REVIEWS PAPERS

Plant hormone cross-talk: the pivot of root growth

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Abstract

Root indeterminate growth and its outstanding ability to produce new tissues continuously make this organ a highly dynamic structure able to respond promptly to external environmental stimuli. Developmental processes therefore need to be finely tuned, and hormonal cross-talk plays a pivotal role in the regulation of root growth. In contrast to what happens in animals, plant development is a post-embryonic process. A pool of stem cells, placed in a niche at the apex of the meristem, is a source of self-renewing cells that provides cells for tissue formation. During the first days post-germination, the meristem reaches its final size as a result of a balance between cell division and cell differentiation. A complex network of interactions between hormonal pathways co-ordinates such developmental inputs. In recent years, by means of molecular and computational approaches, many efforts have been made aiming to define the molecular components of these networks. In this review, we focus our attention on the molecular mechanisms at the basis of hormone cross-talk during root meristem size determination.

Key words: Auxin, cell differentiation, cell division, cytokinin, gibberellin, root meristem.

Introduction

Plants have the extraordinary capability of being potentially ‘immortal’. Indeed, there are plants which have been living for several hundreds of years, and death occurs due to external factors, such as environmental stresses, diseases, or pathogen infection. This long life expectancy is mainly due to their capacity to produce new tissues and organs throughout their life thanks to the activities of long-lasting stem cells. Plant indeterminate growth and development are predominantly post-embryonic processes taking place in the meristems, where stem cells self-renew and produce daughter cells that differentiate and give rise to different tissues and organ structures (reviewed in Heidstra and Sabatini, 2014). As in animals, plant stem cells are organized in stem cell niches (SCNs). In the root of the Arabidopsis thaliana model plant, the SCN is located at the apex of the meristem where stem cells surround the organizer, the quiescence centre (QC), generating daughter cells (Heidstra and Sabatini, 2014). These cells undergo additional division in the proximal meristem (PM), and differentiate at the distal meristem transition zone (TZ) that encompasses the boundary between dividing and differentiating cells in the different cell files (Dello Ioio et al., 2007). For meristem maintenance, and therefore for continuous root growth, the rate of cell differentiation must equal the rate of generation of new cells. The plant root is thus a highly dynamic structure and the mechanisms that ensured its activity need to be finely regulated. Hormones are among the major endogenous regulators of root growth. Each hormone such as auxin, cytokinin, gibberellin, brassinosteroids, abscisic acid, and strigolactones has its specific biosynthetic and signal transduction pathway, and much evidence has now been collected showing that correct root growth depends on their cross-talk. In the last few years many efforts have been made to elucidate these complex networks. In this review, we fist summarize current knowledge on the molecular
mechanism at the basis of hormone biosynthesis, transport, and signalling, and then discuss recent findings on hormonal cross-talk, focusing our attention on primary root growth and in particular on how hormonal cross-talk controls root meristem size.

**Auxin**

‘Auxein’ is the word that the ancient Greeks used for ‘to grow’, and in 1931 Kögl and Haagen-Smit coined the term auxin for the substance they observed to be able to modulate plant growth (Kögl and Haagen-Smit, 1931). Since then many efforts have been made to try to elucidate the molecular players involved in auxin activity during plant development. The bioactive auxin is indole-3-acetic acid (IAA) (Thimann, 1977). IAA is a weak acid (pK=5.4) existing in a plant as a free form or conjugated to a sugar or amino acid for degradation or storage (Ljung et al., 2002). IAA biosynthesis is divided into two different pathways: the tryptophan-dependent and tryptophan-independent pathways. The tryptophan-independent pathway is poorly understood, but indole-3-glycerol phosphate (IGP) or indole are thought to be the primary precursors of IAA biosynthesis (Zhang et al., 2008). On the other hand, the biosynthetic pathway that has the amino acid tryptophan as the IAA precursor has been widely investigated and now the molecular players are clearly elucidated (reviewed in Zhao, 2014). Tryptophan is converted into IAA through an enzymatic cascade in a two-step pathway in which the intermediate is indole-3-pyruvate (IPA) (Stepanova et al., 2008). Local auxin biosynthesis is necessary to control different plant developmental processes. Genes encoding the molecular components of the IAA biosynthetic pathway are expressed in the roots and their activity is necessary to control root development. For example, the quadruple Yue1Yue4Yue10Yue11 mutant in the YUCCA genes encoding a flavin monooxygenase involved in tryptophan-dependent auxin biosynthesis does not develop a root meristem, indicating the importance of correct auxin biosynthesis in the root (Cheng et al., 2006).

