



Universiteit
Leiden
The Netherlands

Components and targets of the PINOID signaling complex in *Arabidopsis thaliana*

Zago, Marcelo Kemel

Citation

Zago, M. K. (2006, June 15). *Components and targets of the PINOID signaling complex in Arabidopsis thaliana*. Retrieved from <https://hdl.handle.net/1887/4436>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4436>

Note: To cite this publication please use the final published version (if applicable).

Chapter 1

Auxin distribution and signaling act to shape the plant

Marcelo Kemel Zago and Remko Offringa

INTRODUCTION

The phytohormone auxin, or indole-3-acetic acid (IAA), is a central regulator of plant development that controls elementary processes such as cell division and -elongation and also directs complex developmental and patterning processes such as embryogenesis, vascular differentiation, phyllotaxis and fruit development (1, 2). More than a century ago, Darwin's observations on the bending of canary grass coleoptiles to unidirectional light led him to conclude that some matter in the upper part of the coleoptile is acted on by light, and then transmits its effects to the lower part of this tissue (3). Around 1930 this matter was identified as indole-3-acetic acid (IAA) and named after the greek word for "to grow" (auxein) (4, 5). More detailed observations by Went and Cholodny on the auxin-mediated orientation of plant growth to unidirectional light (phototropism) or gravity (gravitropism) led to the Cholodny and Went hypothesis (4, 6, 7). This model states that tropic growth is the result of predominant distribution of auxin to the dark or lower side upon light or gravity stimulation, respectively, and that due to differences in sensitivity to auxin, shoot growth is enhanced, whereas root growth is inhibited by the elevated auxin concentrations, ultimately leading to bending of the shoot or the root. In support of this hypothesis, more recent experiments demonstrated asymmetric expression of auxin responsive genes in light and gravity-induced shoots (8-10) and roots (11-13). The early tropic growth experiments clearly demonstrate that auxin action is a result of the interplay between the local auxin concentration – which is determined by biosynthesis, transport, and inactivation - and the sensitivity or responsiveness of cells to this plant hormone.

Below we will review what is known on auxin-mediated plant development with an emphasis on auxin signaling and -transport and the role of the PINOID protein kinase in these processes. The biosynthesis of auxin and its inactivation through catabolism and conjugation, although important, are beyond the scope of this thesis, and for these subjects we refer to recent reviews (14).

Abbreviations: 1-NAA, 1-naphthaleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; ABC/MDR/PGP, ATP binding cassette/multidrug resistance type/P-glycoprotein protein; AEC, auxin efflux carrier; AIC, auxin influx carrier; ARF, auxin response factor; ARF-GEF, ADP-ribosylation factor-GTP exchange factor; AuxRe, auxin responsive element; AXR, auxin resistant; BDL/IAA12, Bodenloss/IAA12 protein; BFA, Brefeldin A; COP, constitutive photomorphogenesis; CSN, COP9 Signalosome; IAA, indole-3-acetic acid; MP, ARF5/Monopteros; NPA, 1-N-naphthylphthalamic acid; NPH4, ARF7/Nonphototropic hypocotyl 4; PAT, polar auxin transport; PBK, PBP2 binding kinesin; PBP, pinoid binding protein; PID, pinoid; PM, plasma membrane; SCF, SKP1/Cullin/F-box

AUXIN SIGNALING

The term auxin signaling is often used to describe the role of proteins that are by default part of a signaling pathway - such as protein kinases - and are known to regulate auxin-action. In this chapter we will use signaling in the strictest meaning of the word, aiming at canonical signaling pathways that perceive the hormone signal, and based on its concentration – the net result of biosynthesis, transport and inactivation – induce primary cellular responses.

Several processes are known to occur within a few minutes after auxin application. These vary from changes in enzymatic activities (15-17) and gene expression (18-20) to changes in transporter activities, leading to increase of the membrane potential (21), rapid increase of the cytosolic calcium levels (22) and acidification of the cell wall (23). For most of these primary responses the signaling pathways are yet unknown, but in the last few years the signaling processes leading to auxin responsive gene expression have been elucidated.

Auxin responsive gene expression: a balance between activators and repressors

Differential screens of cDNA libraries in the 1980s led to the identification of the first auxin responsive genes (24-27). Most of these genes were activated within minutes after auxin stimulation in a process independent of *de novo* synthesis of proteins. Several auxin responsive elements (AuxREs) have been identified in the promoters of these primary auxin response genes (28-30), and Auxin Response Factors (ARFs) were shown to bind to these elements and to activate or to repress transcription (31).

ARFs in general contain four well defined domains: a DNA binding domain (DBD) that binds AuxREs, a middle region domain and domains III and IV (31). Whether an ARF is an activator or repressor depends on the structure of its middle region domain. For example, ARFs with Q-rich middle regions activate transcription, while ARFs with P/S/T-rich middle region repress transcription (31). Domains III and IV were found to mediate homo- or heterodimerization (28).

