Chapter 1

Ca^{2+} signaling in the regulation of auxin transport by developmental and environmental signals

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The signaling molecule auxin is an important regulator of plant development, instructing tissue differentiation and organ development and growth, while translating environmental stimuli into developmental responses. Here we review the role of auxin in a plant's response to developmental and environmental signals, with a focus on the interaction of auxin with the second messenger Ca^{2+}, Ca^{2+} receptors, such as calmodulins, play an important role in this interaction, as these receptors are able to translate signal-induced changes in the cytosolic Ca^{2+} concentrations into a developmental or growth response, e.g. by modulating the direction of polar auxin transport.

1. Auxin is a crucial regulator of plant development

As sessile organisms, plants respond to changes in their environment by adjusting their growth and development through a highly dynamic combination of signal perception and transduction systems. The plant hormone auxin is a central regulator of these adaptive responses to environmental cues. Already around 1880, Charles Darwin predicted the existence of some 'mobile factor' that mediates the bending of canary grass and oat coleoptiles toward a unidirectional light source (Darwin, 1880). This 'factor' was first isolated from coleoptile tips in 1926, and was later identified as the plant hormone indole-3-acetic acid (IAA), which was named auxin after the greek word 'auxein' for 'to grow' (Went and Thimann, 1937). Auxin acts as a crucial regulator of plant development and mediates different cellular responses by its differential distribution between cells, resulting from its biosynthesis and metabolism on the one hand (Zhao, 2010; Sauer et al., 2013), and its polar cell-to-cell auxin transport on the other. Polar auxin transport (PAT) results in local auxin accumulation or -depletion in tissues and organs, which instructs plant growth and development by regulating basic cellular processes such as cell division, -differentiation and -elongation (Tanaka et al., 2006).

2. Polar auxin transport

PAT involves the activity of several families of auxin transporters, of which the "long" members of the PIN auxin efflux carrier family in *Arabidopsis* (PIN1, 2, 3, 4, 7 and possibly 6) determine the direction of transport through their asymmetric localization at the plasma membrane (PM) (Petrasek et al., 2006; Tanaka et al., 2006; Wisniewska et al., 2006; Mravec et al., 2009; Sawchuk et al., 2013). This PIN-driven PAT is essential for a wide array of plant developmental processes, including apical-basal axis formation

during embryogenesis (Friml et al., 2003), postembryonic organogenesis (Okada et al., 1991; Benkova et al., 2003; Reinhardt et al., 2003), root meristem organization (Sabatini et al., 1999; Friml et al., 2002b; Blilou et al., 2005), tropisms (Luschnig et al., 1998; Friml et al., 2002a; Ding et al., 2011), vascular differentiation and tissue regeneration (Sauer et al., 2006; Scarpella et al., 2006; Xu et al., 2006).

Polar PIN localization is dynamic; according to the current model, following their biosynthetic secretion to the PM PIN proteins constitutively undergo cycles of clathrin-dependent endocytosis and subsequent recycling to their correct polar domain (Geldner et al., 2001; Dhonukshe et al., 2007; Dhonukshe et al., 2008; Kleine-Vehn et al., 2011). This trafficking is mediated by ADP ribosylation factors (ARFs) GTPases and their corresponding guanine nucleotide exchange factors (GEFs), of which the brefeldin A (BFA) toxin sensitive ARF GEF GNOM mediates exocytosis to the basal (rootward) or inner-lateral polar domains (Geldner et al., 2001; Geldner et al., 2003; Kleine-Vehn et al., 2009; Ding et al., 2011). The subcellular sorting of PIN proteins is directed by post-translational modification through phosphorylation or ubiquitination (Habets and Offringa, 2014). The PINOID (PID) serine/threonine protein kinase has been identified as one of the key determinants in this process (Benjamins et al., 2001; Friml et al., 2004). *Arabidopsis pinoid* mutants share the phenotypic defects in the inflorescences of the *pin-formed* mutant, which develop pin-like structures that lack lateral organs due to defective polar auxin transport (Bennett et al., 1995; Christensen et al., 2000). Loss of *PINOID* (*PID*) function causes an apical-to-basal shift in PIN polarity, correlating with defects in embryo and shoot organogenesis. In contrast, PID gain-of-function results in an opposite basal-to-apical PIN polarity shift, which leads to auxin depletion from the root meristem, ultimately causing collapse of the meristem (Friml et al., 2004). Together with the close homologs WAG1, WAG2 and AGC3-4, PID forms the AGC3 clade of the plant-specific AGC-VIII protein kinases in *Arabidopsis*. For PID, WAG1 and WAG2 it has been shown that they phosphorylate serines in three conserved TPRXS motifs in the central hydrophilic loop (HL) of "long" PINs, thereby recruiting these PINs into the apical recycling pathway, directing cotyledon development, and regulating root meristem size and gravitropic responses (Dhonukshe et al., 2010; Huang et al., 2010; Ding et al., 2011). The three AGC3 kinases act antagonistically with trimeric PP2A-/PP6-type phosphatases on the phosphorylation state of the PIN HL (Michniewicz et al., 2007; Dai et al., 2012; Ballesteros et al., 2013). Phosphorylated PINs are sorted to the GNOM-independent recycling pathway, which is apical for epidermal cells in meristems and for pro-vascular cells, outer-lateral for

endodermis cells, and lobe-polarity for leaf pavement cells (Friml et al., 2004; Kleine-Vehn et al., 2009; Ding et al., 2011).

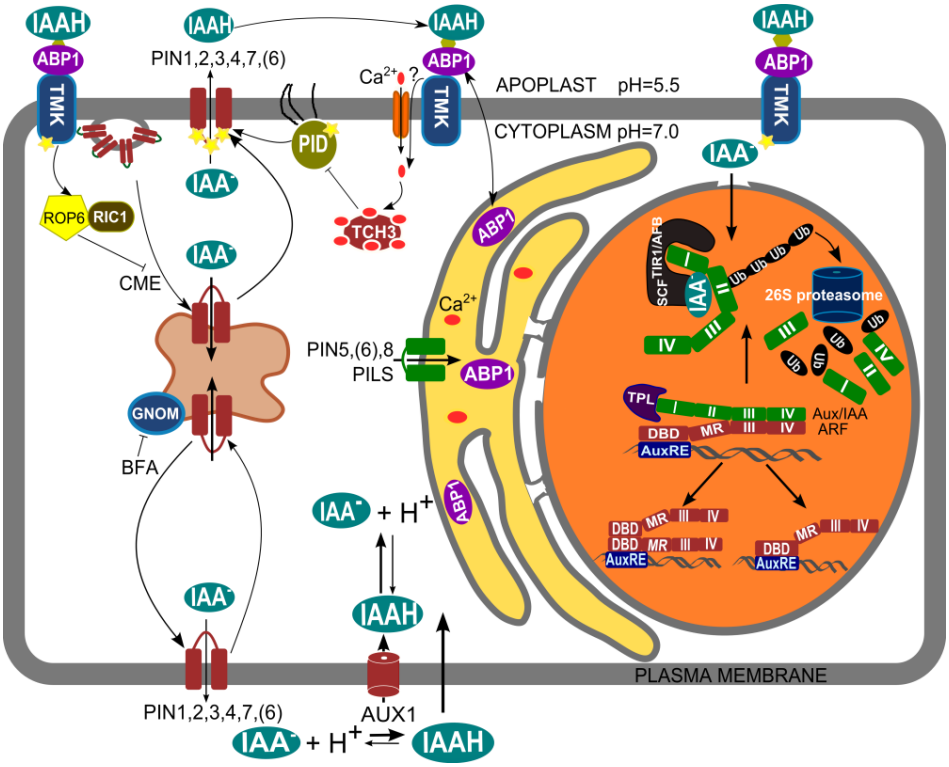

Figure1 The auxin transport and signaling network.

Auxin, or indole-3-acetic acid, is mostly in its protonated form (IAAH) in the relatively acidic apoplast, and can pass the plasma membrane (PM) by diffusion, or can enter the cell via the AUX1/LAX influx carriers. In the more basic cytosol, auxin becomes trapped as deprotonated anion (IAA-) that requires "long" PIN auxin efflux carriers (1, 2, 3, 4, 7 and possibly 6) for its transport across the PM or short PINs (5, 6, 8) for transport across the endoplasmatic reticulum (ER, yellow) membrane. Long PINs determine the direction of polar cell-to-cell auxin transport through their asymmetric localization at the PM, which is determined by phosphorylation of their central hydrophilic loop by the PM-associated PINOID (PID) kinase. The Ca^{2+}-dependent interaction of PID with the calmodulin-like protein TOUCH3 (TCH3) negatively regulates PID activity.

Plant cells sense auxin either by the apoplastic auxin-binding protein 1 (ABP1), or by the nuclear

TRANSPORT INHIBITOR RESISTANT 1 (TIR1)/AUXIN SIGNALING F-BOX (AFB) and Aux/IAA co-receptors. Binding of apoplastic auxin to ABP1 inhibits its activity in promoting clathrin-mediated endocytosis (CME), leading to PIN stabilization at the plasma membrane, and resulting in its recruitment by the Transmembrane Kinase 1 (TMK1), which triggers the activation of the ROP2/RIC4 and ROP6/RIC1 pathways that antagonistically regulate polar cell expansion. The ABP1-TMK1 complex is might also be responsible for the auxin-induced elevation of Ca^{2+} levels by activation of Ca^{2+} channels in the PM. In the nucleus, auxin stabilizes the interaction between the TIR1/AFB and Aux/IAA co-repectors, leading to SKP1-CULLIN-F-Box (SCF)-mediated ubiquitination (Ub) and proteasome degradation of the Aux/IAA repressor proteins, In the the absence of the Aux/IAA-TOPLESS (TPL) repressor complex, the AUXIN RESPONSE FACTORs (ARFs) can dimerize at the DNA binding domain (DBD) to efficiently activate gene transcription.

