New insights into root gravitropic signalling

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Abstract

An important feature of plants is the ability to adapt their growth towards or away from external stimuli such as light, water, temperature, and gravity. These responsive plant growth movements are called tropisms and they contribute to the plant's survival and reproduction. Roots modulate their growth towards gravity to exploit the soil for water and nutrient uptake, and to provide anchorage. The physiological process of root gravitropism comprises gravity perception, signal transmission, growth response, and the re-establishment of normal growth. Gravity perception is best explained by the starch–statolith hypothesis that states that dense starch-filled amyloplasts or statoliths within columella cells sediment in the direction of gravity, resulting in the generation of a signal that causes asymmetric growth. Though little is known about the gravity receptor(s), the role of auxin linking gravity sensing to the response is well established. Auxin influx and efflux carriers facilitate creation of a differential auxin gradient between the upper and lower side of gravistimulated roots. This asymmetric auxin gradient causes differential growth responses in the gravisresponding tissue of the elongation zone, leading to root curvature. Cell biological and mathematical modelling approaches suggest that the root gravitropic response begins within minutes of a gravity stimulus, triggering genomic and non-genomic responses. This review discusses recent advances in our understanding of root gravitropism in \textit{Arabidopsis thaliana} and identifies current challenges and future perspectives.

Key words: \textit{Arabidopsis thaliana}, auxin, calcium, differential growth, gravitropic response, root growth, tropism.

Root gravitropism

Plants adapt to environmental signals such as light, water, temperature, and gravity by modifying their growth. Directional growth responses are termed tropisms. The first detailed studies of tropisms were performed by Charles Darwin and his son Christopher, who described that etiolated grass coleoptiles grew towards the source of light when they were illuminated from one side (\textit{Darwin}, 1880). Plant roots provide another example of a tropism as they respond to changes in orientation (Fig. 1). Upon seed germination, roots immediately orient themselves to grow downwards along the gravity vector in the soil, in order to secure water, nutrients, and anchorage (\textit{Bailey et al.}, 2002).

In roots, gravity is perceived in the columella cells in the root cap, but the gravitropic response takes place in the elongation zone cells. Thus, there is a clear physical separation between the site of gravity perception and the site of gravitropic response (Fig. 2). Root gravitropism can be divided into three spatially and temporally distinct phases: gravity perception, transmission of the gravitropic signal, and ultimately the growth response itself (\textit{Swarup and Bennett}, 2009).

This review will primarily focus on our current understanding of root gravitropism in the model plant \textit{Arabidopsis thaliana}, reviewing the gravitropic response chronologically.

Gravity perception (Fig. 2)

The root cap is the primary site of gravity perception in higher plants, as early experiments involving surgical removal of the root cap resulted in agravitropic roots (\textit{Barlow}, 1974;
The root cap is comprised of central columella cells that are surrounded by lateral root cap cells (Dolan et al., 1993). More sophisticated experiments including laser ablation (Blancaflor et al., 1998), genetic ablation (Tsugeki and Fedoroff, 1999), or heavy-ion microbeam irradiation (Tanaka et al., 2002) of individual root cap cells showed that within the entire root cap the columella cells are the site of gravity perception. In *Arabidopsis thaliana*, there are normally four layers of columella cells termed the S1–S4 cell layers, of which S1 and S2 appear to be most important for root gravitropism (Blancaflor et al., 1998). Columella cells contain starch-filled amyloplasts and as per the starch–statolith hypothesis (Haberlandt et al., 1900; Nèmeč, 1900) sedimentation of amyloplasts triggers a signal transduction cascade that results in generation of a signal that is then transmitted to the graviresponsive tissues of the elongation zone to achieve gravitropic bending (Leitz et al., 2009; Band et al., 2012a; Baldwin et al., 2013; Blancoflor, 2013; Swarup et al., 2013).