Some of the primary auxin response genes were found to encode small short-lived proteins, named Aux/IAA proteins, that resemble bacterial repressors (20, 32). Aux/IAA proteins contain four distinct domains, of which domain I has been shown to have transcription repression activity (33), domain II is involved in destabilization of Aux/IAA proteins, and may be target for ubiquitination (34), and domains III and IV have protein-protein interaction properties, allowing Aux/IAA proteins to homodimerize or to heterodimerize with ARFs or other Aux/IAA proteins (35).

Apart from being identified in screens for auxin responsive genes, the Aux/IAA encoding genes have also been identified through gain-of-function mutations that

lead to auxin insensitivity. Aux/IAA proteins are generally short lived, and all gain-of-function mutations in the *Aux/IAA* genes led to specific amino acid changes in domain II that stabilize the encoded protein, and thus lead to phenotypes that relate to auxin insensitivity (36).

Surprisingly, *aux/iaa* loss-of-function mutants provide very little information compared to gain-of-function ones. In fact, all of these knock-out mutant plant lines analyzed to present display very subtle phenotypes, indicating that there is functional redundancy between Aux/IAAs. By contrast, loss-of-function mutations have been informative for three ARFs: ARF3/ETTIN, ARF5/MONOPTEROS (MP) and ARF7/NONPHOTOTROPIC HYPOCOTYL 4 (NPH4). ARF3/ETTIN was characterized for playing a role in floral organ development since the *arf3/ettin* mutant displays abnormal apical-basal gynoecium development (37). Mutations in the gene *ARF5/MP* interfere with the formation of vascular strands at all stages and also with the initiation of the body axis in the early embryo (38). The mutant *arf7/nph4* shows non-phototropic response, resistance to the auxin transport inhibitor 1-*N*-naphthylphthalamic acid (NPA), impaired hypocotyls gravitropism, altered apical hook maintenance, and epinastic or hyponastic leaves. In general terms, *arf7/nph4* is impaired in differential growth responses in aerial tissues (36, 39).

The Arabidopsis genome encodes 29 AUX/IAA proteins and 23 ARFs, which can combine to translate the auxin signal into a gene expression response. For example, it has been shown in yeast two-hybrid assays that specific combinations of ARFs and Aux/IAAs are preferred interaction partners (40, 41). Expression and functional specificity has been demonstrated for several ARFs and Aux/IAAs, further demonstrating that specific interactions between such proteins should occur (42, 43). The specificity in these interactions seems essential to differentiate auxin responses in different cell types.

Auxin perception leads to enhanced degradation of the Aux/IAA repressors

The identification of Aux/IAAs as primary response proteins (20) and the observation that they are instable, especially, undergoing rapid degradation upon auxin stimulation (44), placed the proteolysis machinery as key player in auxin signaling. More specifically, the proteolysis components are an E1 ubiquitin-activating enzyme, which transfers the ubiquitin component to an E2 ubiquitin conjugating protein. The E2-ubiquitin complex then binds to an E3 ubiquitin ligase complex, which mediates transfer of ubiquitin to the target protein. Ubiquitination is generally thought to label these target proteins for degradation by the 26S proteasome (Figure 1A), but strangely enough, auxin-induced ubiquitination of Aux/IAA proteins has not yet been demonstrated. The E3 ligase complex that

participates in auxin signaling is the SCF (SKP1/Cullin/F-box) complex comprising ASK1 (the Arabidopsis SKP1-like protein), CUL1 (Cullin 1) the F-Box protein TIR1 (Transport Inhibitor Response 1), and the E2-interacting RING domain protein RBX1 (Figure 1A) (45). Interestingly, three of the components of the SCF^{TIR1} complex have been identified through Arabidopsis mutants with a defective auxin response (46-49). Aux/IAA proteins were shown to interact with the F-box protein TIR1 (44), and the recent finding that auxin-binding to TIR1 enhances this interaction with and thus leads to enhanced degradation of Aux/IAs, uncovered TIR1 as the long sought auxin receptor (Figure 1A) (50, 51).

Several regulatory components of SCF E3 ligases have been identified. For example, it has been found that the CUL1 subunit of the SCF complex is modified by the addition of the ubiquitin-like protein RUB1/NEDD8 in a process mediated by the regulatory protein RCE1 which binds to RBX1 (49, 52). Prior to that process, RUB1/NEDD8 is activated by the subcomplex AXR1-ECR1, which catalyzes the transfer of RUB1 to RCE1 (Figure 1A) (53). Knock-out mutations in most of these regulatory components lead to auxin resistant phenotypes, and the double mutant *axr1/rce1* causes embryonic defects similar to *mp*, leading to the hypothesis that RUB modification positively regulates SCF activity (53-56). The RUB-conjugated state of the SCF complex is regulated by the COP9 Signalosome (CSN), a protein complex that shares reasonable similarity to the lid of the 26S proteasome (57). CSN action has been demonstrated to be necessary for both auxin response and RUB1 removal from CUL1 (58), which probably destabilizes the SCF complex after its function so that new complexes can be formed (Figure 1) (59, 60). The CSN is also known to interact with other types of E3 ligases, such as the photomorphogenesis related COP1, and to be required for the nuclear import of this RING finger protein (61-63). COP1 and the CSN have been shown to promote degradation of HY5 (64, 65), a transcription factor that positively regulates photomorphogenesis, and loss-of-function mutations in *COP1* or in the single CSN-subunit encoding genes causes a constitutive photomorphogenesis (*cop*) phenotype (66). Finally, the protein CAND1 has been demonstrated to specifically bind the CUL1/RBX1 core complex and to dissociate from it upon RUB modification of CUL1, allowing the F-Box/ASK1 substrate receptor to interact. It is therefore hypothesized that CAND1 regulates SCF complex assembly by making the interaction of CUL1 with F-Box/ASK1 dependent on RUB modification (Figure 1B) (67). The role for CAND1 in auxin signaling is apparent, since *cand1* loss-of-function mutants display clear auxin resistant features (67).