The role of the fourth member of the AGC3 clade, AGC3-4, is until now unclear. Apart from the AGC3 kinases, the D6 protein kinases (D6PK) and the D6PK-like1 (D6PKL1), D6PKL2 and D6PKL3, belonging to the AGC1 clade of the *Arabidopsis* AGCVIII kinases, have been reported to promote PAT (Zourelidou et al., 2014). Two recent reports suggest that D6PK phosphorylates a partially overlapping set of serine residues in the PIN HL, leading to activation rather than changing the polarity of PAT (Barbosa et al., 2014; Zourelidou et al., 2014).

3. Auxin perception and signaling

PAT-generated differential auxin distribution is translated into developmental or growth responses by a complex signaling network. Based on our current knowledge, there are at least three types of auxin receptors. One is the family of TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALING F-BOX (TIR1/AFB) proteins, which together with the Aux/IAA repressor proteins act as auxin co-receptors to regulate the AUXIN RESPONSE FACTOR (ARF)-mediated gene expression in the nucleus (Dharmasiri et al., 2005a; Dharmasiri et al., 2005b; Kepinski and Leyser, 2005; Tan et al., 2007). The TIR1/AFB family consists of 6 members in *Arabidopsis* that use auxin as a molecular glue to recruit Aux-IAA proteins for ubiquitination and subsequent degradation as part of the SKP1 – CULLIN1 – F-box SCF ubiquitin E3 ligase complex (Gray et al., 2001; Dharmasiri et al., 2005a; Dharmasiri et al., 2005b). Arabidopsis has 29 Aux/IAAs proteins and each Aux/IAA protein has four highly conserved domains (Liscum and Reed, 2002). The domain I has an ERF-associated amphiphilic repressor (EAR) motif which is crucial for its role in transcription repression (Tiwari et al., 2004), as it binds

the TOPLESS (TPL) corepressor (Szemenyei et al., 2008). Domain II interacts with TIR1, resulting in the ubiquitination and subsequent degradation of AUX/IAAs by the 26S proteasome, thereby releasing ARFs to activate transcription (Gray et al., 2001; Dharmasiri et al., 2005a; Kepinski and Leyser, 2005; Maraschin Fdos et al., 2009). The domains III and IV are also found in Aux/IAAs and mediate dimerization between Aux/IAAs and ARFs (Liscum and Reed, 2002; Tiwari et al., 2004). The ARFs consist of a family of 23 proteins in *Arabidopsis* that depending on their middle domain act as activators or repressors (Liscum and Reed, 2002; Tiwari et al., 2004). Through their N-terminal DNA binding domain (DBD) ARFs interact with Auxin Response Elements (AuxRE) in the promoters of auxin responsive genes (Guilfoyle and Hagen, 2007). ARFs have been reported to act as monomers or dimers, but the recent resolution of the crystal structure of ARF5/MONOPTEROS(MP) and ARF1 has revealed that ARFs most likely act as homo- or heterodimers, and that dimerization occurs through their DNA binding domain (Boer et al., 2014).

The second type of auxin receptor is the plant-specific protein AUXIN BINDING PROTEIN 1 (ABP1). ABP1 is retained in the endoplasmic reticulum (ER) by a KDEL-motif, but is also secreted to the extracellular space (Jones and Herman, 1993; Henderson et al., 1997). The *abp1* null mutant is embryo lethal, showing that ABP1 plays an important role in embryogenesis (Chen et al., 2001), but conditional knock downs and weak mutant alleles show that ABP1 is also important for postembryonic shoot and root development (Braun et al., 2008; Tromas et al., 2009; Xu et al., 2010; Li et al., 2011). Recent studies show that ABP1 promotes the recruitment of clathrin to the PM, thereby promoting clathrin-mediated PIN endocytosis (Robert et al., 2010). When auxin binds to ABP1, it inhibits this action and thus prevents clathrin-mediated endocytosis (Robert et al., 2010). Auxin was found to promote binding of ABP1 to the leucine rich repeat TransMembrane Kinase (TMK) receptor-like kinases (Xu et al., 2014). One developmental process where the role of ABP1 has been demonstrated is the interdigitated pattern of leaf pavement cell expansion by coordinated activation of the antagonistic Rho of Plants 2 (ROP2) and ROP6 GTPases (Xu et al., 2010). At the position of the lobe, binding of apoplastic auxin to ABP1 leads to TMK-mediated activation of the ROP2-RIC4 pathway, promoting diffuse F-actin formation that allows PM expansion during lobe formation, whereas at the indentation the auxin-ABP1-TMK1 complex activates the ROP6-RIC1 pathway, promoting microtubules activity that suppresses cell expansion (Xu et al., 2010). This interdigitated patterning is enhanced by ROP2-RIC4-mediated PIN1 localization at the lobes, since

PIN1 loss-of-function or a shift of PIN1 localisation to the indentations by reduced PP2A phosphatase activity or *PID* overexpression led to a significant reduction in pavement cell interdigitation (Jurado et al., 2010; Li et al., 2011).

Recent studies show that ABP1 is a negative regulator of the $SCF^{TIR1/AFB}$ pathway. *ABP1* knock-down results in enhanced degradation of AUX/IAA repressors, which appears to be independent of the effect of ABP1 on endocytosis (Tromas et al., 2009).

A third potential auxin receptor is the S-PHASE KINASE-ASSOCIATED PROTEIN 2A (SKP2A), which is an F-box protein that regulates proteolysis of cell cycle transcription factors. Direct and specific binding of auxin to SKP2A induces its ubiquitin-dependent degradation, together with the proteolysis of a transcription repressor and the promotion of cell division in the root meristem. The results suggest that SKP2A is a positive regulator of cell division (Jurado et al., 2010).

4. Ca^{2+}, a general second messenger in plant signaling

Ca^{2+} is a second messenger that is involved in many developmental and stress signaling processes in plants. Developmental and stress signals can be translated into transient or oscillating changes in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$). These changes differ per signal and are therefore called stimulus-specific Ca^{2+} signatures that elicit specific physiological responses by altering enzyme activities, cell structures and/or gene expression profiles (Webb et al., 1996; Sanders et al., 1999; Kaplan et al., 2006; Kudla et al., 2010).

Structural changes in the cell wall as well as in membranes are maintained using Ca^{2+} as structural component (Hepler, 2005; Reddy et al., 2011). Ca^{2+} also plays an important role in the symbiosis process in legume root hair cells, where biphasic changes in the $[Ca^{2+}]_{cyt}$ can be induced by rhizobial-derived nodulation (Nod) factors that induce an initial Ca^{2+} influx and a subsequent long-term Ca^{2+} oscillation in the perinucleus (Shaw and Long, 2003; Levy et al., 2004; Kudla et al., 2010). Also during tip growth in pollen tubes and root hair cells, a high $[Ca^{2+}]_{cyt}$ is maintained at the tip by extracellular influx (Sanders et al., 1999; Hepler et al., 2001; Kudla et al., 2010).

Elevation of $[Ca^{2+}]_{cyt}$ can be induced by environmental stimuli by regulation of Ca^{2+} channels and transporters. Ca^{2+} is toxic to plants cells at high intracellular concentrations, and therefore Ca^{2+} is actively pumped from the cytosol either to the apoplast or to intracellular compartments, such as the endoplasmic reticulum and the vacuole, to maintain the $[Ca^{2+}]_{cyt}$ at 10^{-7} M. In the apoplast, Ca^{2+} concentrations are high, varying around 10^{-3} M (McCormack and Braam, 2003). Several Ca^{2+} channels and transporters have been identified so far, such as energy-dependent Ca^{2+} ATPases and

transporters that pump Ca^{2+} out of the cytosol, and Ca^{2+}-permeable channels that allow Ca^{2+} influx into the cytosol based on the electrochemical potential. Rapid and dramatic (10- to 100-fold) increases in local $[Ca^{2+}]_{cyt}$ can be achieved by gating Ca^{2+} channels (McAinsh and Pittman, 2009) . Based on the activation mechanism, Ca^{2+} influx channels can be classified as voltage-dependent, voltage-independent/ligand-dependent, and stretch-activated Ca^{2+} channels (Cosgrove and Hedrich, 1991; White et al., 2002; White and Broadley, 2003; Dutta and Robinson, 2004; Nakagawa et al., 2007; Kudla et al., 2010).

5. Ca^{2+} sensors: Ca^{2+} receptors for different signaling pathways

Ca^{2+} signaling is mediated by Ca^{2+} sensors, which are proteins that can monitor the Ca^{2+} concentration changes by affinity-dependent binding of Ca^{2+} to specific receptor domains. Each Ca^{2+} binding site consists of a Ca^{2+} binding loop with negatively charged residues flanked by two helices, which are named E and F helices, and the Ca^{2+} binding pocket is named 'EF hand' after of the hand-like shape of the helix-loop-helix structure (Strynadka and James, 1989). After Ca^{2+} binding, the EF hands generally undergo a conformational change, leading to activation of the EF-hand containing protein, or to an enhanced interaction with other proteins that are in turn activated or repressed (Trav é et al., 1995; Sanders et al., 1999; Luan et al., 2002). There are two groups of Ca^{2+} sensors: one is the sensor relays, including calmodulins (CaMs) and calcineurin B-like proteins (CBLs), which do not have any intrinsic activity and have to transmit the Ca^{2+} binding-induced modification to downstream target proteins. The other group comprises the sensor protein kinases, including the Ca^{2+}-dependent protein kinases (CDPKs) and the Ca^{2+} and calmodulin-dependent protein kinases (CCaMKs), which can be directly activated upon Ca^{2+} binding (Sanders et al., 2002).

CDPKs have an N-terminal protein kinase domain, connected by a junction sequence to a CaM-like domain that keeps the protein inactive through a pseudosubstrate-binding mechanism. CCaMKs have a kinase domain at the N-terminus of the protein, followed by two regulatory domains. One is a CaM-binding domain that overlaps with an auto-inhibitory region. The other is a visinin-like domain containing 3 EF-hands. This specific structure allows the protein to be regulated by both Ca^{2+} and Ca^{2+}/CaM (Sathyanarayanan and Poovaiah, 2004; A.Pareek, 2010). At basal Ca^{2+} concentrations, Ca^{2+} binding to two EF-hands keeps the CCaMK in the inactive state. Elevated $[Ca^{2+}]_{cyt}$ during Ca^{2+} spiking results in activation of the kinase by Ca^{2+} binding to the third EF hand and CaM binding to the autoinhibitory domain (Miller et al., 2013).