From its site of biosynthesis auxin moves within the Arabidopsis root through two types of transport. The protonated form of the IAA (IAAH) passively diffuses across the plasma membrane, while the anionic form (IAA-) of the auxin is transported outside and inside the cells through an active transport that requires efflux and influx carriers, respectively. Auxin influx is mediated by AUX1/LAX (AUXIN 1/LIKE AUX1) carriers, in particular by the AUXIN PERMEASE1 (AUX1) (Swarup et al., 2001). Efflux transporters are PIN FORMED (PIN) and ATP-BINDING CASSETTE GROUP B (ABCB/MDRPGP) (Balzan et al., 2014). PIN proteins are polarly localized in cell membranes through the cell endomembrane system and play a crucial role in polar auxin transport (PAT) responsible for the formation of an auxin gradient along the root (Bilou et al., 2005; Ljung et al., 2005; Grieveisen et al., 2007). According to this gradient, a peak of auxin is established at the root apex and provides positional information essential for maintenance of correct cell division, polarity, and fate (Sabatini et al., 1999).

In the cell, IAA molecules are perceived by specific receptor proteins, the TIR1/AFB (TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALING F-BOX). TIR1 and AFB1–AFB5 contain the F-box that takes part in the ubiquitin ligase complex SCFTIR1 (Kepinski and Leyser, 2005). More recently, ABP1 (AUXIN BINDING PROTEIN 1), an extracellular localized protein able to bind IAA, has been proposed as another auxin receptor involved in auxin inhibition of clathrin-mediated endocytosis of the polar auxin transporters PIN1 and PIN2 through the activation of a specific ROP-GTPase (Rho of plants) (Chen et al., 2012). Auxin signal transduction strongly depends on the auxin intracellular concentration. A high IAA concentration acts to stabilize the binding of the TIR1/AFB receptors to repressor auxin/IAA (Aux/IAA) proteins causing Aux/IAA ubiquitination and degradation via the proteasome. Aux/IAA proteins work through heterodimerizing with AUXIN RESPONSE FACTORS (ARFs), thus repressing their transcriptional activity (reviewed in Wang and Estelle, 2014). Thus, Aux/IAA degradation causes ARF activation that modulates transcription of auxin-responsive target genes, binding the AUXIN RESPONSIVE ELEMENT (ARE: 5′-TGTCTC-3′) (Boer et al., 2014). At low IAA concentration, the ubiquitin ligase complex SCFTIR1 displays less affinity for Aux/IAA proteins, so the amount of stable Aux/IAA protein increases, causing the formation of Aux/IAA–ARF heterodimers which inhibit ARF-mediated regulation of target gene transcription (reviewed in Peer, 2013).

**Cytokinin**

Cytokinins are plant hormones, known to be N6-prenylated adenine derivatives that control many aspects of plant growth and development. In higher plants, bioactive cytokinins are isoprenoid cytokinins such as isopentenyladenine (ip), trans-zeatin (iz), cis-zeatin, and dihydrozeatin (reviewed in Del Biondo et al., 2013). The most abundant forms found in Arabidopsis are ip and iz (Sakakibara, 2006). Cytokinin homeostasis is a multistep process regulated by the balance between biosynthesis and catabolism (reviewed in Frébort et al., 2011). The correct plant growth depends on spatial and temporal co-ordination of this process (Miyawaky et al., 2004; Sakakibara, 2006). The initial, rate-limiting step in cytokinin biosynthesis is catalysed by the ADP/ATP-ISOSENTRYLTRANSFERASE (IPT) gene family. In Arabidopsis, there are seven IPT genes. Moreover, the expression domain of each IPT gene is tissue and organ specific (Miyawaky et al., 2004). IPTs catalyse the transfer of an isopentenyl group to an adenine nucleotide (iP nucleotide). Two cytochrome P450 monoxygenases, CYP735A1 and CYP735A2, convert the iP nucleotides to tZ nucleotides (Takei et al., 2004). An additional step is necessary to convert the cytokinin from its inactive to its active form. This step is conducted by enzymes encoded by the LONELY GUY (LOG) gene family (Kuroha et al., 2009). Analysis of the expression pattern domain of AtLOG revealed that, like the IPT genes, these genes are expressed throughout the plant...
during development, although with different and overlapping expression domains (Kuroha et al., 2009). The final step involves catabolic genes that ensure the maintenance of a correct concentration of active cytokinin. Enzymes encoded by the CYTOKININ OXIDASE (CKX) gene family (Schmulling et al., 2003) mediate cytokinin degradation. The CKX enzymes catalyse irreversible inactivation of cytokinin through oxidative cleavage of its N6 side chain, resulting in the formation of adenine (or its corresponding derivative for N6-substituted cytokinin) and a side chain-derived aldehyde (Frebort et al., 2011).