An integrative role of protein kinases in auxin signaling

With the molecular basis of auxin signaling - from the perception of this hormone to the induction of auxin responsive gene expression - largely uncovered, it is

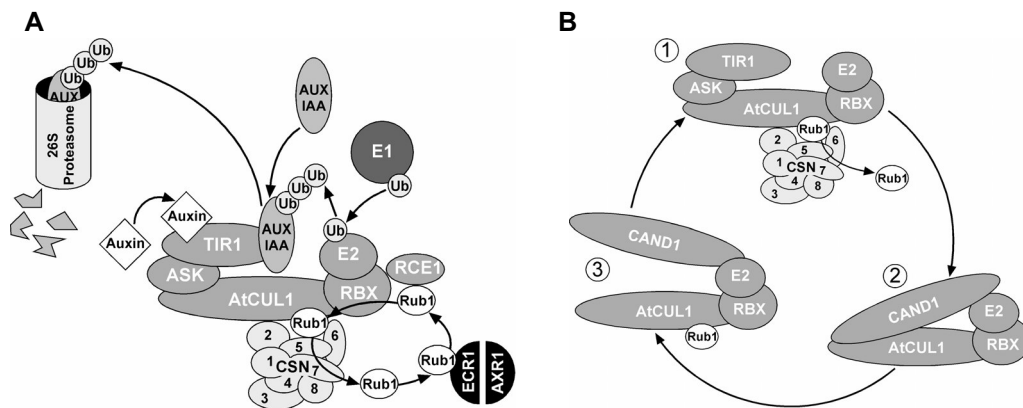


Figure 1. The SCF^{TIR1} E3 ubiquitin ligase is the core of the protein degradation machinery that regulates auxin responses in *Arabidopsis thaliana*. (A) Target proteins are labeled for proteolysis by ubiquitination. This process is mediated by the ubiquitin activating enzyme E1, the ubiquitin conjugating enzyme E2 and the ubiquitin ligase E3. E1 transfers the ubiquitin component to E2, that in turn binds to and acts in concert with E3 to ubiquitinate the substrate protein. Once targets are ubiquitinated, they are degraded by the 26S proteasome. SCF^{TIR1}, the E3 ligase complex that participates in auxin signaling, consists of CULLIN1 (CUL1), the Arabidopsis SKP1 homolog ASK1, and the F-box protein TIR1. Auxin-binding to TIR1 enhances its interaction with the AUX/IAAs which leads to enhanced degradation of these proteins. Regulatory subunits of SCF^{TIR1} include RCE1, which modifies CUL1 by adding RUB1 that is previously activated by the AXR1-ECR1 subcomplex. The COP9 signalosome (CSN) removes RUB1 from CUL1, leading to subsequent dissociation of the SCF^{TIR1} complex. (B) CAND1 and CSN are modulators of the cyclic assembly and dissociation of the SCF complex. The active SCF complex recruits CSN, which cleaves RUB1 from CUL1 (1). This enables CAND1 to bind CUL1 and eventually strip away ASK1 and the F-box protein TIR1, thereby sequestering CUL1 in an inactive state (2). RUB1 modification of CUL1 weakens the affinity of CAND1 for the CUL1-complex (3), and an incoming ASK1-F-box heterodimer is able to displace CAND1 to yield an active SCF complex (1) (Cope *et al.*, 2003).

intriguing to note that there is limited evidence for the involvement of canonical signaling pathways, comprising a membrane bound receptor and a protein kinase cascade. Phosphorylation events have occasionally been reported to be involved in auxin signaling. Aux/IAA proteins have been shown to be phosphorylated by phytochromes *in vitro*, suggesting that light signaling acts on auxin responsive gene expression by influencing the stability of Aux/IAA proteins (68). Also the Mitogen Activated Protein Kinase (MAPK) cascade has been implied in the modulation of auxin response. Roots of *Arabidopsis* seedlings treated with auxin showed an increase in MAPK activity and this activation was inhibited in the auxin resistant *axr4* mutant (69). It has also been shown that the MAPKKK NPK1 that activates stress responses, represses auxin induced gene expression (70). For now we conclude that phosphorylation events are important modulators of the auxin

response pathway, that serve to integrate other signals, such as light or stresses, with auxin signaling (71).