Despite the support for the starch–statolith hypothesis, alternative models for gravitropic sensing have been suggested. The protoplast pressure hypothesis is based on changes in pressure exerted by the cytoplasm on the plasma membrane in response to a gravity stimulus (Wayne et al., 1990; Wayne and Staves, 1996), and also explains the rather weak agravitropic phenotype of starchless mutants (Kiss and Sack, 1989; Kiss et al., 1996; MacCleery and Kiss, 1999; Fitzelle and Kiss, 2001; Band et al., 2012a). Barlow (1995)...

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**Fig. 1.** Gravitropic bending of an *Arabidopsis thaliana* root. Sequential imaging of a bending root with colour codes at different time points.

**Fig. 2.** Gravity perception in *Arabidopsis thaliana*. At time point 0, roots grow vertically. After a 90° turn, the following series of events take place: (1) At 10 s, statoliths are still at the old bottom of the cell. After 3 min, statoliths move towards the new bottom of the cell to be uniformly distributed at 5 min (Leitz et al., 2009); (2) PIN3 and PIN7 relocalization is achieved 2 min after the gravity stimulus and, in consequence, a lateral auxin gradient is generated between the upper and lower side of the root (thin and thick orange arrows respectively) (Friml et al., 2002b); (3) Development of differential extracellular pH levels between the upper (acidic) and lower (alkaline) side of the gravistimulated root (Monshaussen et al., 2011).
suggested that over the period of evolution plants may have evolved multiple gravity-sensing mechanisms, a view shared by Perbal (1999), who suggests that both protoplast pressure and starch–statolith hypotheses may operate side by side. In addition, Wolverter et al. (2002a, b) proposes that there might be additional minor gravity-sensing sites in the root cap outside the columella cells.

Following gravity perception in the central columella cells, there is a change in the cytosolic pH of root cap cells from 7.2 to 7.6 in wild-type roots but not in the starchless pgm mutants (Fasano et al., 2001). This observation directly links these pH changes to statolith sedimentation (Scott and Allen, 1999; Fasano et al., 2001; Hou et al., 2004), which is supported by the finding that acidifying or alkalinizing agents can alter the gravitropic response. Also gravitropic arg1 mutants have a defect in the pH changes following gravity perception (Boonsirichai et al., 2003).

Much research and speculations have focused on how a physical signal (statolith sedimentation) can be translated into a moving physiological signal. It has been proposed that an interaction between the sedimenting amyloplasts and proteins belonging to the endoplasmic reticulum or the plasma membrane may be involved in the graviperception. This hypothesis is based on observations in rhizoids of the green algae Chara. As the rhizoids are single cells, the gravity perception and its response both occur in the same cell. In parabolic flights, weightless Chara statoliths (barium sulphate crystals) could still sense gravity as long as their statoliths remained in contact with plasma membrane sensitive sites (Limbach et al., 2005). It was therefore suggested that not the pressure (exerted by the statoliths) but the contact of the statolith with some membrane-bound receptors triggered the gravity perception (Braun and Limback, 2006). The fact that root gravitropism defects of starchless mutants can be rescued in hypergravity conditions (Fitzelle and Kiss, 2001) indicates that similar mechanisms may operate in higher plants too. This idea is further supported by the finding that TOC (translocon of outer membrane of chloroplasts) complex-related proteins may be part of a receptor–ligand system (Stanga et al., 2009). The TOC complex is important for the delivery of nuclear-encoded proteins to the chloroplasts and presumably to the amyloplasts. In a screen for enhancers of arg1, mar1 and mar2 mutations were identified. MAR1 and MAR2 encode components of the TOC complex. Mutations in TOC132 enhance the gravitropic defects of a starchless mutant (Strohm et al., 2014), suggesting that plastids, besides functioning as statoliths, may also be part of a signal transduction mechanism, facilitating interaction between a ligand protein at the outer amyloplast envelope and a receptor protein on the endoplasmic reticulum or plasma membrane (Stanga et al., 2009).

