced division of QC cells is accompanied by QC-specific gene expression and the surrounding stem cells remain undifferentiated (Ortega-Martínez *et al.*, 2007). This implies that quiescence is not required for QC cells to send differentiation-inhibiting signals to adjacent stem cells. On the other hand, ethylene has no dramatic effect on stem cell fate and cell division in the PM (Růžička *et al.*, 2007).

Abscisic acid

Abscisic acid (ABA) is involved in responses to environmental stresses, such as cold, salt stress, osmotic stress, and pathogen attack, which trigger various biological processes (i.e. stomatal closure, inhibition of fruit ripening, seed dormancy, and inhibition of cell division) (Melcher *et al.*, 2010). Although ABA is generally recognized as a growth inhibitor (Zeevaart and Creelman, 1988; Finkelstein and Gibson, 2002), it is largely unknown how ABA affects cell division activity in roots. However, there are a few reports that shed light on the mechanism underlying ABA-mediated inhibition of cell division. Wang *et al.* (1998) demonstrated that expression of the *Arabidopsis* CDK inhibitor *KRP1* is highly induced by ABA, thus leading to inhibition of cell division (Fig. 1). In addition, ABA treatment results in down-regulation of *CYCB1* expression, whereas the expression of CDKs remains almost unchanged (Xu *et al.*, 2010). Therefore, ABA seems to inhibit cell division in the PM by modulating the expression of cyclins and CDK inhibitors, but not CDKs themselves (Fig. 1).

In contrast to ethylene, ABA is associated with maintenance of the quiescent state of QC cells (Fig. 1; Han *et al.*, 2010; Zhang *et al.*, 2010). Moreover, ABA suppresses differentiation of stem cells. Overexpression of *WOX5* produces extra cell layers of undifferentiated stem cells in the columella root cap. Exogenously applied ABA further increases the number of WOX5-induced stem cell layers, while fluridone, a widely used inhibitor of ABA biosynthesis, has the opposite effect (Han *et al.*, 2010; Zhang *et al.*, 2010). Considering that ABA is induced by various external stresses, this hormone may contribute to the maintenance of the SCN by preserving undifferentiated stem cells under stressful conditions (Fig. 1).

Strigolactone

Strigolactones are phytohormones that adjust shoot architecture in response to environmental conditions. For example, phosphate starvation enhances strigolactone production, resulting in a decrease in the number of shoot branches (Kohlen *et al.*, 2011). Ruyter-Spira *et al.* (2011) reported that strigolactones are also important in root development; the primary roots of strigolactone-deficient or -insensitive plants are shorter than those of the wild type due to a reduction in the number of meristem cells. On the other hand, treatment with a high concentration of strigolactones decreases the accumulation of PIN1, PIN3, and PIN7 proteins in the provascular tissue of roots (Ruyter-Spira *et al.*, 2011). This suggests that tightly balanced auxin–strigolactone interactions are crucial for controlling root growth.

Phase change from cell division to endoreplication in the transition zone

In the PM of *Arabidopsis*, root cells undergo mitotic cell cycles, whereas in the TZ, they start endoreplication in which genomic DNA is replicated without cell division, leading to an increase in nuclear DNA content and cell size. This process of DNA polyploidization is called the endocycle (Joubès and Chevalier, 2000; Lee *et al.*, 2009; Fox and Duronio, 2013). The boundary between the PM and the TZ is defined as the point where the first elongated cell appears (Benfey *et al.*, 1993; Verbelen *et al.*, 2006; Dello Ioio *et al.*, 2007). Thus, entry into the TZ is equivalent to the transition from the mitotic cell cycle to the endocycle, which is controlled by CK signalling. It is assumed that cell differentiation progresses through the TZ and EDZ, which indicates that endoreplication is associated with cell growth and cell differentiation (Dello Ioio *et al.*, 2007; Ishida *et al.*, 2010; Adachi *et al.*, 2011). However, the physiological role of endoreplication is still largely unknown. Adachi *et al.* (2011) showed that, in *Arabidopsis* roots, early onset of endoreplication is induced by DNA double-strand breaks, suggesting that plants actively convert the mitotic cell cycle to the endocycle to avoid producing daughter cells with incorrect genetic information. This conversion from mitosis to the endocycle may be a plant-specific survival strategy, as animals usually induce apoptosis to cope with severe genotoxic stress (Blank and Shiloh, 2007). Here we focus on the hormonal control of endoreplication in the developmental context, which enables zonation of the PM and the TZ.