AUXIN TRANSPORT

The initial observations by Darwin on tropisms (3) and subsequent more detailed experiments by plant biologists such as Went (7) not only led to the identification of auxin, but also revealed that this hormone is transported to sites of action. Based on transport measurements using radio-labeled auxin, two types of auxin transport are distinguished: a fast and non-directional one occurring through the phloem, and a slow and directional cell-to-cell transport that is referred to as polar auxin transport (PAT). The transport through the phloem was first detected by Morris and Thomas (72) and occurs in both basi- and acropetal directions at approximately 5-20cm/h (73). Experiments performed by Baker (74) indeed revealed significant presence of IAA in the phloem. A connection between the fast transport of auxin and PAT was demonstrated in experiments performed in pea, in which radio-labeled IAA initially present in the phloem was detected later in the polar transport system (75).

In contrast to the phloem-mediated auxin transport, PAT is restricted to free IAA, is unidirectional and occurs in a cell-to-cell manner only. The velocity is much slower, and has been estimated to occur at approximately 5-20mm/h. PAT is initiated in the young growing organs at the plant shoot apex and runs via the plant base down to the root tip (76). At the root tip, PAT is redirected upwards, proceeding basipetally through the root epidermis towards the root elongation zone (77). In the shoot, PAT is believed to also occur laterally for shoot elongation and to inhibit lateral bud outgrowth (72).

Polar auxin transport: the chemiosmotic model

Initial evidence that PAT requires energy, is saturable and sensitive to protein synthesis inhibitors led to the formulation in the 1970s of the chemiosmotic hypothesis for the mechanism behind PAT (78, 79). It was postulated that, due to the acidic extracellular pH of approximately 5.5, part of the IAA molecules in the apoplast is protonated (IAAH) and can easily pass through the plasma membrane by diffusion; once in the more basic cytoplasmic environment (pH close to 7.0), IAAH becomes de-protonated, assuming an anionic form (IAA⁻) that becomes trapped inside the cell. Due to this ion trap, the IAA⁻ anion can only exit the cell via the activity of auxin efflux carriers (AECs), whose asymmetric subcellular distribution determines the direction of PAT. An addition to this model was elaborated in which the presence of auxin influx carriers (AICs) was considered (80). The presence of AICs was later demonstrated to be viable based on experiments showing that the auxin influx capacity of cells is saturable (81, 82). Interestingly,

mathematical modeling of PAT predicts that polar transport can only lead to the observed local auxin accumulation when AICs and AECs act in concert (83).

In *Arabidopsis*, the AUX1/LAX amino-acid permease-like proteins have been identified as candidates for the AICs (84). The *aux1* mutants show a reduced response of the root to gravity, a reduced number of lateral roots and altered IAA distribution in young leaf and root tissues (85). The *aux1* root phenotypes can be rescued by germinating mutant seedlings on the lipophilic and highly diffusible auxin 1-naphthaleneacetic acid (1-NAA), but not with the impermeable auxin analog 2,4-dichlorophenoxyacetic acid (2,4-D), nor with the natural auxin IAA (86). It has also been demonstrated that AUX1 facilitates IAA loading into the vascular transport system (85). Interestingly, *aux1* phenotypes can be mimicked by application of the auxin influx inhibitors 1-naphthoxyacetic acid (1-NOA) and 3-chloro-4-hydroxyphenylacetic acid (CHPAA). Again, rescue is possible by 1-NAA but not by 2,4-D (87). Finally, AUX1 shows an asymmetrical subcellular localization (88, 89), which is in line with the chemiosmotic model for PAT and further supports the role of AUX1 as AIC.

Direction in polar auxin transport through the auxin efflux carriers (AECs)

The most important components for PAT, according to the chemiosmotic model, that provide both the driving force and the direction, are the AECs. To date two families of putative AECs have been identified: the PIN proteins and the ATP binding cassette (ABC), multidrug resistance (MDR)-type, P-glycoprotein (PGP) proteins (90-92).

The *Arabidopsis* PIN family is best characterized and includes the proteins PIN1 to PIN8. The main characteristic of the PIN proteins is the presence of several highly conserved transmembrane domains that, with the exception of PIN5 and PIN8, flank a less conserved central large cytoplasmic loop (93, 94). As predicted for the AECs, PINs mostly localize polarly in the plasma membrane (PM) of the cells in positions that are perfectly correlated with the actual directionality of the efflux of auxin (89, 91, 95, 96). The placement of PINs in cells differs per PIN protein and tissue of expression, and in this chapter the words apical and basal are used to indicate their localization at respectively the upper or lower cell membrane. As each PIN has a particular expression domain, loss-of-function mutations in their corresponding genes results in tissue-specific defects related to the directional transport of auxin (97).

PIN1, for example, is present in the apical pole of epidermal, and basal pole of provascular strand, cells of the shoot apex and in the basal pole of vascular and cortical root cells, therefore it is believed that this protein is essential for IAA transport in shoot and root tissues (89, 94, 98, 99). *pin1* mutants display pin-formed

inflorescences and reduced basipetal PAT in inflorescences (100), although no root defects were observed in such plants, probably implying functional redundancy in this organ.