CBLs have two globular domains containing two conserved EF-hand motifs that are connected by a short linker (Nagae et al., 2003). There are ten members of the CBL family in *Arabidopsis* and rice (Kolukisaoglu et al., 2004). CaMs, like CBLs, have two pairs of Ca^{2+} binding sites that are connected by a linker domain. Ca^{2+} binding to the EF hands at micromolar affinity induces a conformational change that exposes the negative charges in the EF hand, allowing the CaM to bind to positively charged residues in target proteins (Lee et al., 2000; Snedden and Fromm, 2001; McCormack and Braam, 2003; Choi et al., 2005; McCormack et al., 2005; Yoo et al., 2005).

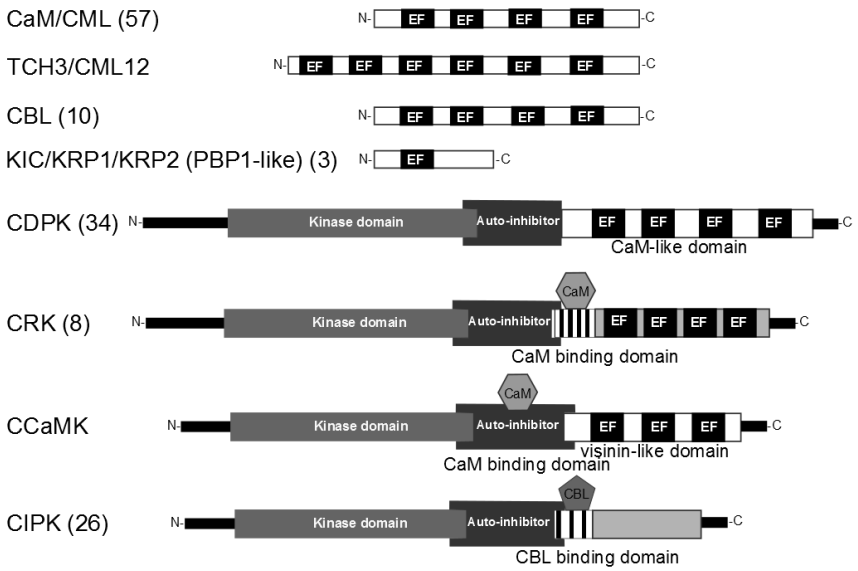

Figure 2 Schematic representation of the structures of plant Ca^{2+} sensors.

Calmodulins and calmodulin-like proteins (CaM/CMLs), calcineurin B-like proteins (CBLs) and KIC/KRP1/KRP2 (PBP1-like) are Ca^{2+} sensor relay proteins that bind Ca^{2+} through their EF hands. Most CaM/CMLs have four predicted EF-hands, although some members of this family are predicted to have 1, 3 or 6 (TCH3/CML12) EF hands. Ca^{2+}-dependent protein kinases (CDPK), CDPK-related protein kinase (CRK), Ca^{2+} and CaM-dependent protein kinases (CCaMKs) and CBL-Interacting Protein Kinases (CIPKs) are sensor protein kinases. The number of family members in *Arabidopsis* is indicated between brackets.

Plants have a large family of CaM and CaM-like (CML) proteins (McCormack et al., 2005). The *Arabidopsis* genome encodes seven CAM isoforms that differ only in one to four amino acids, and 50 CMLs, which are more divergent and contain additional

domains. The seven *CAM* genes share 89% identity to vertebrate CaMs and are considered to be the true type CaMs (McCormack et al., 2005). The 50 CML proteins share at least 16% amino acid identity with CaMs, most of them (31/50) are predicted to have four EF hands, and based on their apparent divergence, they have been classified into nine groups (McCormack and Braam, 2003; McCormack et al., 2005).

Several *CML* genes have been identified as touch-inducible (*TCH*) genes in *Arabidopsis*, whose mRNA levels are rapidly induced by mechanical stimuli, in some cases up to 100-fold within minutes (Braam and Davis, 1990). *TCH3/CML12* is one of these genes, encoding a unique CML with six instead of four predicted EF hands (Antosiewicz et al., 1995).

By using a protein binding microarray, several previously identified and more than 173 novel *in vitro* CaM/CML binding partners have been identified, including Receptor-like Protein Kinases (RLKs), transcription factors, CBL-Interacting Protein Kinases (CIPKs), Ca^{2+}-dependent protein kinases (CDPKs), proteins involved in the cell cycle, and F-box and RNA-binding proteins. Around 25% of the identified target proteins could interact with all CaMs/CMLs, whereas another 25% could only interact with one CaM/CML, indicating that there is both functional redundancy and also specificity among the different CaMs and CMLs (Popescu et al., 2007).

CaMs and CMLs play a significant role in plant defense, transducing the pathogen-induced Ca^{2+} increase to downstream components of defense signaling (Harding et al., 1997; Heo et al., 1999; Chiasson et al., 2005; Takabatake et al., 2007; Zhu et al., 2010; Reddy et al., 2011). CaM binding transcription factors play a role in plant responses to biotic and abiotic signals (Reddy et al., 2011).

6. Auxin transport in plant responses to abiotic signals

Abiotic environmental factors such as gravity, light, and touch play an important role in modulating plant growth via respectively gravitropic, phototropic and thigmotropic growth responses. Abundant studies on these tropic plant growth responses have uncovered a tight relationship between Ca^{2+} and auxin signaling. Below we have summarized the involvement of them in PAT.

Around 1930, Cholodny and Went hypothesized, based on their investigations on light- or gravity-induced bending of seedling coleoptiles and roots, that this bending was the result of asymmetric growth induced by the redistribution of a plant growth regulator to respectively the dark or lower side of plant tissues (Went and Thimann,

1937). Later, after the identification of auxin as the plant growth regulator, others confirmed the lateral movement of auxin to the dark or lower side of root tips, coleoptiles or hypocotyls (Briggs, 1963; Gillespie and Thimann, 1963; Filner et al., 1970; Baskin et al., 1986; Gehring et al., 1990b; Esmon et al., 2006). With the development of the *DR5::GFP* and *35S*::*DII:VENUS* auxin response reporters it became possible to follow the differential auxin response in time and to show with *Arabidopsis* mutants or inhibitor treatments that PAT is essential for both the asymmetric auxin distribution and the resulting growth response (Rashotte et al., 2000; Friml et al., 2002a; Ottenschlager et al., 2003; Ding et al., 2011; Band et al., 2012; Brunoud et al., 2012).

In *Arabidopsis* root tips, the gravitropic response is thought to be initiated in the collumella root cap cells, where the statolites sense the gravity vector by touching the cortical cytoskeleton. Via an unknown signaling pathway, this leads to a relocalization of PIN3 and PIN7 from apolar to polarized to the lower side in gravity-sensing columella root cap cells, resulting in predominant auxin transport to the lower side of the root tip (Friml et al., 2002a; Kleine-Vehn et al., 2010). This higher auxin induces calcium influx and TCH3 expression high expression at the lower side of root tip, thus PID is internalized by calmodulins and results in PIN2 apolarization, leading to higher auxin levels in the lateral root cap- and epidermis cells at the lower side of the root (Band et al., 2012; Brunoud et al., 2012; Baster et al., 2013). The higher auxin levels in these cells lead to stabilization of PIN2 at the PM by inhibition of ABP1-mediated endocytosis, whereas PIN2 is degraded due to reduced auxin levels at the upper side of the root tip (Paciorek et al., 2005; Abas et al., 2006; Robert et al., 2010; Baster et al., 2013).

Phototropic growth of the *Arabidopsis* hypocotyl is initiated by the phototropins phot1 and phot2 under respectively low and medium light fluency conditions (Liscum et al., 2014). These blue light photoreceptors trigger a signaling cascade that leads to the rapid redistribution of PIN3 to the inner lateral side of hypocotyl endodermis cells (Friml et al., 2002; Ding et al., 2011). Recent findings suggest the involvement of the PID kinase in this process. In the dark, expression and activity of the PID kinase results in PIN3 apolar targeting to all cell sides. Light represses *PID* transcription and polarizes the auxin efflux carrier PIN3 cellular localization to the lateral inner side in hypocotyls endodermis cells by GNOM ARF-GEF-dependent trafficking, resulting in auxin distribution changes and differential growth, causing hypocotyls to bend toward the light source (Ding et al., 2011). Besides PIN3, at least eight other auxin transporters (PIN1, 2, 4 and 7, ABCB19, AUX1, LAX2, LAX3) are involved in phototropism.

However, their independent roles have not been defined as clearly as for PIN3 (reviewed in (Liscum et al., 2014)).

Thigmotropism is the plant growth response to mechanical stresses, like touch or contact stimuli (thigmo means "touch" in Greek). Thigmotropism can be induced by the soil or rocks for roots or by wind, raindrops and animals passing by for the shoot. Surprisingly, the role of auxin and PAT in thigmotropic growth has not been studied in a lot of detail, possibly because the induction of the response requires a more delicate experimental set up. The research efforts have been mainly focused on the shoot part, where auxin turn-over by peroxidase-mediated decarboxylation has been proposed as major mechanism leading to touch-induced differential growth of the shoot (reviewed in (Chehab et al., 2009)). Although it is likely that differential auxin distribution plays a role in touch-induced growth responses, the role of PAT in thigmotropism remains to be clarified.