How cytokinin is transported through the plant is still not completely clear. Recently, it has been demonstrated that, besides diffusion, long-distance (root to shoot) cytokinin transport also occurs through the phloem (Bishop et al., 2011). Researchers identified members of the PURINE PERSERASE (PUP) and EQUILIBRATE NUCLEOSIDE TRANSPORT (ENT) families as putatively responsible for cytokinin transport through the phloem (Gillissen et al., 2000; Burkle et al., 2003). Recently, another protein, the Arabidopsis ATP-binding cassette (ABC) transporter G14 (AtABCG14), has been demonstrated to be essential for cytokinin transport through the xylem (Ko et al., 2014; Zhang et al., 2014).

Cytokinin signalling is mediated by a multistep phospho-transfer cascade similar to the bacterial two-component system (reviewed in El-Showk et al., 2013). In Arabidopsis, cytokinins are perceived by transmembrane histidine kinase receptors, named ARABIDOPSIS HIS KINASE 2 (AHK2), AHK3, and AHK4/WOODENLEG (WOL)/CYTOKININ RESPONSE 1 (CRE1) (Hwang and Sheen, 2001; Inoue et al., 2001). Cytokinin–receptor binding activates a phosphorylation cascade ending with the transfer of a phosphate group from members of the ARABIDOPSIS HIS PHOSPHOTRANSFER PROTEIN (AHP) family to the nuclear-localized ARABIDOPSIS RESPONSE REGULATOR (ARR) protein family (Hwang and Sheen, 2001; Hutchison et al., 2006). The ARR proteins are classified into two different types, type-A and type-B. Type-A ARRs (ARR3–ARR9 and ARR15–ARR17) are negative regulators of cytokinin responses and their mechanism of action is poorly understood (Kim, 2008). In contrast, type-B ARRs (ARR1, ARR2, ARR10–ARR14, and ARR18–ARR21) are a family of transcription factors that act as positive regulators of cytokinin-dependent gene expression (Muller, 2011). These proteins are finely regulated by a family of F-box proteins, called KISS ME DEADLY (KMD), that physically interact with type-B ARRs, targeting these proteins for degradation via the proteasome (Kim et al., 2013). The type-B cytokinin transcription factors are believed to act together with another family of cytokinin-dependent transcription factors, the CYTOKININ RESPONSE FACTORS (CRFs), which positively control cytokinin response (Rashotte et al., 2006).

Gibberellin

Gibberellins (GAs) are a large family of tetracyclic diterpenoid plant growth regulators. Biologically active GA forms, GA1, GA4, and GA7, control different plant growth processes including seed germination, stem elongation, leaf expansion, and flower and seed development (Yamaguchi, 2008). Many non-bioactive GAs exist in plants as precursors for the bioactive forms or as deactivated metabolites (Yamaguchi, 2008). The GA homeostasis is maintained by biosynthesis, formation of bioactive GAs, and deactivation pathways. GAs are biosynthesized from geranylgeranyl diphosphate (GGDP), a common C20 precursor of diterpenoids. Three different classes of enzymes are required for the production of bioactive GAs from GGDP: terpene synthases (TPSs), cytochrome P450 monoxygenases (P450s), and 2-oxoglutarate-dependent dioxygenases (2ODDs) (Yamaguchi, 2008). The final step of biosynthesis is catalysed by GA 20-oxidases (GA20oxs) and GA 3-oxidases (GA3oxs) (Chiang et al., 1995; Phillips et al., 1995). These enzymes, by a series of oxidation steps, synthesize the bioactive GAs, in particular GA2 and GA4. The bioactive GA homeostasis is sustained by feedback mechanisms that also depend on catabolic genes. One of these genes encodes the GA 2-oxidase (GA2ox) which mediates the deactivation of bioactive GAs by 2β-hydroxylation (Schomburg et al., 2003; Rieu et al., 2008). GAs are synthesized in the root meristem; in fact the activity of biosynthetic genes is higher in the meristem and lower in other cell types (Silverstone, 1997; Birnbaum et al., 2003). GAs accumulate in the endodermis of the root elongation zone (Shani et al., 2013), but the mechanism through which GAs move along the root is poorly understood.