PIN2 is present at the basal cell pole in cortical cells and in the apical pole in epidermal cells of the root and functions primarily in the redistribution of auxin involved in root gravitropism (93, 101, 102). Accordingly, *pin2* mutants show agravitropic root response as a result of the reduced basipetal PAT in this organ (93, 101, 102).

PIN3 is mainly present at the apical hook, around the hypocotyl vasculature, where it seems to be baso-laterally localized, and in the root pericycle and columella, where it is apolarly localized and appears to function in the lateral redistribution of auxin upon gravistimulation (8). *pin3* mutants are defective in tropic growth responses (8).

PIN4 is localized in the root meristem cells surrounding the quiescent center, where its subcellular localization is responsible for the establishment of an auxin sink below the quiescent center (QC) of the root apical meristem (103). *pin4* mutants are defective in establishment and maintenance of endogenous auxin gradients, fail to canalize externally applied auxin, and display slight patterning defects in both embryonic and seedling roots (103).

Finally, PIN7 localizes polarly in embryonic cells and plays a role in forming and maintaining apical–basal auxin gradients that are essential for the establishment of embryonic polarity. PIN7 also functions in root acropetal auxin transport (104). In *pin7* mutants, specification of the apical daughter cell of the zygote is compromised and occasionally *pin7* embryos fail to establish the pro-embryo (104).

Despite the specific roles of each PIN in auxin distribution throughout the plant, their functions have been shown to be overlapping. For example, PIN1 is localized at the basal cell pole in vascular root tip cells, but the *pin1* loss-of-function mutant does not show a clear root phenotype. In addition, the phenotypic defects of the *pin4* loss-of-function mutant are very weak, and loss of QC establishment is only found in triple and quadruple mutant background with *pin1*, *pin3* and *pin7* (98, 103, 104). It has been demonstrated that some PINs have their expression either enhanced and/or broadened to different cell files in the root tip in other *pin* loss-of-function backgrounds (105). This explains in part the observed functional redundancy among the different *PIN* genes (91, 98, 106).

Evidence that PINs really are AECs is accumulating. Initially, the activity of PIN2 was tested in a heterologous yeast system and this showed that yeast cells expressing this protein become more resistant to the toxic IAA-derivative 5-fluoro-IAA (12, 107). Only recently, a more extensive study in both plant, animal and yeast cells confirmed the direct involvement of PIN proteins in catalyzing cellular auxin efflux (108). This together with the strong correlation between their subcellular

localization and the direction of auxin transport (95, 96, 104) assigns to these proteins the status of AECs.

On the other hand, there is also substantial evidence that proteins of the MDR/PGP family of ABC transporters function as auxin transporters. Initially, loss-of-function mutants of two of these proteins, AtMDR1 and AtPGP1, which were originally identified as being functionally related to anion channels, have been characterized for their reduced auxin transport capabilities (109). The finding that AtMDR1 and AtPGP1 are able to bind NPA reinforces their role as auxin transporters (109, 110). The most striking evidence supporting the participation of such proteins in the transport of auxin, however, comes from experiments showing that AtPGP1, a protein localized apolarly in the cells of shoot and root apices, catalyzes auxin efflux from Arabidopsis protoplasts and in yeast and human cells (111). Interestingly, transport assays have indicated that the MDR/PGP family member PGP4 enhances auxin uptake of plant cells (112). The MDR/PGP proteins should therefore be classified as auxin transporters, rather than AECs.

Recently, it has been reported that PINs co-purify with MDR/PGP proteins (97). These data point to a scenario where the PIN and MDR/PGP proteins represent two PAT pathways, and that the PIN and MDR proteins interact in cells and tissues where both pathways overlap to mediate and direct PAT (97).

PIN polarity is maintained through GNOM ARF GEF-controlled vesicle trafficking

The polarity of PINs is determining the direction of polar auxin transport (95), and the polar localization of PIN1 appears to depend on the cytoskeleton. Treatment with the microtubule depolymerizing agent oryzalin suggested the presence of a microtubule-dependent cytokinesis pathway that localizes PIN1 at the cell plate of dividing cells (99). In interphase cells, however, asymmetric localization of PIN1 at the PM is reduced in response to treatment with actin depolymerizing drugs. Interestingly, this treatment impairs PAT, corroborating the importance of F-actin and polar localization of PIN1 for this process (113). Actin depolymerization also prevents the internalization of PIN1 to endosomal compartments upon treatment with the vesicle trafficking inhibitor Brefeldin A (BFA), and the restoration of PIN1 localization after BFA wash-out, indicating that F-actin provides tracks for vesicle movement between the endosomal compartments and the PM (99). In support of these data, it has been shown that the ADP-Ribosylation Factor-GTP Exchange Factor (ARF-GEF) GNOM is the BFA sensitive component that is required for recycling of PIN1 to the PM (114, 115). It remains to be established, however, whether GNOM is the polarity determinant in the recycling of PIN vesicles.

Based on the current data, two different functions for the PIN cycling could be hypothesized: i) to maintain PIN polarity and thus keep the directionality of auxin

efflux, as blocking of the cycling seems to suppress this characteristic; ii) to allow rapid changes in cell polarity, as the cycling of auxin efflux complexes would provide important flexibility for rapid changes in polarity of PM localization and thereby for the redirection of auxin efflux.