7. Auxin and Ca^{2+} in plant responses to abiotic signals

In 1956 Bennet-Clark proposed that Ca^{2+} might act antagonistically with auxin during the phototropic response in oat coleoptiles (College. et al., 1956). In 1973, dela Fuente and Leopold showed that the Ca^{2+} chelator EGTA could reduce auxin transport in sunflower stem sections, which could be restored after washing with Ca^{2+}, indicating that Ca^{2+} is an important regulator of PAT (dela Fuente and Leopold, 1973). Ten years later, Lee and coworkers showed that lateral PAT was promoted by a Ca^{2+} gradient in maize root tips (Lee et al., 1983), and that PAT inhibitors not only prevented the gravity response of these roots, but also the gravity-induced Ca^{2+} gradient (Lee et al., 1984). Detailed investigations of the effect of exogenously applied Ca^{2+} on the kinetics of gravitropism has shown that the root elongation rate could be significantly reduced by high concentrations of Ca^{2+}, similar to treatment with low auxin concentrations, suggesting that auxin induces an increase in $[Ca^{2+}]_{cyt}$, which in turn affects plant growth (Hasenstein and Evans, 1986; Mulkey and Vaughan, 1986). In 1987, Gross and Sauter published on experiments with corn coleoptiles, suggesting that Ca^{2+} ions move to lower auxin concentrations in a lateral auxin concentration gradient, whereas auxin moves to the lower Ca^{2+} concentration side when exposed to a lateral Ca^{2+} concentration gradient, proposing a link between Ca^{2+} and auxin fluxes (Gross and Sauter, 1987). Although this result suggests an antagonistic role for Ca^{2+} and auxin, later experiments have shown a more synergistic role for Ca^{2+} in auxin responses. By monitoring $[Ca^{2+}]_{cyt}$ using double-barrelled ion-sensitive microelectrodes, it was observed that 1 μM

exogenously applied IAA could induce Ca^{2+} oscillations within a period of 20 to 30 minutes in epidermal cells of maize (*Zea mays* L.) coleoptiles (Felle, 1988). Similarly, by using the Ca^{2+} indicator fluo-3, it was shown that the synthetic auxin analogue 2,4-dichlorophenoxyacetic acid (2,4-D) could induce an increase in $[Ca^{2+}]_{cyt}$ within 4 minutes after application in epidermal cells of parsley hypocotyls and roots and dark-grown corn coleoptiles (Gehring et al., 1990a). IAA and Ca^{2+} could enhance adventitious root initiation on sunflower (*Helianthus annuus* L.) hypocotyls, but when a Ca^{2+} channel blocker was used instead of Ca^{2+}, the IAA-induced rooting response was inhibited, suggesting that auxin induces a net influx of Ca^{2+}, which is required for root initiation (Kalra and Bhatia, 1998).

Ca^{2+} signaling has also been reported to mediate the phototropic bending response (Harada et al., 2003; Stoelzle et al., 2003). By using a fluorescent Ca^{2+} probe fluo-3, a rapid light induced increase in $[Ca^{2+}]_{cyt}$ was observed on the shaded side of maize coleoptiles. This provided the first direct link between rapid $[Ca^{2+}]_{cyt}$ increases and the cell elongation induced by light (Gehring et al., 1990a). A rapid transient $[Ca^{2+}]_{cyt}$ increase could be induced by photostimulation of PHOT1(Baum et al., 1999).

Based on electrophysiology studies it has been reported that the $[Ca^{2+}]_{cyt}$ in epidermis cells of the elongation zone is increased upon gravistimulation and that the $[Ca^{2+}]_{cyt}$ peaks coincide with the basipetal movement of auxin from the root tip to the elongation zone at the lower side of the root tip (Lee et al., 1984; Toyota et al., 2008).

The development of the FRET-based Ca^{2+} sensor yellow cameleon YC3.6 has allowed to more accurately follow the temporal and spatial dynamics of $[Ca^{2+}]_{cyt}$ changes in whole plant tissues. By using this marker it could be demonstrated that exogenous auxin application or gravitropism-induced enhancement of auxin levels triggers a rapid Ca^{2+} influx in *Arabidopsis* root epidermal cells within seconds. These experiments further demonstratedthat Ca^{2+} acts downstream of auxin to translate local auxin levels into appropriate physiological responses (Plieth and Trewavas, 2002; Nagai et al., 2004; Monshausen et al., 2008; Monshausen et al., 2011).

Gravistimulation by rotating apo-aequorin expressing *Arabidopsis* seedlings induced a two-peaked $[Ca^{2+}]_{cyt}$-increase in root epidermis cells, which lasted for several minutes. The auxin transport inhibitor TIBA and vesicle trafficking inhibitor BFA could attenuate the increased $[Ca^{2+}]_{cyt}$ peak amplitudes, indicating that the $[Ca^{2+}]_{cyt}$ response during gravitropism is tightly coupled to auxin transport (Toyota et al., 2008). By using parabolic flight-induced microgravity conditions it could be demonstrated that gravistimulation is responsible for the second sustained increase in $[Ca^{2+}]_{cyt}$ (Toyota et

al., 2013).

Mechanosensing in roots induces calcium responses leading to reactive oxygen species and pH (Monshausen et al., 2009). The mechnical stress-induced calcium channels identified in *Arabiodpsis* (Nakagawa et al., 2007) provide a likely mechanism for the rapid touch-induced elevation of $[Ca^{2+}]_{cyt}$. Based on analogy with gravitropism, it is tempting to speclate that the differential growth response induced by touch is mediated by PAT-mediated differential auxin distribution. The exact mechanism, however, remains to be established.

How auxin-induced Ca^{2+} signals are actually translated into physiological responses has remained unclear for a long time. In 1990, Janet Braam identified at least four touch-induced (*TCH*) genes of which the transcription levels were increased in ten to thirty minutes by wind, touch, water spray, subirrigation, wounding or darkness. Several of these genes appeared to encode CMLs, suggesting that Ca^{2+} ions and CaM are involved in translating touch signals into plant growth responses (Braam and Davis, 1990). The first molecular players were identified through the finding that one of the key regulators of PAT, the PID protein kinase, interacts with two Ca^{2+} binding proteins: TOUCH3 (TCH3) and PID-BINDING PROTEIN 1 (PBP1) (Benjamins et al., 2003). As discussed above, PID regulates the direction of PAT by triggering the recruitment of PIN proteins into the GNOM-independent trafficking pathway through direct phosphorylation of the PIN HL (Friml et al., 2004; Huang et al., 2010). The interaction of PID with Ca^{2+} binding proteins potentially makes this kinase responsive to changes in $[Ca^{2+}]_{cyt}$, which would induce changes in the direction of PAT. The PID-Ca^{2+} binding protein module is an important candidate in the signaling pathway that modulates the subcellular PIN localization by environmental and developmental cues, as has been observed for several developmental processes (Friml et al., 2002a; Benkova et al., 2003; Friml et al., 2003; Reinhardt et al., 2003; Scarpella et al., 2006; Michniewicz et al., 2007). Another player in Ca^{2+} signalling in animals cells is inositol 1,4,5-trisphosphate ($InsP_3$), which can trigger Ca^{2+} release into the cytoplasm from intracellular stores (Mikoshiba, 2007). In plants IP3 has also been suggested to act as a signalling molecule, even in modulating PIN polarity (Blatt et al., 1990; Gilroy et al., 1990; Krinke et al., 2007; Tang et al., 2007; Zhang et al., 2011) It is very unlikely however, that IP3 is a signaling molecule in plants, as the levels of the precursor PIP2 in plant membranes are extremely low, and also since candidate genes for the IP3 receptor and the downstream calcium-activated kinase PKC have not been identified in sequenced plant genomes (Wheeler and Brownlee, 2008; Munnik, 2014).

8. Auxin and Ca^{2+} in responses to endogenous mechanical stress

During plant development, also endogenous mechanical stresses on cells have an effect on their growth. This has been mainly studied in relation to the regular patterns of organ initiation at the shoot apical or inflorescence meristems, referred to as phyllotaxis. It is well established that PAT-generated auxin maxima form initiation points for the development of new organs (reviewed in (Sassi and Vernoux, 2013)). More recently, reports on the role of mechanical stresses in determining phyllotactic patterns have appeared, indicating an important role for the cell wall and the microtubule cytoskeleton (reviewed in (Sassi and Vernoux, 2013)).

Cellulose microfibrils which are the main component of the plant cell wall, are synthesized by hexameric complexes of cellulose synthesizing proteins called CESA at the PM. A tight association of these cellulose microfibrils with a matrix of proteins and polysaccharides, like pectins and hemicelluloses, make the cell wall extreme stiff, and by modulating these interactions the stiffness of the wall and the dynamics and mechanical properties can be varied (Cosgrove, 2005; Uyttewaal et al., 2010). Uptake of osmotic water gives the plants a strong internal pressure, which is named turgor pressure, causing the cells to swell. The extremely rigid cell wall prevents the cell from bursting (Hamant et al., 2010). In response to this turgor pressure, cells yield to it through cell wall synthesis and remodeling, resulting in an increase in cell size and shape changes. The forces produced in one cell can quickly spread to the surrounding cells. In this way mechanical forces act as a motor of growth and shape change. The feedback between cell wall expansion and turgor pressure is important for plant morphogenesis. Another mechanical stress is from the differential growth between neighboring cells in the tissues where cells adhere to each other.

Assuming that the epidermis is under tension and limiting for growth, the shoot apical meristem can be compared to a pressure vessel (Kutschera and Niklas, 2007; Uyttewaal et al., 2010). Assuming that the epidermis is stiffer than ground tissues, turgor is mainly supported by epidermal walls. Interestingly, the predicted force pattern in this system strongly correlates with the direction of microtubules. Microtubules align along the predicted stress directions in the SAM, suggesting that microtubule orientation could be controlled by mechanical stress (Uyttewaal et al., 2010). At the shoot apical meristem, cortical microtubules show characteristic patterns: dynamic at the tip, transverse arrays on the flanks, and arrays parallel to the crease at the boundary between the emerging organ and the meristem tip (Hamant et al., 2008; Hamant et al., 2010; Uyttewaal et al., 2010). Further manipulation of the meristem by compression or

cell ablation induces microtubule reorientation along stress patterns, confirming that cortical microtubules tend to be parallel to the direction of maximal force (Hamant et al., 2008; Hamant et al., 2010; Uyttewaal et al., 2010). The ensuing reinforcement by cellulose microfibrils might also help delimiting the boundary between a primordium and the meristem tip (Hamant et al., 2008; Corson et al., 2009; Hamant et al., 2010; Uyttewaal et al., 2010). This mechanical feedback can be viewed as a cell-autonomous readout of the meristem shape, providing directional information (Hamant et al., 2010). Interestingly, PIN1 localisation in shoot meristem cells negative correlated with the direction of the microtubule arrays, and wounding experiments suggested that PIN1 polarity and thus auxin transport patterns are modulated by mechanical forces (Heisler et al., 2010). The mechano-sensitive calcium channels that have been identified in *Arabidopsis*, could directly translate mechanical stress into a Ca^{2+} response. The prominent role of PID in organ-initiation in the inflorescence meristem (Christensen et al., 2000; Benjamins et al., 2001; Friml et al., 2004) makes it tempting to speculate that mechanical stress induced peaks in $[Ca^{2+}]_{cyt}$ could affect PIN1 polarity through the calcium-dependent interaction between the CML12/TCH3 calcium receptor and the PIN polarity regulator PID (Benjamins et al., 2003).