GAs are perceived in the cell through a simple circuit, which is regulated by the nuclear-localized growth-repressing DELLA proteins (DELLAs), a subgroup of the GRAS family of putative transcription regulators (Bolle, 2004; reviewed in Daviere and Achard, 2013). In Arabidopsis, there are five DELLA: GA-INSENSITIVE (GAI), REPRESSOR OF GA (RGA), RGA-LIKE1 (RGL1), RGL2, and RGL3. These proteins are the intracellular negative regulators of the GA response (Peng et al., 1997; Silverstone et al., 1998). The GA signalling pathway starts when GA molecules are perceived by a soluble GA receptor with homology to human-sensitive lipase, GA-INSENSITIVE DWARF1 (GID1) (Ueguchi-Tanaka et al., 2005). The direct binding between GAs and GID1 induces the interaction between GID1 and the DELLA-domain of DELLAs (Willige et al., 2007), allowing the formation of the GA–GID1–DELLA complex. Consequently, the GA–GID1–DELLA complex is targeted by a specific SCF (SK1, CULIN, F-BOX) E3 ubiquitin-ligase complex, through the binding between DELLA protein and the F-BOX protein SLEEPY (SLY). In turn, SCFSLY promotes the ubiquitinylation and subsequent degradation of DELLA by the 26S proteasome, triggering the GA response (Fu et al., 2004). In the root, the tissue committed to GA response is the endodermis where it has been shown that it is sufficient to manipulate GAI degradation to control root meristem size (Ubeda-Tomas et al., 2008, 2009).

Brassinosteroids

Brassinosteroids (BRs) are involved in the regulation of cell elongation and division during plant organ formation and are
implicated in abiotic and biotic stresses responses. Moreover BRs are also involved in the control of photomorphogenesis, bending, development of reproductive organs, and vascular development. The most bioactive form of BR, brassinolide (BL), is synthesized from campesterol as a primary precursor upstream of different biosynthetic pathways named early and late C-6 oxidation, C-22, and C-23 oxidation pathways converging on castasterone production which is finally converted to BL (Noguchi et al., 2000).

It has been suggested that BRs are inactivated by different reactions, such as hydroxylation, glycosylation, demethylation and side chain cleavage at multiple positions (Baiguz et al., 2007).

The BR signal cascade is activated when BR binds to the BRASSINOSTEROID INSENSITIVE1 (BR1) receptor, a leucine-rich repeat (LRR)-receptor kinase located at the plasma membrane (She et al., 2011). From the cell surface, BR signal reaches the nucleus following a multistep process in which degradation of the GSK3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2) determines the activation of the two key transcription factor homologues, BRASSINAZOLE RESISTANT1 (BZR1) and BR1-EMS-SUPPRESSOR1 (BES1)/BZR2, by a dephosphorylation step mediated by PP2A phosphatase (Clouse, 2011; Tang et al., 2011). BZR1 and BES1 are consequently stable and accumulate in the nucleus where they form homo- and heterodimers that bind DNA of specific target genes recognizing brassinosteroid-responsive elements (BRREs) (Sun et al., 2010; Yu et al., 2011). In this way, the BR signalling pathway generates a homeostatic feedback loop also controlling its own biosynthesis. However, despite recent progress in our comprehension of BRs signalling, many aspects of BR homeostasis, transport, and functions need to be elucidated.