Recently, Paciorek and co-workers (116) demonstrated that auxin itself is an important component in the regulation of PIN cycling, since this hormone appears to inhibit PIN endocytosis. By performing this task, auxin increases PIN levels at the PM, thereby stimulating its own efflux by a vesicle-trafficking dependent mechanism.

PIN polarity is controlled by protein kinase activity

How is the directionality of PAT regulated or, in other words, what determines the asymmetric sub-cellular localization of PIN proteins? A key component in PIN polar targeting was identified through the *pinoid* loss-of-function mutant, that phenocopies the pin-like inflorescences of the *pin formed/pin1* mutant (117). Cloning of the *PINOID* gene revealed that it encodes a plant specific protein kinase (118), overexpression of which results in phenotypes such as agravitropic growth and collapse of the main root meristem. The root meristem collapse could be rescued by PAT inhibitors, suggesting that the PINOID (PID) protein kinase is a regulator of PAT (119). Recently, it was shown that the apico-basal subcellular polarity of PIN proteins is determined by threshold levels of PID. PID overexpression in the root tip, an organ where PID is not expressed, causes basally localized PINs (PIN1, 2 and 4) to be re-localized apically. Conversely, reduced PID activity in the epidermis of the inflorescence apex, an organ where PID activity is normally high, causes apically localized PIN1 to be re-localized to the basal PM (120). These data imply that regular levels of cellular PID are required in order to maintain proper PIN and PAT polarity.

Interestingly, a mutant has been identified in a gene encoding the regulatory A subunit of a trimeric protein phosphatase 2A, that displays root curling in response to NPA (*rcn1*) (121). The *rcn1* mutant displays increased root basipetal auxin transport, reduced gravitropic response and a delay in the establishment of differential auxin-induced gene expression across a gravity-stimulated root tip, aspects that were restored to normal upon NPA treatment (13). Although a direct link between RCN1 and PID has not been established yet, it is tempting to speculate that 2A-type protein phosphatases are involved in directing PAT, by counteracting the activity of the PID protein kinase.

Recently, Dai and co-workers (122) provided a first indication that PAT is also regulated by a MAPK cascade. Overexpression of the MAPKK BUD1/MKK7 resulted in defective auxin response and transport, while MKK7 repression caused enhanced PAT, indicating that this protein is a negative regulator of PAT (122).

Unraveling the PINOID signaling pathway

The demonstration that overexpression of the wild type PID kinase (35S::PID), but not of the negative kinase mutant MPID, leads to severe phenotypes that are the result of defective PIN localization, corroborate that PID-mediated phosphorylation is an important factor in the regulation of PAT (119, 120).

In an effort to unravel the phosphorylation targets of PID, and consequently the link between PID, PINs and PAT, several PID interactors were identified. Two of them, PINOID Binding Protein 1 (PBP1) and TOUCH3 (TCH3), are calmodulins which *in vitro* seem to up- or downregulate PID activity in the presence of Ca²⁺, respectively. This result is corroborated by assays in which 35S::PID seedlings treated with calcium influx and calmodulin inhibitors were found to have enhanced PID activity, and by the fact that neither PBP1 nor TCH3 seem to be PID phosphorylation targets (123).

A third interactor of PID is the PINOID Binding Protein 2 (PBP2). PBP2 contains two protein-protein interaction domains, the BTB/POZ- ('Bric-a-brac, Tramtrack and Broad Complex/Pox virus and Zinc finger) and the TAZ (Transcriptional Adaptor putative Zinc Finger) domain. PBP2 has previously been identified as a calmodulin binding transcriptional regulator AtBT1 (124). Moreover, BTB domain proteins have been implicated in proteolysis processes, as several of them were shown to interact with CULLIN3 (CUL3) and to recruit target proteins for degradation. Although PBP2 was not found to interact with CUL3 in the yeast two-hybrid system (124, 125), *in vitro* pull down with CUL3 has been reported (126). Our observations suggest, however, that PBP2 acts as a regulator of PID activity. PBP2 has a repressive effect on PID auto-phosphorylation activity *in vitro* (127). Moreover, the fact that the GFP-PBP2 fusion protein shows a cytoskeleton-like localization in onion cells (127), suggests that PBP2 provides a possible link between the established roles of PID and the cytoskeleton in regulating PAT.

The fourth identified PID binding protein is COP9/CSN8, one of the subunits of the COP9 Signalosome (CSN) (127). Considering the role of COP9 in proteolysis, the finding that PID interacts with the CSN suggests that PID plays a role in the protein degradation machinery, possibly by regulating the activity of CSN itself. Recent work by Abas and co-workers (128) indicated that PIN2 levels and localization are modulated by proteasome-dependent degradation. This finding also suggests that an hypothetical association of PID with proteolysis could influence the regulation of some PINs.

Although the PID interactors are very interesting proteins that unravel unexpected facets of PID action, none of them is a substrate for PID-mediated phosphorylation (127). The identification of substrates of the PID kinase will be a crucial step in unraveling the signaling pathways that lead to the asymmetry in PIN localization and thus determine the direction of auxin flow.