Auxin inhibits the onset of endoreplication

As described above, auxin plays an important role in the maintenance of mitotic activity in the PM. However, it is also involved in controlling the transition from mitosis to the endocycle. In *Arabidopsis* roots, a high level of auxin signalling is required to repress the onset of the endocycle (Fig. 1). Reduction of auxin signalling by the auxin antagonist α-(phenyl ethyl-2-one)-IAA rapidly decreases the expression of several core cell cycle genes and promotes transition to the endocycle (Ishida *et al.*, 2010). This early onset of endoreplication is partially suppressed by overexpression of the mitotic cyclin *CYCA2;3*, which inhibits endocycle onset and promotes the termination of endoreplication (Fig. 2; Imai *et al.*, 2006; Boudolf *et al.*, 2009). Therefore, auxin signalling is critical for determining the timing of the transition to the endocycle. However, it is still unknown whether auxin has a direct role in endocycle onset or principally regulates mitotic activity in the PM.

A nuclear-localized SUMO E3 ligase, *HIGH PLOIDY 2* (*HPY2*), is predominantly expressed in proliferating cells in the PM, and loss of *HPY2* results in a premature transition from the mitotic cell cycle to the endocycle (Ishida *et al.*, 2009). The expression levels of CDKB1 and CDKB2, which are critical for G_2 to M progression, are reduced both transcriptionally and post-transcriptionally in the *hpy2* mutant, suggesting that HPY2-mediated sumoylation promotes cell proliferation in the PM by inducing CDKB1/2 expression (Ishida *et al.*, 2009). Although *HPY2* expression is modulated downstream of PLTs, the molecular link between them remains unknown (Fig. 2). It is also an open question as to whether CDKBs are direct targets of HPY2-mediated sumoylation. However, it is likely that auxin up-regulates mitotic regulators (e.g. CDKBs) through sumoylation in the PM and, consequently, inhibits the onset of endoreplication.

Cytokinins induce endoreplication and antagonize auxin signalling

In *Arabidopsis*, CCS52A1 is an activator of the anaphase-promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase which promotes degradation of mitotic regulators such as cyclins (Boudolf *et al.*, 2009). The *ccs52a1* mutants have enlarged root meristems as a consequence of delayed onset of endoreplication. This indicates that CCS52A1 promotes the transition to the endocycle (Vanstraelen *et al.*, 2009). This proposed function is supported by the distinct expression pattern of *CCS52A1* in the TZ and the EDZ, but not in the PM (Vanstraelen *et al.*, 2009). Recently, Takahashi *et al.* (2013) reported that *CCS52A1* expression is up-regulated by the B-type response regulator ARR2, which is activated by CK signalling (Fig. 2). On the other hand, auxin does not affect *CCS52A1* expression in roots (Takahashi *et al.*, 2013). Therefore, CKs play a direct role in promoting endoreplication by inducing *CCS52A1*, thereby determining root meristem size (Figs 1, 2).