Abscisic acid

Plants, which are sessile organisms, evolved highly efficient environmental adaptive strategies. External environmental stresses are perceived by plants and translated into internal signals activating specific stress-responsive genes. A phytohormone playing a crucial rule in this process is the iso-prenoid hormone abscisic acid (ABA). ABA was originally identified in the 1960s as a hormone involved in seed dormancy regulation and organ abscission (Liu and Carns, 1961). It has been shown that exogenous ABA application induces the expression of specific stress-related genes, the same genes that are up-regulated under environmental stress conditions (Shinozaki and Yamaguchi-Shinozaki, 2000). Therefore, plants respond to environmental stresses by overproducing ABA and activating the ABA signalling pathway. ABA biosynthesis occurs in plastids of all cell types, but predominantly in the vascular tissues. It is characterized by a series of oxidation and isomerization of carotenoids leading to the cleavage of C40 carotenoid that causes the production of xanthoxin that is then converted in ABA aldehyde and finally oxidized to ABA. There is also a parallel pathway starting from ABA aldehyde that is converted to ABA alcohol and transformed into ABA after oxidation by a P450 monooxygenase (Nambara and Marion-Poll, 2005). ABA homeostasis involves catabolic processes including oxidative and conjugative reactions. Hydroxylation of three different methyl groups of ABA (7’, 8’, and 9’) leads to different oxidation pathways. The hydroxylation reaction results in the production of phaseic acid (PA) that is transformed to dihydrophaseic acid (DPA) which is a biologically inactive form of ABA. On the other hand, a conjugative reaction is responsible for the production of the ABA-glucose ester (ABA-GE) derived from ABA upon covalent conjugation with sugar (Jiang and Hartung, 2008). ABA-GE is a long-distance transported form and a storage form of ABA, as demonstrated by the fact that cleavage of the conjugate, by β-glucosidases, releases free ABA (Lee et al., 2006).

ABA perception starts when this hormone is perceived by the PYRABACTIN RESISTANCE (PYR)/PYR1-LIKE (PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR) family of ABA receptors (Santiago et al., 2009; Mosquera et al., 2011). ABA binding to PYR/PYL/RCAR receptors blocks the phosphatase activity of type 2C protein phosphatases (PP2Cs). These are negative regulators of ABA signalling that activate the transcription of specific ABA-responsive genes, containing ABA-responsive elements (ABREs; PyACGTGGC) in the promoter (Giraudat et al., 1994; Busk and Pages, 1998).

As ABA is the major hormone involved in stress responses, such as salinity, drought, and temperature stresses, a full comprehension of ABA signalling and perception would be useful to increase crop productivity.

Strigolactones

Recent studies identified a class of terpenoid lactones called strigolactones (SLs) as new plant hormone (Gomez-Roldan et al., 2008). The first data collected assigned a role for SLs in parasitic and symbiotic interaction responses. Subsequently, in 2008, two groups discovered a fundamental role for SLs in shoot branching regulation (Gomez-Roldan et al., 2008; Umehara et al., 2008). Recent work identified SLs as positive regulators of primary root elongation and negative regulators of adventitious root formation (Ruyter-Spira et al., 2011; Rasmussen et al., 2012). Thus, SLs are new important components involved in plant growth and development. Little is known about the SL biosynthetic pathway, but recent data suggested a possible involvement of carotenoid as a precursor of SLs. Indeed, carotenoid-deficient mutants show absence of SLs and, in plants treated with fluridone, a carotenoid biosynthesis inhibitor, SL levels are reduced (Matusova et al., 2005). Alder et al. (2012) identified carlactone as a possible SL biosynthetic intermediate. Some genes involved in SL biosynthesis have been identified, and among them there are two carotenoid cleavage dioxygenases (CCD7 and CCD8) and one cytochrome P450, MORE AXILLARY GROWTH1 (MAX1) (Gomez-Roldan et al., 2008; Umehara et al., 2008). Localization of biosynthetic gene expression patterns is useful to obtain preliminary information about sites of SL
biosynthesis in the plant. In rice root, CCD7 (HTD1) and CCD8 (D10) are expressed throughout the vascular parenchyma cells, while in Arabidopsis MAX1 is active in root vascular tissue and CCD8 (MAX4) is expressed in primary and lateral roots in the columella root cap (Bainbridge et al., 2005; Booker et al., 2005; Arite et al., 2007). SL receptors have not been identified yet, but some proteins have been proposed as possible candidates. One of them is the MAX2 protein which is a member of the SCF-type ubiquitin ligase complex (Stirnberg et al., 2007). More recently, evidence has been collected showing that DWARF 14 (D14), an α/β hydro-lase protein, connects SL perception and signalling through protein–protein interactions with MAX2-type proteins (Arite et al., 2009; Waters et al., 2012; Kagiyama et al., 2013).