Thesis outline

The role of PID in the regulation of PAT has now been elucidated (120), but the signaling components downstream of this kinase are still elusive. The effort to unravel such PID-signaling related proteins began with the identification of several PID interactors, namely PBP1, PBP2, TCH3 and COP9 (127). Although the interaction of PID with PBP2 and COP9 revealed unexpected aspects of the functionality of this kinase, their role as part the PID-signaling complex remained unclear. The research described in this thesis therefore focused on uncovering the function of PBP2 and COP9, and on the identification of PID phosphor-targets through an 'estimated-guess' approach.

The PID partner PBP2 was characterized for being a putative protein complex organizer, an observation that opened great possibilities for putative complexes eventually formed between PID, PBP2 and PBP2 binding proteins. Consequently, the research described in this thesis starts with a detailed characterization of the interaction between PID and PBP2 and the identification of PBP2 interactors (Chapter 2). The data indicate that PBP2 is a scaffold protein with multiple functions, one of which is to be recruited to the PID signaling complex to regulate PIN polar targeting.

Chapter 3 addresses in more detail the interaction between PBP2 and two paralogous microtubule motor proteins PBP2 Binding Kinesin 1 and 2 (PBK1 and 2, respectively), and their possible relationship with PID. The analyses provide evidence that PBK1 and 2 may be involved in the PBP2-mediated repression of PID, possibly by transporting PBP2 to specific sub-cellular locations.

In the view of the clear relationship between PID and PINs, we performed *in vitro* phosphorylation assays involving PID and the large cytoplasmic loops of several PIN proteins, to test whether these cytosolic domains can be phosphorylated by PID (Chapter 4). The results suggest that PID regulates the trafficking and subcellular localization of PIN proteins by direct modification of these auxin transporters at conserved serine residues in their large cytoplasmic domains.

Finally, Chapter 5 describes the interaction between PID and components of auxin signaling such as subunits of the CSN and the AUX/IAA protein BDL/IAA12. The analyses show that, although PID interacts with one subunit of the CSN, the COP9/CSN8, it phosphorylates a second CSN subunit, the COP15/CSN7. Further observations that PID phosphorylates BDL/IAA12 *in vitro* and that both proteins functionally interact *in vivo* open the possibility that PID controls auxin signaling by affecting proteolysis processes.

REFERENCE LIST

1. Davies, P. J. (1995) in *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, ed. Davies, P. J. (Kluwer, Boston), pp. 13-38.
2. Leyser, O. (2005) *Cell* **121**, 819-822.
3. Darwin, C. & Darwin, F. (1881) *The Power of Movement in Plants* (London).
4. Went, F. W. (1926) *Proc K Akad Wet Amsterdam* **30**, 10-19.
5. Kogl, F. & Haagen-Smit, A. (1931) *Proc. Sect. Sci.* **34**, 1411-1416.
6. Cholodny, N. (1927) *Biol Zent* **47**, 604-626.
7. Went F. & Thimann, K. (1937) *Phytohormones* (MacMillan, New York).
8. Friml, J., Wisniewska, J., Benkova, E., Mendgen, K. & Palme, K. (2002) *Nature* **415**, 806-809.
9. Li, Y., Hagen, G. & Guilfoyle, T. J. (1991) *Plant Cell* **3**, 1167-1175.
10. Esmon, C. A., Tinsley, A. G., Ljung, K., Sandberg, G., Hearne, L. B. & Liscum, E. (2006) *Proc Natl. Acad. Sci. U S. A* **103**, 236-241.
11. Larkin, P. J., Gibson, J. M., Mathesius, U., Weinman, J. J., Gartner, E., Hall, E., Tanner, G. J., Rolfe, B. G. & Djordjevic, M. A. (1996) *Transgenic Res.* **5**, 325-335.
12. Luschign, C., Gaxiola, R. A., Grisafi, P. & Fink, G. R. (1998) *Genes Dev.* **12**, 2175-2187.
13. Rashotte, A. M., DeLong, A. & Muday, G. K. (2001) *Plant Cell* **13**, 1683-1697.
14. Woodward, A. W. & Bartel, B. (2005) *Ann. Bot. (Lond)* **95**, 707-735.
15. Morre, D. J., Morre, D. M. & Ternes, P. (2003) *In Vitro Cell Dev. Biol Plant* **39**, 368-376.
16. Droog, F., Hooykaas, P. & van der Zaal, B. J. (1995) *Plant Physiol* **107**, 1139-1146.
17. Bilang, J. & Sturm, A. (1995) *Plant Physiol* **109**, 253-260.
18. Theologis, A. (1986) *Ann. Rev. Plant Physiol.* **37**, 407-438.
19. Guilfoyle, T. (1986) *CRC Crit Rev Plant Sci* **4**, 247-276.
20. Abel, S. & Theologis, A. (1996) *Plant Physiol* **111**, 9-17.
21. Karcz, W. & Burdach, Z. (2002) *J Exp. Bot* **53**, 1089-1098.
22. Gehring, C. A., Irving, H. R. & Parish, R. W. (1990) *Proc Natl. Acad. Sci. U S. A* **87**, 9645-9649.
23. Grebe, M. (2005) *Science* **310**, 60-61.
24. Theologis, A., Huynh, T. V. & Davis, R. W. (1985) *J Mol. Biol* **183**, 53-68.