Another view is that stretch-activated mechanosensitive ion channels may be involved in graviperception (Sievers et al., 1991; Perbal and Driss-Ecole, 2003). These ion channels may be activated by the force exerted by sedimenting amyloplasts on the plasma membrane and/or endoplasmic reticulum. Electron tomography experiments that show that sedimenting amyloplasts bend and distort the endoplasmic reticulum at the point of contact may support this view (Leitz et al., 2009). Although inhibitors of mechanosensitive ion channels inhibit gravitropism (Caldwell et al., 1998), genetic studies so far have failed to pinpoint their role in gravitropism (Nakagawa et al., 2007; Haswell et al., 2008). It can, however, not be ruled out that as yet unidentified plant-specific mechanosensitive ion channels are involved in root gravitropism.

There has been a lot of controversy on the role of the actin cytoskeleton in root gravitropism, and the results were often contradictory (Blancaflor and Hasenstein, 1997; Staves, 1997; Yamamoto and Kiss, 2002; Friedman et al., 2003; Hou et al., 2003, 2004; Palmieri and Kiss, 2005; Mancuso et al., 2006). In recent years, it is generally accepted that actin negatively regulates root gravitropism (Blancaflor, 2013; Swarup et al., 2013). However, Blancaflor (2013) proposes a fine-tuning role for actin in root gravitropism and argues that actin may positively regulate gravitropism as breaking the actin connection will result in faster sedimentation of the amyloplasts and hence more compressive forces on the cortical endoplasmic reticulum, and thus enhanced gravitropic curvature.

The finding that some of the key proteins involved in the early stages of gravity perception, such as ARG1 (Boonsirichai et al., 2003) and the pea homologue of the MAR2 part of the TOC complex (Jouhet and Gray, 2009), can both bind to actin provides a direct link between statolith sedimentation and the actin cytoskeleton. The finding that the chloroplast outer envelope protein CHUP1 (CHLOROPLAST UNUSUAL POSITIIONING1) binds to actin (Oikawa et al., 2003; Schmidt von Braun and Schleiff, 2008) further supports this link. If similar proteins were present in the outer amyloplast membrane as well, they could potentially act as a ligand for some plasma membrane or endoplasmic reticulum receptor for gravitropic signalling, thus linking actin to gravitropic signalling.

Gravity signal transduction (Fig. 3)  
Gravity is perceived in the columella cells, but the actual graviresponse is achieved in the root elongation zone. To explain this, Cholodny (1927) and Went (1926) proposed their famous hypothesis that, following gravity perception, a signal is generated and transduced from the sedimenting amyloplasts to the cells in the root elongation zone, the actual site of the graviresponse. Pharmacological, genetic, and cell biology-based approaches have shown that this signal is auxin. The use of the auxin response reporters DR5 and IAA2, and the auxin sensor DII 28 (Brunoud et al., 2012) shows a differential accumulation of auxin between the lower and upper side of a gravistimulated root (Ottenschläger et al., 2003; Swarup and Bennett, 2009; Band et al., 2012a).

Indole-3-acetic acid (IAA), the major form of auxin in higher plants, is a weak acid and, at the intracellular pH, exists in its membrane-impermeable (IAA ) form. However, at the more acidic apoplastic pH, IAA will exist in both IAAH (membrane-permeable) and IAA- forms. Auxin moves from cell to cell in a directional manner and this polarity of auxin movement is well explained by the chemiosmotic hypothesis (Rubery and Sheldrake, 1974; Raven, 1975) that not only proposed the existence of specialized auxin carriers
but also hypothesized that the asymmetric localization of auxin transporters provides the basis for the directionality of auxin movement. Genetic and cell biology approaches later confirmed that auxin transport is carrier mediated and facilitated by specific auxin influx and efflux carriers (Bennett et al., 1996; Gälweiler et al., 1998; Swarup et al., 2014).

The auxin influx carriers are encoded by a small gene family comprised of four members (AUX1 and Like AUX1 or LAX genes). AUX1 is expressed in columella, lateral root cap, and epidermal cells, tissues that are involved in gravity perception, transmission, and response, respectively (Swarup et al., 2001, 2004, 2005, 2014). Mutation in the AUX1 gene results in severely agravitropic roots, pointing to the importance of AUX1 in root gravitropism (Bennett et al., 1996; Swarup and Peret, 2012; Swarup et al., 2014). Using a trans-activation-based approach, Swarup et al. (2005) mapped the tissues required to transport auxin during a root gravitropic response. They showed that auxin moves via the lateral root cap cells to the cells in the elongation zone, which can serve as direct evidence for auxin being the primary gravitropic signal and linking the site of gravity perception to the graviresponse.