Another B-type response regulator, ARR1, directly promotes the expression of *SHY2*, which encodes a member of the Aux/IAA family that inhibits the auxin response by forming heterodimers with ARF transcription factors (Dello Ioio *et al.*, 2008). SHY2 down-regulates the auxin transport facilitator *PIN* genes. Thus, CK-activated ARR1 causes auxin redistribution and enhances cell differentiation (Figs 1, 2). On the other hand, auxin promotes degradation of the SHY2 protein via the SKIP-CULLIN-FBOX and TIR1 (SCF^{TIR1}) ubiquitin-ligase complex (Mockaitis and Estelle, 2008), thus sustaining PIN activity and cell division in the PM (Fig. 2; Dello Ioio *et al.*, 2008). ARR12, which also up-regulates *SHY2* (Moubayidin *et al.*, 2010), and ARR1 are expressed around the TZ (Dello Ioio *et al.*, 2007). Therefore, CK-induced SHY2 accumulation principally occurs in the TZ in order to antagonize auxin signalling and promote cell differentiation. Taken together, CKs play a key role in determining the root meristem size via two pathways; namely (i) control of the switch from cell proliferation to cell differentiation by repressing auxin signalling; and (ii) control of the transition from the mitotic cell cycle to the endocycle by enhancing degradation of mitotic regulators (Fig. 2). While ARR1 and ARR12 do not control *CCS52A1* expression, ARR2 up-regulates not only *CCS52A1*, but also *SHY2*. This suggests that the functions of B-type ARRs are divergent and that ARR2 fine-tunes root meristem size by controlling both cell differentiation (via the *SHY2* pathway) and endoreplication (via the *CCS52A1* pathway).

The following two conditions are essential for precise control of root meristem size: (i) ARR1, ARR2, and ARR12 are specifically expressed in the TZ; and (ii) CKs exist in sufficient quantity in the TZ to activate the ARRs. Expression of *ARR1*, *ARR2*, and *ARR12* occurs predominantly around the TZ (Moubayidin *et al.*, 2010; Takahashi *et al.*, 2013). As the CK level or signalling does not affect the expression levels of B-type ARRs, some unknown mechanism may regulate their spatial expression in the TZ. Therefore, it is important to identify such regulatory mechanisms in order to uncover the primary determinant controlling the transition from cell division to differentiation (endoreplication). It was recently reported that ARR1, the expression of which is directly repressed by SCR in the QC, stimulates auxin synthesis in the SCN, and this synthesized auxin up-regulates *ARR1* expression in the TZ in a non-cell-autonomous manner (Moubayidin *et al.*, 2013). This indicates that *ARR1* expression and CK signalling in the QC must be adequately repressed in order to maintain meristem size.

Previous reports have indicated that active CKs are likely to be synthesized in both the PM and the TZ, as genes for LONELY GUY (LOG), a key enzyme that produces active CKs, are expressed in distinct regions over the root tip (Miyawaki *et al.*, 2004; Kuroha *et al.*, 2009). Dello Ioio *et al.* (2012) found that the HD-ZIPIII transcription factor PHABULOSA enhances CK biosynthesis in the PM by directly inducing the expression of *IPT7*, whose product catalyses the initial step of CK biosynthesis. CKs produced in the PM then move to the TZ and activate B-type ARRs. Indeed, CKs are transported through the phloem in roots (Bishopp *et al.*, 2011). However, as some *IPT* genes are also expressed in the TZ, it remains to be determined whether the CKs that activate B-type ARRs in the TZ are synthesized *de novo* in the PM or in the TZ.

CKs inhibit the endocytotic trafficking of PIN1, but not that of other PINs (e.g. PIN2, PIN3, and PIN7), and promote its lytic degradation in the vacuole (Marhavy *et al.*, 2011). Therefore, it is likely that auxin signalling in the TZ is antagonized not only through the ARR1/12–SHY2 pathway, but also by active degradation of PIN1. However, the mechanism by which CKs control PIN1 endocytotic trafficking needs to be resolved. In young seedlings, high GA levels repress the expression of *ARR1*, but not *ARR12*. This reduces *SHY2* expression and results in a relatively large root meristem with relatively high mitotic activity (Figs 1, 2; Moubayidin *et al.*, 2010).

Hormonal control of cell growth in the elongation/differentiation zone

Rapid cell elongation in the EDZ is one of the determinants for root growth. In the TZ, endoreplication is mainly involved

in cell elongation, while a combination of many factors is assumed to control cell growth in the EDZ. For example, the uptake of water, which is stored in the vacuole, and the irreversible extension of the cell wall are involved in rapid cell growth (Dolan and Davies, 2004). Several phytohormones and their cross-talk play important roles in controlling such processes, and their actions are distinct from those in the PM or TZ.