**Plant hormones: the importance of cross-talk in controlling root meristem size**

Data collected in the last few years demonstrate that coherent root growth is a finely regulated process requiring that each hormone communicates with the others, giving rise to a complex network of hormone interactions.

Root meristem development starts post-embryonically when the root SCN is activated, leading to radicle protrusion during seed germination processes. In this phase of root development, the division rate of stem cell daughters overcomes their differentiation, allowing meristem building (Moubayidin et al., 2010). At 5 days post-germination (dpg), the meristem sets its final size, establishing a dynamic equilibrium between cell division and cell differentiation. Meristem size is then maintained constant over time (Dello Ioio et al., 2007). The overall events ensuring meristem building and maintenance need to be finely regulated, and a pivotal role in this process is played by plant hormones and their cross-talk. The Aux/IAA SHORT HYPOCOTYL2 (SHY2) gene, a repressor of auxin signalling, has a crucial role in controlling meristem size and development. Indeed, the loss-of-function shy2-31 mutant displays an enlarged root meristem due to a delay in cell differentiation that causes loss of the balance between cell division and cell differentiation, while high levels of SHY2 during the root meristem growth phase are sufficient to stop root growth and reduce meristem size (Dello Ioio et al., 2008; Moubayidin et al., 2010). It has been shown that SHY2 specifically acts at the vascular tissue transition zone to regulate PIN expression and auxin distribution. Cross-talk of many hormones converges on this gene, fine-tuning its level and thus controlling SHY2 abundance in a time-dependent manner during root development (Fig. 1).

The optimal SHY2 level is reached only when both the ARR1 and ARR12 cytokinin-dependent genes activate SHY2 transcription (Moubayidin et al., 2010) (Fig. 1B). During the meristem growth phase, a high amount of GA causes ARR1 repression through degradation of the DELLA protein REPRESSOR OF GA (RGA) (Moubayidin et al., 2010) (Fig. 1A). In this way SHY2 levels are kept low because its activation depends only on ARR12. A low SHY2 level results in auxin signalling activation that positively regulates the auxin transport facilitator PIN genes promoting auxin distribution and cell division (Dello Ioio et al., 2008; Moubayidin et al., 2010) (Fig. 1A). At the same time, BREVIS RADIX (BRX), a protein implicated in the BR pathway, also controls polar auxin transport, specifically inducing PIN3 expression (Scacchi et al., 2010) (Fig. 1A). Recently, it has been shown that ARR12 controls root growth by activating the transcription of a member of the ARF family, AUXIN RESPONSE FACTOR19 (ARF19), together with SHY2, promoting cell differentiation of meristematic cells at the TZ (Perilli et al., 2013).

At 5 dpg, the level of RGA increases, probably because of a decrease in amount of GA, inducing ARR1 activation. ARR1 activates SHY2 together with ARR12, increasing its level of expression, repressing PIN activity, and enhancing cell differentiation (Moubayidin et al., 2010) (Fig. 1B). The increase in SHY2 level also depends on SL activity. Indeed, it has been shown that SLs induce SHY2 expression, thus interfering with auxin flux (Koren et al., 2013) (Fig. 1B). It has been demonstrated that BRX expression is controlled by auxin; thus, a reduction in auxin distribution, caused by PIN repression, determines a decrease in BRX level and a consequent PIN3 inhibition. This inhibition limits auxin transport and distribution, thus contributing to the increase of the cell differentiation rate (Scacchi et al., 2010) (Fig. 1B).

Modulation of auxin flux also depends on the activity of the BR receptor, BRASSINOSTEROID INSENSITIVE 1 (BR1), which is expressed in the epidermis and from this tissue post-transcriptionally regulates PIN proteins (Hacham et al., 2011, 2012).

Another hormone involved in polar auxin transport regulation is ABA. Indeed, ABSCISIC ACID INSENSITIVE4 (ABI4), a protein encoding an ABA-regulated AP2 domain transcription factor whose expression is induced by ABA and cytokinin, represses PIN1 expression (Shkolnik-Inbar and Bar-Zvi, 2011) (Fig. 1B). Recently, it has been demonstrated that another gene involved in ABA signalling, ABSCISIC ACID INSENSITIVE5 (ABI5), which encodes a transcription factor belonging to the basic leucine zipper (bZIP) family, represses polar auxin flux through the inhibition of PIN1, thereby controlling root growth (Yuan et al., 2014) (Fig. 1B).