Though AUX1 is important for root gravitropism, the directionality of auxin movement is provided by asymmetric localizations of the PINFORMED (PIN) family of auxin efflux carriers (Grunewald and Friml, 2010). Several efflux carrier proteins have been localized in the root apex (Grunewald and Friml, 2010; Swarup et al., 2014). Of these, PIN3, PIN4, and PIN7 are localized in the columella cells, whereas PIN2 is expressed in the lateral root cap, and epidermal and cortical cells (Luschnig et al., 1998; Müller et al., 1998; Friml et al., 2002a, b). Friml et al. (2002b) showed that upon a gravitropic stimulus PIN3 is relocated within 2 min to the lateral face of the columella cells, providing a very elegant mechanism for the creation of an auxin gradient between the upper and the lower side of a gravistimulated root (Friml et al., 2000b). However, pin3 mutants show only a weak gravitropism defect (Friml et al., 2002b). Kleine-Vehn et al. (2010) showed that PIN7 is also asymmetrically localized in columella cells in response to a gravity stimulus, and pin3pin7 double mutants are significantly more agravitropic than pin3 or pin7 single mutants, strongly suggesting some degree of redundancy. Recent studies, using a novel auxin sensor, DII-Venus, in combination with a mathematical modelling approach, indicate that auxin asymmetry between the lower and upper side of the root can develop within minutes after a gravity stimulus (Band et al., 2012a). Thus, reorientation of PIN3 and PIN7 in the plasma membrane of columella cells facilitates the establishment of a lateral auxin gradient across the root cap upon gravistimulation. Once the auxin asymmetry is initiated by PIN3 and PIN7, auxin is transported by AUX1/PIN2 in a shootward direction through the lateral root cap to the epidermal cells in the elongation zone.

The directionality of this auxin movement is provided by the asymmetric localization of PIN2. In the lateral root cap, PIN2 is localized on the shootward face of the cells, thus channelling auxin into the elongation zone. Interestingly, in the distal elongation zone, PIN2 is localized on the shootward face of epidermal cells but on the rootward face of the cortical cells, which creates an auxin reflux loop that has been shown to be important for root gravitropism (Biilou et al., 2005).

Genetic and pharmacological approaches show that PIN abundance and localization at the plasma membrane are carefully regulated and controlled. Treatments with the vesicular trafficking inhibitor brefeldin A (BFA) affect PIN recycling and cause severe auxin-related developmental defects (Geldner et al., 2004). Treatment with the trafficking modulator TEnin1 (TE1) causes accumulation of several plasma membrane proteins, including PIN2, in pre-vacuolar compartments, which results in agravitropic roots (Paudyal et al., 2014). The small secretory peptide GOLVEN (GLV) has also been shown to affect auxin transport by regulating the distribution of PIN2 in the plasma membrane (Whitford et al., 2012). Even auxin itself appears to play a key role in regulating PIN2 plasma membrane abundance. Abas et al. (2006) showed that auxin promotes its own efflux by regulating PIN2 turnover. They observed an increased rate of PIN2 endocytosis and degradation on the upper side of a gravistimulated root compared with the lower side. This differential accumulation of PIN2 between the lower and upper sides of a gravistimulated root appears to be crucial for maintaining the differential auxin asymmetry initiated by PIN3, as a single missense amino acid substitution in the wav6-52 allele of PIN2 makes PIN2 resistant to degradation, resulting in agravitropic roots. Besides auxin, gibberellic acid (GA) can also regulate the plasma membrane distribution of PIN proteins. Löffke et al. (2013) showed that high GA can result in more PIN proteins in the plasma membrane, whereas low GA results in the internalization of PIN proteins.
In addition, it is well documented that post-translational modifications, such as (de)phosphorylation, also regulate polar PIN localization and therefore are involved in the graviresponse. For example, the protein-serine/threonine kinase PINOID (PID) regulates root gravitropism by affecting PIN2 plasma membrane localization (Sukumar et al., 2009; Huang et al., 2010). Changes in the phosphorylation status of PIN3 have also been reported to affect several PIN3-regulated processes, including root gravitropism (Ganguly et al., 2012). In addition, the D6 family of protein kinases (D6 PROTEIN KINASE; D6PK) may also regulate gravitropism by regulating the phosphorylation status of PINs (Barbosa et al., 2014). D6PKs are membrane-bound protein kinases and auxin can promote internalization of D6PKs from the plasma membrane to the endomembranes, providing further evidence that the abundance of PINs at the plasma membrane is regulated by post-translational modifications such as phosphorylation (Barbosa et al., 2014).