9. Outline of this thesis

As described above, the protein kinase PID works as a binary switch in directing the asymmetric localization of PIN proteins at the PM, and thereby the polarity of auxin transport, by phosphorylating long PIN proteins on serines in three conserved TPRXS motifs present in the PIN HL (Friml et al., 2004; Michniewicz et al., 2007; Dhonukshe et al., 2010). Previous studies have shown that the calmodulin-like protein CML12 /TCH3 interacts with PID in a yeast two hybrid assay and *in vitro* (Benjamins et al., 2003). The research described in this thesis focused on the PID-TCH3 interaction *in vivo* and *in planta* and on the effect of this interaction on the downstream processes, including auxin transport-dependent processes in the root- (**Chapter 2**) and shoot meristem (**Chapter 4**), e.g. gravitropism or phyllotaxis, respectively. Since the *tch3-3* loss-of-function mutant did not show a strong phenotype, we investigated whether other CaMs or CMLs also interact with PID and mapped the CaM-binding site on the PID kinase (**Chapter 3**).

Chapter 2 shows that the PM-associated PID kinase interacted with TCH3 in a Ca^{2+}-dependent manner, and that this interaction resulted in dissociation of PID from the PM to the cytosol, away from its phosphorylation targets, the PIN proteins. Auxin

treatment induced rapid dissociation of PID from the PM of root epidermis cells. We showed that this was mediated by Ca^{2+}-dependent interaction with TCH3 and leads, after the initial apical (shootward) PIN2 stabilization by auxin, to PIN2 apolarity. We investigated the effect of TCH3 mediated PID internalization during gravitropism, inducing a shift from apical (shootward) to apolar PIN2 localisation 3-5 hours after the start of gravistimulation. Of the other three AGC3 kinases, only WAG2 could interact with TCH3.

In **Chapter 3** we showed that CML10 and the calmodulin CAM2, two proteins closely related to TCH3, were also able to recruit PID from the PM. To overcome the redundant action between the calmodulins (CaMs) and CMLs in our functional analysis of the PID-calmodulin signaling complex, we fine-mapped the PM associated domain and the CML/CaM binding domain in PID. Our analysis revealed that PID CML/CaM binding and PM association converges on an amphipathic alpha helix/IQ-like motif in the PID insertion domain, and that the two functionalities cannot be separated. This dual interaction domain allows an elegant novel mechanism of protein kinase activity regulation, by which $[Ca^{2+}]_{cyt}$ determine whether the kinase is available to phosphorylation of substrate proteins.

In **Chapter 4** we show that PIN1 phosphorylation by the three AGC3 kinases is important for regular phyllotaxis in *Arabidopsis*, and that *pidwag1*, *pidwag2* or *pidwag1wag2* loss-of-function, or PIN1 loss-of-phosphorylation, or to a lesser extent also gain-of-phosphorylation, leads to irregular phyllotaxis, varying from absence of primordium initiation to a switch from the normal spiral (137.5 °angle) to a decussate (alternating 180 °and 90 °angles) pattern. Auxin, mechanical stress and TCH3 overexpression trigger Ca^{2+}-dependent internalization of PID in the inflorescence meristem, probably involving the calmodulin-like protein (CML) TCH3 based on the research from Chapter 2, but also can from other calmodulins as the redundancy. The enhanced variation in the divergence angle between flowers at *TCH3* overexpression or *tch3* loss-of-function mutant inflorescences suggests that a dynamic recruitment of the kinase by TCH3 is required for a regular spiral phyllotaxis. Our results suggest the involvement of the AGC3 kinase-CML signalling complex in modulating of the phyllotactic pattern in response to auxin and mechanical stress.

References

A.Pareek, S.K.S., H.J.Bohnert and Govindjee. (2010). Abiotic stress adaptation in plants:physiological, molecular and genomic foundation**,** 75-90.

Abas, L., Benjamins, R., Malenica, N., Paciorek, T., Wisniewska, J., Moulinier-Anzola, J.C., Sieberer, T., Friml, J., and Luschnig, C. (2006). Intracellular trafficking and proteolysis of the *Arabidopsis* auxin-efflux facilitator PIN2 are involved in root gravitropism. Nat Cell Biol **8,** 249-256.

Antosiewicz, D.M., Polisensky, D.H., and Braam, J. (1995). Cellular localization of the Ca^{2+} binding TCH3 protein of *Arabidopsis*. Plant J **8,** 623-636.

Ballesteros, I., Domínguez, T., Sauer, M., Paredes, P., Duprat, A., Rojo, E., Sanmartín, M., and Sánchez-Serrano, J.J. (2013). Specialized functions of the PP2A subfamily II catalytic subunits PP2A-C3 and PP2A-C4 in the distribution of auxin fluxes and development in Arabidopsis. Plant J **73,** 862-872.

Band, L.R., Wells, D.M., Larrieu, A., Sun, J., Middleton, A.M., French, A.P., Brunoud, G., Sato, E.M., Wilson, M.H., Peret, B., Oliva, M., Swarup, R., Sairanen, I., Parry, G., Ljung, K., Beeckman, T., Garibaldi, J.M., Estelle, M., Owen, M.R., Vissenberg, K., Hodgman, T.C., Pridmore, T.P., King, J.R., Vernoux, T., and Bennett, M.J. (2012). Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. Proc Natl Acad Sci U S A **109,** 4668-4673.

Barbosa, I.C., Zourelidou, M., Willige, B.C., Weller, B., and Schwechheimer, C. (2014). D6 PROTEIN KINASE activates auxin transport-dependent growth and PIN-FORMED phosphorylation at the plasma membrane. Dev Cell **29,** 674-685.

Baskin, T.I., Briggs, W.R., and Iino, M. (1986). Can lateral redistribution of auxin account for phototropism of maize coleoptiles? Plant Physiol **81,** 306-309.

Baster, P., Robert, S., Kleine-Vehn, J., Vanneste, S., Kania, U., Grunewald, W., De Rybel, B., Beeckman, T., and Friml, J. (2013). $SCF^{TIR1/AFB}$-auxin signalling regulates PIN vacuolar trafficking and auxin fluxes during root gravitropism. EMBO J **32,** 260-274.

Baum, G., Long, J.C., Jenkins, G.I., and Trewavas, A.J. (1999). Stimulation of the blue light phototropic receptor NPH1 causes a transient increase in cytosolic Ca^{2+}. Proc Natl Acad Sci U S A **96,** 13554-13559.

Benjamins, R., Ampudia, C.S., Hooykaas, P.J., and Offringa, R. (2003). PINOID-mediated signaling involves calcium-binding proteins. Plant Physiol **132,** 1623-1630.

Benjamins, R., Quint, A., Weijers, D., Hooykaas, P., and Offringa, R. (2001). The PINOID protein kinase regulates organ development in *Arabidopsis* by enhancing polar

auxin transport. Development **128,** 4057-4067.

Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G., and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell **115,** 591-602.

Bennett, S.R.M., Alvarez, J., Bossinger, G., and Smyth, D.R. (1995). Morphogenesis in *pinoid* mutants of *Arabidopsis thaliana*. Plant J. **8,** 505-520.

Blatt, M.R., Thiel, G., and Trentham, D.R. (1990). Reversible inactivation of K^+ channels of *Vicia* stomatal guard cells following the photolysis of caged inositol 1,4,5-trisphosphate. Nature **346,** 766-769.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K., and Scheres, B. (2005). The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature **433,** 39-44.

Boer, D.R., Freire-Rios, A., van den Berg, W.A., Saaki, T., Manfield, I.W., Kepinski, S., Lopez-Vidrieo, I., Franco-Zorrilla, J.M., de Vries, S.C., Solano, R., Weijers, D., and Coll, M. (2014). Structural basis for DNA binding specificity by the auxin-dependent ARF transcription factors. Cell **156,** 577-589.

Braam, J., and Davis, R.W. (1990). Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. Cell **60,** 357-364.

Braun, N., Wyrzykowska, J., Muller, P., David, K., Couch, D., Perrot-Rechenmann, C., and Fleming, A.J. (2008). Conditional repression of AUXIN BINDING PROTEIN1 reveals that it coordinates cell division and cell expansion during postembryonic shoot development in *Arabidopsis* and tobacco. Plant Cell **20,** 2746-2762.

Briggs, W.R. (1963). Mediation of phototropic responses of corn coleoptiles by lateral transport of auxin. Plant Physiol **38,** 237-247.

Brunoud, G., Wells, D.M., Oliva, M., Larrieu, A., Mirabet, V., Burrow, A.H., Beeckman, T., Kepinski, S., Traas, J., Bennett, M.J., and Vernoux, T. (2012). A novel sensor to map auxin response and distribution at high spatio-temporal resolution. Nature **482,** 103-106.

Chehab, E.W., Eich, E., and Braam, J. (2009). Thigmomorphogenesis: a complex plant response to mechano-stimulation. J Exp Bot **60,** 43-56.

Chen, J.G., Ullah, H., Young, J.C., Sussman, M.R., and Jones, A.M. (2001). ABP1 is required for organized cell elongation and division in *Arabidopsis* embryogenesis. Genes Dev **15,** 902-911.