All the hormonal pathways described co-operate to keep polar auxin transport low, while at the same time auxin counteracts this repression, directing SHY2 degradation, and thus allowing the maintenance of PIN activity and the consequent induction of cell division (Dello Ioio et al., 2008).

This complex network of hormone interactions allows cell differentiation to balance cell division, consequently setting the final root meristem size.

To ensure coherent root growth, cell differentiation at the TZ needs to be co-ordinated not only with cell division of meristematic cells, but also with the activity of stem cells. Recently the molecular mechanism involved in the spatial co-ordination between the TZ activity and the SCN has been partially clarified. Co-ordination between these two zones is regulated by the SCARECROW (SCR) gene (Sabatini et al., 2003; Moubayidin et al., 2013). In the QC, SCR directly binds to and negatively regulates ARR1, which in turn, controls...
auxin production by modulating the expression of the auxin biosynthesis gene *ASB1*. SCR, by controlling the level of auxin production in the QC, exerts a long-distance control of ARR1 in the TZ. The auxin produced by ASB1 in the QC and distributed along the meristem by PINs is sufficient to activate *ARR1* expression in the TZ, thus sustaining cell differentiation via SHY2 (Dello Ioio *et al.*, 2008; Moubayidin *et al.*, 2013).

**Conclusions and perspectives**

In this review, we provide an overview on the plant hormones involved in the regulation of root meristem size. In particular, we integrated recent data showing molecular details of hormonal interaction. The emerging picture is that auxin is the pivot of the dynamic regulation of root meristem size. A lot of data indicate that auxin is not a common plant hormone, but it acts as a morphogen instructing cell fate in a dose-dependent manner (reviewed in Benkova *et al.*, 2009).

Hormonal cross-talk converges on the regulation of genes involved in auxin signalling and/or transport to ensure a correct auxin graded distribution, allowing, in this case, correct root meristem size determination. Therefore, the finely regulated spatio-temporal interactions between hormones ultimately modulate the distribution and the perception of auxin.

The increasing amount of molecular data contributes to broaden our knowledge, at the same time introducing an additional level of complexity, as the outputs of such networks are non-intuitive. Mathematical modelling has already proved to be a powerful tool to integrate data at multiples levels, allowing for the dissection of hormonal cross-talk involved in the control of a particular system. For example, a computational approach has been used to demonstrate that auxin–cytokinin interaction is responsible for the formation of a stable pattern within a growing vascular tissue (De Rybel *et al.*, 2014).

We are confident that further modelling development will provide insights into the role of the complex hormonal circuit involved in root meristem size determination. (A, B) Confocal microscope images of the *Arabidopsis* root tip with different developmental zones indicated, stem cell niche (SCN), proximal meristem (PM), transition zone (TZ), and elongation and differentiation zone (EDZ), flanked by a schematic model of hormonal interaction at 3 days post-germination (dpg) (A) and at 5 dpg (B). At the bottom a schematic picture of communicating vessels is used to represent the ratio between cell division and cell differentiation at 3 dpg (A) and at 5 dpg (B). (A) At 3 dpg, hormonal cross-talk guarantees a low level of cell differentiation, allowing meristem growth. (B) At 5 dpg, a complex network of hormonal interactions balances cell differentiation with cell division, setting the meristem size. In both cases, hormone cross-talk converges on the *SHY2* gene whose levels are kept low at 3dpg (A) and higher at 5 dpg (B). Red arrowheads indicate the TZ of the cortex tissue, placed at the boundary between the last meristematic cell and the first differentiating cell. A colour code has been used to indicate different pathways: in blue pathways under the control of auxin (IAA), in red those under the control of cytokinin (CK), in yellow those controlled by gibberellin (GA), in orange those controlled by brassinosteroi (BR), in green those controlled by abscisic acid (ABA), and in grey that controlled by strigolactones (SLs).
responsible for pattern formation and growth in the root meristem, predicting emerging properties that could not be observed solely by means of ‘wet’ biology.

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