Besides phosphorylation, several other pathways have also been proposed to regulate PIN plasma membrane abundance. For example, a ROP GTPase-dependent pathway has been implicated for PIN2. Mutations in ROP6-GTPase, its effector RIC1, as well as a DHR2-Dock family of Rho guanine nucleotide exchange factor SPIKE1 (SPK1) affect auxin-mediated suppression of PIN2 internalization and PIN2 plasma membrane localization (Lin et al., 2012). Furthermore, SPK1 is required for auxin induction of ROP6 activation, and a Rho GTPase-based auxin signallng pathway regulates PIN2 plasma membrane abundance (Lin et al., 2012). There is some evidence suggesting a role for phospholipase Dζ2 in regulating PIN2 cycling, as mutations in the PHOSPHOLIPASE Dζ2 gene or treatment with the PLD-inhibitor 1-butanol affect PIN2 cycling and reduce root gravitropism (Li and Xue, 2007). In addition, maintenance of polar distribution of PIN at the plasma membrane also seems dependent on cellulose-based connections to the cell wall (Feraru et al., 2011).

In summary, these results clearly suggest that regulation of PIN trafficking is crucial for fine-tuning auxin transport and creating auxin gradients. However, despite the importance of PIN2 in providing directionality of auxin transport during root gravitropism, pin2 mutants are not severely agravitropic (Chen et al., 1998; Luschnig et al., 1998; Blakeslee et al., 2007). Two members of the p-glycoprotein (PGP) family of auxin efflux transporters, AtPGP1 and AtPGP19, are also expressed in the root elongation zone (Blakeslee et al., 2007). Though single or double ppg1/pgp19 mutants do not have a very strong agravitropic phenotype, pin2ppg1/ppg19 triple mutants are severely agravitropic (Blakeslee et al., 2007), suggesting that both PIN and PGP families of auxin efflux carriers are required for root gravitropism.

In contrast to PIN proteins, which provide directionality to auxin movement, AUX/LAX proteins appear to be important for maintaining the auxin gradient and for contributing to the pattern of auxin distribution at the root tip (Band et al., 2014). Despite the importance of AUX1 in root gravitropism, there has been a general misconception that because protonated IAA is membrane permeable, influx carriers play only a supplemental role. Using confocal microscopy and fluorescent pH sensors, Monshausen et al. (2011) recently showed that there is an increase in the surface pH on the lower side of gravistimulated wild-type but not in aux1 roots. One important implication of this finding is that the increase in the root apoplastic pH will result in more IAA in its ionic IAA form. Since this form is not membrane permeable it will require a carrier (AUX1)-mediated uptake. Computer simulation studies estimate that this carrier-mediated IAA uptake is 15 times greater than diffusion (Swarup et al., 2005; Kramer and Bennett, 2006). These studies suggested that epidermal-expressed AUX1 along with PIN2 minimize the effect of radial diffusion while facilitating shootward auxin transport (Swarup et al., 2005).