Chiasson, D., Ekengren, S.K., Martin, G.B., Dobney, S.L., and Snedden, W.A. (2005). Calmodulin-like proteins from *Arabidopsis* and tomato are involved in host defense against *Pseudomonas syringae* pv. *tomato*. Plant Mol Biol **58,** 887-897.

Choi, M.Y., Partridge, A.W., Daniels, C., Du, K., Lukacs, G.L., and Deber, C.M. (2005). Destabilization of the transmembrane domain induces misfolding in a phenotypic mutant of cystic fibrosis transmembrane conductance regulator. J Biol Chem **280,** 4968-4974.

Christensen, S.K., Dagenais, N., Chory, J., and Weigel, D. (2000). Regulation of auxin response by the protein kinase PINOID. Cell **100,** 469-478.

College., W., Wain, R.L., and Wightman, F. (1956). The chemistry and mode of action of plant growth substances; proceedings of a symposium held at Wye College, University of London, July 1955. Edited by R.L. Wain and F. Wightman. (London: Butterworths Scientific Publications).

Corson, F., Hamant, O., Bohn, S., Traas, J., Boudaoud, A., and Couder, Y. (2009). Turning a plant tissue into a living cell froth through isotropic growth. Proc Natl Acad Sci U S A **106,** 8453-8458.

Cosgrove, D.J. (2005). Growth of the plant cell wall. Nat Rev Mol Cell Biol **6,** 850-861.

Cosgrove, D.J., and Hedrich, R. (1991). Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of Vicia faba L. Planta **186,** 143-153.

Dai, M., Zhang, C., Kania, U., Chen, F., Xue, Q., McCray, T., Li, G., Qin, G., Wakeley, M., Terzaghi, W., Wan, J., Zhao, Y., Xu, J., Friml, J., Deng, X.W., and Wang, H. (2012). A PP6-type phosphatase holoenzyme directly regulates PIN phosphorylation and auxin efflux in *Arabidopsis*. Plant Cell **24,** 2497-2514.

Darwin, C. (1880). The power of movement in plants. London: John Murray.

dela Fuente, R.K., and Leopold, A.C. (1973). A role for calcium in auxin transport. Plant Physiol **51,** 845-847.

Dharmasiri, N., Dharmasiri, S., and Estelle, M. (2005a). The F-box protein TIR1 is an auxin receptor. Nature **435,** 441-445.

Dharmasiri, N., Dharmasiri, S., Weijers, D., Lechner, E., Yamada, M., Hobbie, L., Ehrismann, J.S., Jurgens, G., and Estelle, M. (2005b). Plant development is regulated by a family of auxin receptor F box proteins. Dev Cell **9,** 109-119.

Dhonukshe, P., Aniento, F., Hwang, I., Robinson, D.G., Mravec, J., Stierhof, Y.D.,

and Friml, J. (2007). Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in *Arabidopsis*. Curr Biol **17,** 520-527.

Dhonukshe, P., Huang, F., Galvan-Ampudia, C.S., Mahonen, A.P., Kleine-Vehn, J., Xu, J., Quint, A., Prasad, K., Friml, J., Scheres, B., and Offringa, R. (2010). Plasma membrane-bound AGC3 kinases phosphorylate PIN auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. Development **137,** 3245-3255.

Dhonukshe, P., Grigoriev, I., Fischer, R., Tominaga, M., Robinson, D.G., Hasek, J., Paciorek, T., Petrasek, J., Seifertova, D., Tejos, R., Meisel, L.A., Zazimalova, E., Gadella, T.W., Jr., Stierhof, Y.D., Ueda, T., Oiwa, K., Akhmanova, A., Brock, R., Spang, A., and Friml, J. (2008). Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. Proc Natl Acad Sci U S A **105,** 4489-4494.

Ding, Z., Galvan-Ampudia, C.S., Demarsy, E., Langowski, L., Kleine-Vehn, J., Fan, Y., Morita, M.T., Tasaka, M., Fankhauser, C., Offringa, R., and Friml, J. (2011). Light-mediated polarization of the PIN3 auxin transporter for the phototropic response in *Arabidopsis*. Nat Cell Biol **13,** 447-452.

Dutta, R., and Robinson, K.R. (2004). Identification and characterization of stretch-activated ion channels in pollen protoplasts. Plant Physiol **135,** 1398-1406.

Esmon, C.A., Tinsley, A.G., Ljung, K., Sandberg, G., Hearne, L.B., and Liscum, E. (2006). A gradient of auxin and auxin-dependent transcription precedes tropic growth responses. Proc Natl Acad Sci U S A **103,** 236-241.

Felle, H. (1988). Auxin causes oscillations of cytosolic free calcium and pH in *Zea mays* coleoptiles. Planta **174,** 495-499.

Filner, B., Hertel, R., Steele, C., and Fan, V. (1970). Some aspects of geotropism in coleoptiles. Planta **94,** 333-354.

Friml, J., Wisniewska, J., Benkova, E., Mendgen, K., and Palme, K. (2002a). Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature **415,** 806-809.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., and Jurgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. Nature **426,** 147-153.

Friml, J., Benkova, E., Blilou, I., Wisniewska, J., Hamann, T., Ljung, K., Woody, S., Sandberg, G., Scheres, B., Jurgens, G., and Palme, K. (2002b). AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell **108,** 661-673.

Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P.B., Ljung, K., Sandberg, G., Hooykaas, P.J., Palme, K., and Offringa, R. (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science **306,** 862-865.

Gehring, C.A., Irving, H.R., and Parish, R.W. (1990a). Effects of auxin and abscisic acid on cytosolic calcium and pH in plant cells. Proc Natl Acad Sci U S A **87,** 9645-9649.

Gehring, C.A., Williams, D.A., Cody, S.H., and Parish, R.W. (1990b). Phototropism and geotropism in maize coleoptiles are spatially correlated with increases in cytosolic free calcium. Nature **345,** 528-530.

Geldner, N., Friml, J., Stierhof, Y.D., Jurgens, G., and Palme, K. (2001). Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature **413,** 425-428.

Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Muller, P., Delbarre, A., Ueda, T., Nakano, A., and Jurgens, G. (2003). The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell **112,** 219-230.

Gillespie, B., and Thimann, K.V. (1963). Transport & distribution of auxin during tropistic response. I. The lateral migration of auxin in geotropism. Plant Physiol **38,** 214-225.

Gilroy, S., Read, N.D., and Trewavas, A.J. (1990). Elevation of cytoplasmic calcium by caged calcium or caged inositol triphosphate initiates stomatal closure. Nature **346,** 769-771.

Gray, W.M., Kepinski, S., Rouse, D., Leyser, O., and Estelle, M. (2001). Auxin regulates SCF^{TIR1}-dependent degradation of AUX/IAA proteins. Nature **414,** 271-276.

Gross, J., and Sauter, M. (1987). Are auxin and calcium movements in corn coleoptiles linked processes? Plant Science **49,** 189-198.

Guilfoyle, T.J., and Hagen, G. (2007). Auxin response factors. Curr. Opin. Plant Biol. **10,** 453-460.

Habets, M.E., and Offringa, R. (2014). PIN-driven polar auxin transport in plant developmental plasticity: a key target for environmental and endogenous signals. New Phytol **203,** 362-377.

Hamant, O., Traas, J., and Boudaoud, A. (2010). Regulation of shape and patterning in plant development. Curr Opin Genet Dev **20,** 454-459.

Hamant, O., Heisler, M.G., Jonsson, H., Krupinski, P., Uyttewaal, M., Bokov, P.,

Corson, F., Sahlin, P., Boudaoud, A., Meyerowitz, E.M., Couder, Y., and Traas, J. (2008). Developmental patterning by mechanical signals in *Arabidopsis*. Science **322,** 1650-1655.

Harada, A., Sakai, T., and Okada, K. (2003). phot1 and phot2 mediate blue light-induced transient increases in cytosolic Ca^{2+} differently in *Arabidopsis* leaves. Proc Natl Acad Sci U S A **100,** 8583-8588.

Harding, S.A., Oh, S.H., and Roberts, D.M. (1997). Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species. EMBO J **16,** 1137-1144.

Hasenstein, K.-H., and Evans, M.L. (1986). Calcium dependence of rapid auxin action in maize roots. Plant Physiol **81,** 439-443.

Heisler, M.G., Hamant, O., Krupinski, P., Uyttewaal, M., Ohno, C., Jonsson, H., Traas, J., and Meyerowitz, E.M. (2010). Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport. PLoS Biol **8,** e1000516.

Henderson, J., Bauly, J.M., Ashford, D.A., Oliver, S.C., Hawes, C.R., Lazarus, C.M., Venis, M.A., and Napier, R.M. (1997). Retention of maize auxin-binding protein in the endoplasmic reticulum: quantifying escape and the role of auxin. Planta **202,** 313-323.

Heo, W.D., Lee, S.H., Kim, M.C., Kim, J.C., Chung, W.S., Chun, H.J., Lee, K.J., Park, C.Y., Park, H.C., Choi, J.Y., and Cho, M.J. (1999). Involvement of specific calmodulin isoforms in salicylic acid-independent activation of plant disease resistance responses. Proc Natl Acad Sci U S A **96,** 766-771.

Hepler, P.K. (2005). Calcium: a central regulator of plant growth and development. Plant Cell **17,** 2142-2155.

Hepler, P.K., Vidali, L., and Cheung, A.Y. (2001). Polarized cell growth in higher plants. Annu Rev Cell Dev Biol **17,** 159-187.

Huang, F., Zago, M.K., Abas, L., van Marion, A., Galvan-Ampudia, C.S., and Offringa, R. (2010). Phosphorylation of conserved PIN motifs directs *Arabidopsis* PIN1 polarity and auxin transport. Plant Cell **22,** 1129-1142.

Jones, A.M., and Herman, E.M. (1993). KDEL-Containing auxin-binding protein is secreted to the plasma membrane and cell wall. Plant Physiol **101,** 595-606.

Jurado, S., Abraham, Z., Manzano, C., Lopez-Torrejon, G., Pacios, L.F., and Del Pozo, J.C. (2010). The *Arabidopsis* cell cycle F-box protein SKP2A binds to auxin.