As described above, CKs promote the onset of endoreplication and the resultant cell elongation in the TZ. However, this does not necessarily mean that CKs enhance the ability of cells to grow. Beemster and Baskin (2000) showed that CK application diminished the magnitude of cell elongation in the EDZ and reduced the final cell length by 20%. This indicates that cell elongation in the EDZ is negatively controlled by CKs (Fig. 1), although the molecular mechanism of this negative control remains unknown.

Screening of *Arabidopsis* mutants with defects in ethylene response identified genes for ethylene metabolism or signalling, as well as those that control the action of auxin (Roman *et al.*, 1995). Recently, a mechanistic model for the ethylene–auxin interaction in roots has been proposed; namely, ethylene enhances auxin biosynthesis and upward transport from the root tip, and the resultant increase in auxin level inhibits cell elongation in the EDZ (Fig. 1; Růžička *et al.*, 2007; Swarup *et al.*, 2007; Perrot-Rechenmann, 2010). Furthermore, ethylene is known to inhibit GA accumulation in the endodermis, thus suppressing GA-induced cell growth (Shani *et al.*, 2013). The expression of GA biosynthetic genes is particularly high in the meristem, but GAs accumulate mainly in endodermal cells in the EDZ (Silverstone *et al.*, 1997; Mitchum *et al.*, 2006; Shani *et al.*, 2013). This suggests that GAs move from the PM to the EDZ. Therefore, ethylene may inhibit this GA movement and consequently suppress GA-induced cell elongation in the EDZ (Fig. 1).

When *Arabidopsis* plants are treated with ABA, the intracellular Ca^{2+} concentration becomes elevated due to activation of Ca^{2+} channels. The resultant disturbance of calcium homeostasis inhibits cell elongation in the EDZ (Fig. 1; Bai *et al.*, 2009*a*, b). The mutation of *PERK4*, which encodes a member of the proline-rich extensin-like receptor kinase family, disrupts ABA-induced activation of Ca^{2+} channels, suggesting that PERK4 mediates the ABA signalling associated with Ca^{2+} homeostasis (Bai *et al.*, 2009*a*). PERK4 also modulates the expression of genes related to cell wall components and ABA signalling (Bai *et al.*, 2009*a*). Therefore, PERK4 may be associated with multiple ABA-triggered pathways that inhibit cell elongation.

Perspective

Here, we summarized each hormone's role in each root zone and described how the interplay between hormones is engaged in the dynamic process of root growth. However, the classical question still remains: how do auxin and CK control cell proliferation? *CCS52A1* is the only identified gene that is involved in cell cycle regulation and is directly controlled by a B-type response regulator (Takahashi *et al.*, 2013). Other genes that are regulated by CKs seem to be indirect targets of CK signalling. Therefore, we are still missing important modules that link hormonal signalling pathways to the core cell cycle machinery. Identification of such modules will provide insight into the regulatory mechanisms underlying cell division and endocycle-associated cell growth during root development. In the last two decades, molecular-level information about root growth has been obtained primarily through studies of *Arabidopsis*, an excellent system in which the sequential process of cell division and differentiation in each cell file can be easily followed. However, to understand further the complicated networks of hormones, it is important to pay more attention to other plant species. For example, the TZ is not necessarily present in all plant species, and DNA polyploidization does not occur in some plant species, such as rice and trees (Arumuganathan and Earle, 1991; Mellerowicz and Riding, 1992). Nevertheless, in such plants, the transition from cell division to cell differentiation (cell elongation) occurs in a similar manner to *Arabidopsis*, demonstrating that some unknown process triggers this transition. To uncover such a pivotal process, it is essential to study cell division and elongation processes in the distinct root zones of different species. Technical advances in bioimaging and omics data analysis, as well as the combination of modelling and experimental approaches to systems biology, will greatly aid our understanding of hormonal regulation of cell division in plant tissues. Further studies of the molecular mechanisms underlying root growth and development will highlight plant-specific features in the control of cell division and differentiation, and may thus provide clues to understanding totipotency, the most characteristic feature of plant cells.

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