**Gravitropic response (Fig. 4)**

Genetic and pharmacological evidence suggests that the gravitropic response takes place in the elongation zone. Using a transactivation-based approach, Swarup et al. (2005) showed that the root epidermis represents the primary site of the gravity response, since expression of a dominant auxin response repressor axr3, blocking the auxin response specifically in the epidermal cells, resulted in the loss of gravitropic bending. This crucial role of epidermal cells is further supported by computer simulation studies that indicated that a lateral auxin gradient accumulates 10- to 20-fold more in the epidermis than in the underlying tissues (Swarup et al., 2005). Their work clearly established the importance of auxin as a primary gravitropic signal that acts directly in the expanding cells in the elongation zone.
Though the mechanistic aspects of root gravitropism are not very well understood, it has been observed that auxin somehow activates cytosolic Ca\(^{2+}\) waves, which in turn promotes differential apoplastic pH changes at both sides of the gravistimulated root. The Ca\(^{2+}\)-dependent acidification of the cell wall can increase the epidermal cell elongation rate in the upper side of the root, while alkalinization of the apoplast then reduces the cell elongation rate at the lower side (Ishikawa and Evans, 1993; Mullen et al., 1998). This leads to root curvature that becomes evident 10 min after the onset of the gravistimulus. Because of the rapidity of the gravitropic response, it has been suggested that the initial phase of the gravitropic response cannot rely on newly formed proteins and therefore must be non-genomic (Swarup et al., 2012). Using high-resolution confocal imaging, Monshausen et al. (2011) observed pH changes at the surface of the root elongation zone cells within minutes after a gravistimulus. These changes in surface pH were AUX1 dependent, but they were also seen in single or multiple auxin receptor mutant backgrounds, supporting a non-genomic response during early stages of root gravitropism. Gene expression studies showed that peak transcript levels of several auxin-inducible genes such as \(IAA1\), \(IAA2\), and \(ARF19\) are seen only 15 min after a gravistimulus (Band et al., 2012; Brunoud et al., 2012), which is after the first visible signs of actual bending. Knowing that genes downstream of these early auxin-responsive genes are expressed even later, this timing would also support a role for an initial non-genomic phase preceding the genomic response phase. Although at present it is not well understood how a non-genomic auxin response can be achieved in the early stages of gravitropism, involvement of ABP1 (Auxin Binding Protein1) appears to be promising. The role of ABP1 has long been a subject of great debate, but in recent years it is non-genomic auxin response can be achieved (Effendi et al., 2011).

Besides auxin there are several other signals, such as Ca\(^{2+}\), inositol 1,4,5-trisphosphate (InsP\(_3\)), nitric oxide (NO), and reactive oxygen species (ROS), that have all been implicated in root gravitropism, and these may also be involved in the initial non-genomic phase of root gravitropism. For example, asymmetric Ca\(^{2+}\) gradients have been reported in gravistimulated roots (Lee et al., 1984), and the application of Ca\(^{2+}\) chelators results in a loss of gravitropic sensitivity (Lee et al., 1983). Using an elegant cell biological approach, Monsenhausen et al. (2011) showed that application of auxin at the root apex results in a shootward wave of Ca\(^{2+}\) that is not observed when treated with Ca\(^{2+}\) channel blockers. They proposed that the auxin-mediated increase in cytosolic Ca\(^{2+}\) could be due to activation of a Ca\(^{2+}\) channel that in turn activates a plasma membrane H\(^+\)/OH\(^-\) conductance. This would then result in alkalinization of the apoplast. Changes in cell wall pH have been implicated in cell expansion (Cosgrove, 1998, 2000), perhaps by impacting cell wall integrity and affecting intermolecular cross-links (Brady and Fry, 1997) by cell wall remodelling proteins, such as expansins and xyloglucan endotransglucosylase/hydrolases (XTHs), whose actions are pH dependent (Cosgrove, 2005; Nishitani and Vissenberg, 2007; Maris et al., 2009, 2011).

Treatment with U73122, an inhibitor of InsP\(_3\) biosynthesis, results in an attenuated gravity response (Andreeva et al., 2010), indicating the involvement of InsP\(_3\) in regulating root gravitropism. Moreover, concentrations of InsP\(_3\) have been observed to oscillate and then to increase in the lower side of oat, maize, and \(A.\ thaliana\) stems following gravistimulation (Perera et al., 1999, 2001, 2006), suggesting that a similar mechanism could be active in the root.