Plant Cell **22,** 3891-3904.

Kalra, G., and Bhatia, S. (1998). Auxin-calcium interaction in adventitious root formation in the hypocotyl explants of sunflower (*Helianthus annuus* L.). J. Plant Biochem. Biotechnol. **7,** 107-110.

Kaplan, B., Davydov, O., Knight, H., Galon, Y., Knight, M.R., Fluhr, R., and Fromm, H. (2006). Rapid transcriptome changes induced by cytosolic Ca^{2+} transients reveal ABRE-related sequences as Ca^{2+}-responsive *cis* elements in *Arabidopsis*. Plant Cell **18,** 2733-2748.

Kepinski, S., and Leyser, O. (2005). The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature **435,** 446-451.

Kleine-Vehn, J., Ding, Z., Jones, A.R., Tasaka, M., Morita, M.T., and Friml, J. (2010). Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells. Proc Natl Acad Sci U S A **107,** 22344-22349.

Kleine-Vehn, J., Huang, F., Naramoto, S., Zhang, J., Michniewicz, M., Offringa, R., and Friml, J. (2009). PIN auxin efflux carrier polarity is regulated by PINOID kinase-mediated recruitment into GNOM-independent trafficking in *Arabidopsis*. Plant Cell **21,** 3839-3849.

Kleine-Vehn, J., Wabnik, K., Martiniere, A., Langowski, L., Willig, K., Naramoto, S., Leitner, J., Tanaka, H., Jakobs, S., Robert, S., Luschnig, C., Govaerts, W., Hell, S.W., Runions, J., and Friml, J. (2011). Recycling, clustering, and endocytosis jointly maintain PIN auxin carrier polarity at the plasma membrane. Mol Syst Biol **7,** 540.

Kolukisaoglu, U., Weinl, S., Blazevic, D., Batistic, O., and Kudla, J. (2004). Calcium sensors and their interacting protein kinases: genomics of the Arabidopsis and rice CBL-CIPK signaling networks. Plant Physiol **134,** 43-58.

Krinke, O., Novotna, Z., Valentova, O., and Martinec, J. (2007). Inositol trisphosphate receptor in higher plants: is it real? J Exp Bot **58,** 361-376.

Kudla, J., Batistic, O., and Hashimoto, K. (2010). Calcium signals: the lead currency of plant information processing. Plant Cell **22,** 541-563.

Kutschera, U., and Niklas, K.J. (2007). The epidermal-growth-control theory of stem elongation: an old and a new perspective. J Plant Physiol **164,** 1395-1409.

Lee, J.S., Mulkey, T.J., and Evans, M.L. (1983). Gravity-induced polar transport of calcium across root tips of maize. Plant Physiol **73,** 874-876.

Lee, J.S., Mulkey, T.J., and Evans, M.L. (1984). Inhibition of polar calcium movement and gravitropism in roots treated with auxin-transport inhibitors. Planta **160,**

536-543.

Lee, S.H., Johnson, J.D., Walsh, M.P., Van Lierop, J.E., Sutherland, C., Xu, A., Snedden, W.A., Kosk-Kosicka, D., Fromm, H., Narayanan, N., and Cho, M.J. (2000). Differential regulation of Ca^{2+}/calmodulin-dependent enzymes by plant calmodulin isoforms and free Ca^{2+} concentration. Biochem J **350,** 299-306.

Levy, J., Bres, C., Geurts, R., Chalhoub, B., Kulikova, O., Duc, G., Journet, E.P., Ane, J.M., Lauber, E., Bisseling, T., Denarie, J., Rosenberg, C., and Debelle, F. (2004). A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. Science **303,** 1361-1364.

Li, H., Lin, D., Dhonukshe, P., Nagawa, S., Chen, D., Friml, J., Scheres, B., Guo, H., and Yang, Z. (2011). Phosphorylation switch modulates the interdigitated pattern of PIN1 localization and cell expansion in *Arabidopsis* leaf epidermis. Cell Res **21,** 970-978.

Liscum, E., and Reed, J.W. (2002). Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol Biol **49,** 387-400.

Liscum, E., Askinosie, S.K., Leuchtman, D.L., Morrow, J., Willenburg, K.T., and Coats, D.R. (2014). Phototropism: growing towards an understanding of plant movement. Plant Cell **26,** 38-55.

Luan, S., Kudla, J., Rodriguez-Concepcion, M., Yalovsky, S., and Gruissem, W. (2002). Calmodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants. Plant Cell **14,** S389-400.

Luschnig, C., Gaxiola, R.A., Grisafi, P., and Fink, G.R. (1998). EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. Genes Dev. **12,** 2175-2187.

Maraschin Fdos, S., Memelink, J., and Offringa, R. (2009). Auxin-induced, SCF^{TIR1}-mediated poly-ubiquitination marks AUX/IAA proteins for degradation. Plant J **59,** 100-109.

McAinsh, M.R., and Pittman, J.K. (2009). Shaping the calcium signature. New Phytol **181,** 275-294.

McCormack, E., and Braam, J. (2003). Calmodulins and related potential calcium sensors of Arabidopsis. New Phytol. **159,** 585-598.

McCormack, E., Tsai, Y.C., and Braam, J. (2005). Handling calcium signaling: *Arabidopsis* CaMs and CMLs. Trends Plant Sci **10,** 383-389.

Michniewicz, M., Zago, M.K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I.,

Heisler, M.G., Ohno, C., Zhang, J., Huang, F., Schwab, R., Weigel, D., Meyerowitz, E.M., Luschnig, C., Offringa, R., and Friml, J. (2007). Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. Cell **130,** 1044-1056.

Mikoshiba, K. (2007). IP3 receptor/Ca^{2+} channel: from discovery to new signaling concepts. J Neurochem **102,** 1426-1446.

Miller, J.B., Pratap, A., Miyahara, A., Zhou, L., Bornemann, S., Morris, R.J., and Oldroyd, G.E. (2013). Calcium/Calmodulin-dependent protein kinase is negatively and positively regulated by calcium, providing a mechanism for decoding calcium responses during symbiosis signaling. Plant Cell **25,** 5053-5066.

Monshausen, G.B., Messerli, M.A., and Gilroy, S. (2008). Imaging of the Yellow Cameleon 3.6 indicator reveals that elevations in cytosolic Ca^{2+} follow oscillating increases in growth in root hairs of Arabidopsis. Plant Physiol **147,** 1690-1698.

Monshausen, G.B., Bibikova, T.N., Weisenseel, M.H., and Gilroy, S. (2009). Ca^{2+} regulates reactive oxygen species production and pH during mechanosensing in *Arabidopsis* roots. Plant Cell **21,** 2341-2356.

Monshausen, G.B., Miller, N.D., Murphy, A.S., and Gilroy, S. (2011). Dynamics of auxin-dependent Ca^{2+} and pH signaling in root growth revealed by integrating high-resolution imaging with automated computer vision-based analysis. Plant J. **65,** 309-318.

Mravec, J., Skupa, P., Bailly, A., Hoyerova, K., Krecek, P., Bielach, A., Petrasek, J., Zhang, J., Gaykova, V., Stierhof, Y.D., Dobrev, P.I., Schwarzerova, K., Rolcik, J., Seifertova, D., Luschnig, C., Benkova, E., Zazimalova, E., Geisler, M., and Friml, J. (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature **459,** 1136-1140.

Mulkey, T.J., and Vaughan, M.A. (1986). Auxin and root gravitropism: the state of our knowledge. In Plant Growth Substances 1985, M. Bopp, ed (Springer Berlin Heidelberg), pp. 241-246.

Munnik, T. (2014). PI-PLC: Phosphoinositide-Phospholipase C in Plant Signaling. In Phospholipases in Plant Signaling, X. Wang, ed (Springer Berlin Heidelberg), pp. 27-54.

Nagae, M., Nozawa, A., Koizumi, N., Sano, H., Hashimoto, H., Sato, M., and Shimizu, T. (2003). Crystallization and preliminary X-ray characterization of a novel calcium-binding protein AtCBL2 from *Arabidopsis thaliana*. Acta Crystallogr D Biol Crystallogr **59,** 1079-1080.

Nagai, T., Yamada, S., Tominaga, T., Ichikawa, M., and Miyawaki, A. (2004).

Expanded dynamic range of fluorescent indicators for Ca^{2+} by circularly permuted yellow fluorescent proteins. Proc Natl Acad Sci U S A **101,** 10554-10559.

Nakagawa, Y., Katagiri, T., Shinozaki, K., Qi, Z., Tatsumi, H., Furuichi, T., Kishigami, A., Sokabe, M., Kojima, I., Sato, S., Kato, T., Tabata, S., Iida, K., Terashima, A., Nakano, M., Ikeda, M., Yamanaka, T., and Iida, H. (2007). *Arabidopsis* plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots. Proc Natl Acad Sci U S A **104,** 3639-3644.

Okada, K., Ueda, J., Komaki, M.K., Bell, C.J., and Shimura, Y. (1991). Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell **3,** 677-684.

Ottenschlager, I., Wolff, P., Wolverton, C., Bhalerao, R.P., Sandberg, G., Ishikawa, H., Evans, M., and Palme, K. (2003). Gravity-regulated differential auxin transport from columella to lateral root cap cells. Proc Natl Acad Sci U S A **100,** 2987-2991.

Paciorek, T., Zazimalova, E., Ruthardt, N., Petrasek, J., Stierhof, Y.D., Kleine-Vehn, J., Morris, D.A., Emans, N., Jurgens, G., Geldner, N., and Friml, J. (2005). Auxin inhibits endocytosis and promotes its own efflux from cells. Nature **435,** 1251-1256.

Petrasek, J., Mravec, J., Bouchard, R., Blakeslee, J.J., Abas, M., Seifertova, D., Wisniewska, J., Tadele, Z., Kubes, M., Covanova, M., Dhonukshe, P., Skupa, P., Benkova, E., Perry, L., Krecek, P., Lee, O.R., Fink, G.R., Geisler, M., Murphy, A.S., Luschnig, C., Zazimalova, E., and Friml, J. (2006). PIN proteins perform a rate-limiting function in cellular auxin efflux. Science **312,** 914-918.