NO may also play a role in root gravitropism since it has been shown to accumulate on the lower side of a gravistimulated root (Hu et al., 2005), which may interfere with auxin transport (Fernández-Marcos et al., 2011). This differential accumulation can be blocked by treatment with the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA), but is restored with asymmetric application of auxin. Though it is not well understood how NO could participate in root gravitropism, it appears that NO signalling is mediated via cGMP (Hu et al., 2005). It has been further suggested that NO can directly modify key proteins involved in root gravitropism either via nitrosylation (Durner and Klessig, 1999; Terrile et al., 2012) or by elevating cGMP levels (Durner et al., 1998).

Like NO, ROS accumulate at the lower side of gravistimulated roots (Joo et al., 2001, 2005) but, unlike NO, ROS seem to act downstream of auxin as application of hydrogen peroxide induces curvature even in roots treated with the auxin transport inhibitor NPA (Joo et al., 2005). De Cnodder et al. (2005) have reported that ROS can mediate cross-linking of structural cell wall proteins during 1-aminoacyclopropene-1-carboxylic acid (ACC)-induced cell elongation arrest of \(A.\ thaliana\) root epidermal cells. It is therefore tempting to speculate that similar differential cross-linking events could influence cell elongation rates at the lower and upper side of the root during root gravitropism.

In contrast to our limited understanding of the non-genomic phase of root gravitropism, the genomic phase is much better understood. Mutations in several auxin signalling components result in agravitropic roots and have led to the identification of several key molecular players. In the cell, auxin is perceived by its receptor the TIR1/AFB family of F-box proteins that then facilitates degradation of auxin repressor AUX/IAA proteins via the ubiquitin–proteasome pathway (Gray et al., 2001). As a result, auxin response factors (ARFs) are de-repressed and initiate expression of auxin-regulated genes. In \(A.\ thaliana\), AUX/IAAs and ARFs are part of large gene families with 29 and 23 members, respectively, and the output of the TIR1/AFB pathway is dependent on the dynamic equilibrium of the abundance of these two classes of proteins. Genetic studies suggest that among all ARFs, ARF7 and ARF19 appear to be most important for root gravitropism, and AXR2, AXR3, SLR1, and SHY2 are the key AUX/IAA proteins that mediate root gravitropism. The genomic auxin response probably involves the differential expression of genes at the upper and lower side of the
root, and transcriptome analysis suggested that several cell wall-related genes are under ARF7 and ARF19 regulation in expanding root epidermal cells, including AtXTH18 and AtXTH19. The fact that AtXTH18 and AtXTH19 encode cell wall enzymes that modify hemicellulosic tethers between adjacent cellulose microfibrils, and therefore can help to regulate expansion, led Swarup et al. (2013) to speculate that ‘initially through its non-genomic effect, auxin brings about rapid differential cell expansion, whilst later on auxin regulates cell wall biosynthesis and remodelling through its genomic effects’.

**Restoration of the symmetrical auxin flow (Fig. 5)**

Recent studies provide some insight into important questions such as how long the PIN3/PIN7-facilitated auxin asymmetry is maintained, whether it lasts throughout the whole bending period, and how the normal auxin gradient is restored. Using the DII-Venus auxin sensor (Brunoud et al., 2012) in combination with mathematical modelling studies, Band et al. (2012a) suggested that the auxin asymmetry persists for ~100 min or roughly up to the point when the root tip angle reaches 40°. Cell biology studies showed that at this angle, because of the specific morphology of the central columella cells, the statoliths reposition to the new physiological bottom of the cell. This led Band et al. (2012a) to propose a tipping point mechanism to explain restoration of auxin symmetry. Statoliths reposition in the columella cells when the root tip reaches 40°. As a consequence this triggers the restoration of PIN3/PIN7 location, and hence auxin flow is no longer asymmetric.

**Open questions and future challenges**

Regardless of the major success in uncovering major regulatory physiological and genomic processes and factors, there remain several open questions regulating various stages of gravitropism.