Plieth, C., and Trewavas, A.J. (2002). Reorientation of seedlings in the earth's gravitational field induces cytosolic calcium transients. Plant Physiol **129,** 786-796.

Popescu, S.C., Popescu, G.V., Bachan, S., Zhang, Z., Seay, M., Gerstein, M., Snyder, M., and Dinesh-Kumar, S.P. (2007). Differential binding of calmodulin-related proteins to their targets revealed through high-density *Arabidopsis* protein microarrays. Proc Natl Acad Sci U S A **104,** 4730-4735.

Rashotte, A.M., Brady, S.R., Reed, R.C., Ante, S.J., and Muday, G.K. (2000). Basipetal auxin transport is required for gravitropism in roots of Arabidopsis. Plant Physiol **122,** 481-490.

Reddy, A.S.N., Ali, G.S., Celesnik, H., and Day, I.S. (2011). Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. Plant Cell **23,** 2010-2032.

Reinhardt, D., Pesce, E.R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J., and Kuhlemeier, C. (2003). Regulation of phyllotaxis by polar auxin transport. Nature **426,** 255-260.

Robert, S., Kleine-Vehn, J., Barbez, E., Sauer, M., Paciorek, T., Baster, P., Vanneste, S., Zhang, J., Simon, S., Covanova, M., Hayashi, K., Dhonukshe, P., Yang, Z., Bednarek, S.Y., Jones, A.M., Luschnig, C., Aniento, F., Zazimalova, E., and Friml, J. (2010). ABP1 mediates auxin inhibition of clathrin-dependent endocytosis in *Arabidopsis*. Cell **143,** 111-121.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P., and Scheres, B. (1999). An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell **99,** 463-472.

Sanders, D., Brownlee, C., and Harper, J.F. (1999). Communicating with calcium. Plant Cell **11,** 691-706.

Sanders, D., Pelloux, J., Brownlee, C., and Harper, J.F. (2002). Calcium at the crossroads of signaling. Plant Cell **14 Suppl,** S401-417.

Sassi, M., and Vernoux, T. (2013). Auxin and self-organization at the shoot apical meristem. J Exp Bot **64,** 2579-2592.

Sathyanarayanan, P.V., and Poovaiah, B.W. (2004). Decoding Ca^{2+} signals in plants. CRC Crit Rev Plant Sci **23,** 1-11.

Sauer, M., Robert, S., and Kleine-Vehn, J. (2013). Auxin: simply complicated. J Exp Bot **64,** 2565-2577.

Sauer, M., Balla, J., Luschnig, C., Wisniewska, J., Reinohl, V., Friml, J., and Benkova, E. (2006). Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. Genes Dev **20,** 2902-2911.

Sawchuk, M.G., Edgar, A., and Scarpella, E. (2013). Patterning of leaf vein networks by convergent auxin transport pathways. PLoS Genet **9,** e1003294.

Scarpella, E., Marcos, D., Friml, J., and Berleth, T. (2006). Control of leaf vascular patterning by polar auxin transport. Genes Dev **20,** 1015-1027.

Shaw, S.L., and Long, S.R. (2003). Nod factor elicits two separable calcium responses in *Medicago truncatula* root hair cells. Plant Physiol **131,** 976-984.

Snedden, W.A., and Fromm, H. (2001). Calmodulin as a versatile calcium signal transducer in plants. New Phytol **151,** 35-66.

Stoelzle, S., Kagawa, T., Wada, M., Hedrich, R., and Dietrich, P. (2003). Blue light

activates calcium-permeable channels in *Arabidopsis* mesophyll cells via the phototropin signaling pathway. Proc Natl Acad Sci U S A **100,** 1456-1461.

Strynadka, N.C., and James, M.N. (1989). Crystal structures of the helix-loop-helix calcium-binding proteins. Annu Rev Biochem **58,** 951-998.

Szemenyei, H., Hannon, M., and Long, J.A. (2008). TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. Science **319,** 1384-1386.

Takabatake, R., Karita, E., Seo, S., Mitsuhara, I., Kuchitsu, K., and Ohashi, Y. (2007). Pathogen-induced calmodulin isoforms in basal resistance against bacterial and fungal pathogens in tobacco. Plant Cell Physiol **48,** 414-423.

Tan, X., Calderon-Villalobos, L.I., Sharon, M., Zheng, C., Robinson, C.V., Estelle, M., and Zheng, N. (2007). Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature **446,** 640-645.

Tanaka, H., Dhonukshe, P., Brewer, P.B., and Friml, J. (2006). Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. Cell Mol Life Sci **63,** 2738-2754.

Tang, R.H., Han, S., Zheng, H., Cook, C.W., Choi, C.S., Woerner, T.E., Jackson, R.B., and Pei, Z.M. (2007). Coupling diurnal cytosolic Ca^{2+} oscillations to the CAS-IP_3 pathway in *Arabidopsis*. Science **315,** 1423-1426.

Tiwari, S.B., Hagen, G., and Guilfoyle, T.J. (2004). Aux/IAA proteins contain a potent transcriptional repression domain. Plant Cell **16,** 533-543.

Toyota, M., Furuichi, T., Tatsumi, H., and Sokabe, M. (2008). Critical consideration on the relationship between auxin transport and calcium transients in gravity perception of Arabidopsis seedlings. Plant Signal Behav **3,** 521-524.

Toyota, M., Furuichi, T., Sokabe, M., and Tatsumi, H. (2013). Analyses of a gravistimulation-specific Ca^{2+} signature in Arabidopsis using parabolic flights. Plant Physiol **163,** 543-554.

Trav é, G., Lacombe, P.J., Pfuhl, M., Saraste, M., and Pastore, A. (1995). Molecular mechanism of the calcium-induced conformational change in the spectrin EF-hands. EMBO J **14,** 4922-4931.

Tromas, A., Braun, N., Muller, P., Khodus, T., Paponov, I.A., Palme, K., Ljung, K., Lee, J.Y., Benfey, P., Murray, J.A., Scheres, B., and Perrot-Rechenmann, C. (2009). The AUXIN BINDING PROTEIN 1 is required for differential auxin responses mediating root growth. PLoS One **4,** e6648.

Uyttewaal, M., Traas, J., and Hamant, O. (2010). Integrating physical stress, growth, and development. Curr Opin Plant Biol **13,** 46-52.

Webb, A.A.R., McAinsh, M.R., Taylor, J.E., and Hetherington, A.M. (1996). Calcium ions as intracellular second messengers in higher plants. In Advances in Botanical Research, J.A. Callow, ed (Academic Press), pp. 45-96.

Went, F., and Thimann, K.V. (1937). Phytohormones. New York: The Macmillan Company.

Wheeler, G.L., and Brownlee, C. (2008). Ca^{2+} signalling in plants and green algae--changing channels. Trends Plant Sci **13,** 506-514.

White, P.J., and Broadley, M.R. (2003). Calcium in plants. Ann Bot **92,** 487-511.

White, P.J., Bowen, H.C., Demidchik, V., Nichols, C., and Davies, J.M. (2002). Genes for calcium-permeable channels in the plasma membrane of plant root cells. Biochim Biophys Acta **1564,** 299-309.

Wisniewska, J., Xu, J., Seifertova, D., Brewer, P.B., Ruzicka, K., Blilou, I., Rouquie, D., Benkova, E., Scheres, B., and Friml, J. (2006). Polar PIN localization directs auxin flow in plants. Science **312,** 883.

Xu, J., Hofhuis, H., Heidstra, R., Sauer, M., Friml, J., and Scheres, B. (2006). A molecular framework for plant regeneration. Science **311,** 385-388.

Xu, T., Wen, M., Nagawa, S., Fu, Y., Chen, J.G., Wu, M.J., Perrot-Rechenmann, C., Friml, J., Jones, A.M., and Yang, Z. (2010). Cell surface- and rho GTPase-based auxin signaling controls cellular interdigitation in *Arabidopsis*. Cell **143,** 99-110.

Xu, T., Dai, N., Chen, J., Nagawa, S., Cao, M., Li, H., Zhou, Z., Chen, X., De Rycke, R., Rakusova, H., Wang, W., Jones, A.M., Friml, J., Patterson, S.E., Bleecker, A.B., and Yang, Z. (2014). Cell surface ABP1-TMK auxin-sensing complex activates ROP GTPase signaling. Science **343,** 1025-1028.

Yoo, J.H., Park, C.Y., Kim, J.C., Heo, W.D., Cheong, M.S., Park, H.C., Kim, M.C., Moon, B.C., Choi, M.S., Kang, Y.H., Lee, J.H., Kim, H.S., Lee, S.M., Yoon, H.W., Lim, C.O., Yun, D.J., Lee, S.Y., Chung, W.S., and Cho, M.J. (2005). Direct interaction of a divergent CaM isoform and the transcription factor, MYB2, enhances salt tolerance in *Arabidopsis*. J Biol Chem **280,** 3697-3706.

Zhang, J., Vanneste, S., Brewer, P.B., Michniewicz, M., Grones, P., Kleine-Vehn, J., Lofke, C., Teichmann, T., Bielach, A., Cannoot, B., Hoyerova, K., Chen, X., Xue, H.W., Benkova, E., Zazimalova, E., and Friml, J. (2011). Inositol trisphosphate-induced Ca^{2+} signaling modulates auxin transport and PIN polarity. Dev

Cell **20,** 855-866.

Zhao, Y. (2010). Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol **61,** 49-64.

Zhu, X., Caplan, J., Mamillapalli, P., Czymmek, K., and Dinesh-Kumar, S.P. (2010). Function of endoplasmic reticulum calcium ATPase in innate immunity-mediated programmed cell death. EMBO J **29,** 1007-1018.

Zourelidou, M., Absmanner, B., Weller, B., Barbosa, I.C., Willige, B.C., Fastner, A., Streit, V., Port, S.A., Colcombet, J., de la Fuente van Bentem, S., Hirt, H., Kuster, B., Schulze, W.X., Hammes, U.Z., and Schwechheimer, C. (2014). Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. Elife **3,** e02860.