(i) Little is known about the nature of the gravity receptors controlling gravity perception. Where are they located? Are there more than one kind of gravity receptors? The role of mechanosensitive ion channels such as MCA1, MCA2, MS9, and MSL10 is almost ruled out in root gravitropism (Nakagawa et al., 2007; Haswell et al., 2008) unless plant-specific novel mechanosensitive or gravity-related ion channels are identified. On the other hand, the receptor ligand-based hypothesis is sketchy and needs further investigation. Though the role of auxin in regulating root gravitropism is well established, any possible role of other phytohormones during root gravitropism is not very clear and requires further investigation. As mentioned earlier, GA has been shown to regulate the plasma membrane distribution of PIN proteins (Löfke et al., 2013). Further understanding of GA-regulated PIN localization will provide greater insight into the role of GA in regulating root gravitropism.

(ii) With respect to the gravity signal transduction, current cell live-imaging technologies are not yet fully optimized to monitor intracellular changes of all signalling molecules in vivo. Computer-based systems coupled to confocal laser scanning microscopy (CSLM) and the use of fluorescent dyes have permitted the visualization of either plant organelles or physiological parameters such as Ca²⁺, pH, metal ions, etc. (Pollastri et al., 2012). However, for this group of molecules, the quantification and abundance under a particular developmental or physiological process can be problematic. Hence, development of sensors that can precisely monitor very small or transient concentration changes in Ca²⁺, pH, and also other signalling molecules regulating root gravitropism (InsP₃, NO, and ROS) coupled to new detection methods, such as light sheet or selective plane illumination microscopy (SPIM) (Costa et al., 2013), are very promising. Ideally, their simultaneous visualization and quantification will shed light on how these signalling molecules interact or counteract with one another upon gravistimulation. A spatio-temporal map of their action would be very informative not only for plant tropisms, but also for other biological processes.

(iii) While transcriptomic approaches have proven very valuable to shed light on later events of gravitropism (Zhu et al., 2002; Kimbrough et al., 2004), due to the speed of the gravitropic response, proteomics or chemical biology approaches are likely to be more useful tools to identify novel regulators during early stages of gravitropism (Yamazoe et al., 2005; Young et al., 2006; Na et al., 2011). Also downstream events after the development of the auxin asymmetry remain unclear. How is differential growth regulated? Both the intricate complexity of the
cell wall and the functional redundancy of the diverse cell wall remodelling enzymes make the identification of key cell wall-related enzymes in regulating differential growth challenging. Use of solid-state $^{13}$C-nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) spectroscopy, and Raman microscopy promises to provide a better picture of the composition of the cell wall and will allow comparison of the upper and the lower flank of plant roots.

(iv) Auxin symmetry is restored when the root tip angle reaches 40°, but the question remains as to how the remaining bending is regulated. In addition, the apex of lateral roots should respond to gravity as primary roots. Yet, especially at early stages, lateral roots grow at a certain angle to the primary root (Fig. 6). Whether the gravitropism machinery is working and how this might be over-ruled remains open for debate.

(v) In recent years, mathematical modelling approaches have started to provide more quantitative insight into root gravitropism (French et al., 2009; Band et al., 2012a, b, 2014). These approaches are enabling researchers to develop experimentally testable hypotheses providing deeper insight into root gravitropism (Swarup et al., 2005; Laskowski et al., 2008; Kondrachuk et al., 2011; Band et al., 2012b, 2014). Since the gravitropic response is an interplay between perception, transmission, and actual response, and involves a wide variety of key players (from ions to proteins, hormones, and cell wall-related molecules), the next big challenge is to integrate all these factors in mathematical models and develop mathematically sound and experimentally testable hypothesis.

Addressing these issues will be challenging, but recent advances in genetic, genomic, and imaging technologies in combination with a systems biology approach and improved mathematical models are likely to provide a global more holistic understanding of gravitropism at the molecular, cellular, and tissue level.

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References


Fig. 6. Arabidopsis thaliana root system. Overview of the A. root system redrawn from a 7-day-old seedling, showing the orientation of primary and lateral roots.


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