25. Hagen, G. & Guilfoyle, T. J. (1985) *Mol. Cell Biol* **5**, 1197-1203.
26. Key, J. L., Kroner, P., Walker, J., Hong, J. C., Ulrich, T. H., Ainley, W. M., Gantt, J. S. & Nagao, R. T. (1986) *Philos. Trans. R. Soc. Lond B Biol Sci.* **314**, 427-440.
27. van der Zaal E., Memelink, J., Mennes, A., Quint, A. & Libbenga, K. (1987) *Plant Mol Biol.* **10**, 145-157.
28. Ulmasov, T., Murfett, J., Hagen, G. & Guilfoyle, T. J. (1997) *Plant Cell* **9**, 1963-1971.
29. Guilfoyle, T., Hagen, G., Ulmasov, T. & Murfett, J. (1998) *Plant Physiol* **118**, 341-347.
30. Guilfoyle, T. J., Ulmasov, T. & Hagen, G. (1998) *Cell Mol. Life Sci.* **54**, 619-627.
31. Ulmasov, T., Hagen, G. & Guilfoyle, T. J. (1999) *Proc. Natl. Acad. Sci. U S. A* **96**, 5844-5849.
32. Abel, S., Oeller, P. W. & Theologis, A. (1994) *Proc Natl. Acad. Sci. U S. A* **91**, 326-330.
33. Tiwari, S. B., Hagen, G. & Guilfoyle, T. J. (2004) *Plant Cell* **16**, 533-543.
34. Ramos, J. A., Zenser, N., Leyser, O. & Callis, J. (2001) *Plant Cell* **13**, 2349-2360.
35. Ulmasov, T., Hagen, G. & Guilfoyle, T. J. (1999) *Plant J.* **19**, 309-319.
36. Liscum, E. & Reed, J. W. (2002) *Plant Mol. Biol.* **49**, 387-400.
37. Sessions, A., Nemhauser, J. L., McColl, A., Roe, J. L., Feldmann, K. A. & Zambryski, P. C. (1997) *Development* **124**, 4481-4491.
38. Hardtke, C. S. & Berleth, T. (1998) *EMBO J.* **17**, 1405-1411.
39. Liscum, E. & Briggs, W. R. (1995) *Plant Cell* **7**, 473-485.
40. Kim, J., Harter, K. & Theologis, A. (1997) *Proc. Natl. Acad. Sci. U S. A* **94**, 11786-11791.
41. Hardtke, C. S., Ckurshumova, W., Vidaurre, D. P., Singh, S. A., Stamatiou, G., Tiwari, S. B., Hagen, G., Guilfoyle, T. J. & Berleth, T. (2004) *Development* **131**, 1089-1100.
42. Weijers, D. & Jurgens, G. (2004) *Curr. Opin. Plant Biol.* **7**, 687-693.
43. Weijers, D., Benkova, E., Jager, K. E., Schlereth, A., Hamann, T., Kientz, M., Wilmoth, J. C., Reed, J. W. & Jurgens, G. (2005) *EMBO J.* **24**, 1874-1885.
44. Gray, W. M., Kepinski, S., Rouse, D., Leyser, O. & Estelle, M. (2001) *Nature* **414**, 271-276.
45. Schwechheimer, C. & Deng, X. W. (2001) *Trends Cell Biol.* **11**, 420-426.
46. Hellmann, H., Hobbie, L., Chapman, A., Dharmasiri, S., Dharmasiri, N., del Pozo, C., Reinhardt, D. & Estelle, M. (2003) *EMBO J.* **22**, 3314-3325.
47. Gray, W. M., del Pozo, J. C., Walker, L., Hobbie, L., Risseuw, E., Banks, T., Crosby, W. L., Yang, M., Ma, H. & Estelle, M. (1999) *Genes Dev.* **13**, 1678-1691.

48. Ruegger, M., Dewey, E., Gray, W. M., Hobbie, L., Turner, J. & Estelle, M. (1998) *Genes Dev.* **12**, 198-207.
49. Gray, W. M., Hellmann, H., Dharmasiri, S. & Estelle, M. (2002) *Plant Cell* **14**, 2137-2144.
50. Kepinski, S. & Leyser, O. (2005) *Nature* **435**, 446-451.
51. Dharmasiri, N., Dharmasiri, S. & Estelle, M. (2005) *Nature* **435**, 441-445.
52. del Pozo, J. C. & Estelle, M. (1999) *Proc Natl. Acad. Sci. U S A* **96**, 15342-15347.
53. Dharmasiri, S., Dharmasiri, N., Hellmann, H. & Estelle, M. (2003) *EMBO J* **22**, 1762-1770.
54. Lincoln, C., Britton, J. H. & Estelle, M. (1990) *Plant Cell* **2**, 1071-1080.
55. Bostick, M., Lochhead, S. R., Honda, A., Palmer, S. & Callis, J. (2004) *Plant Cell* **16**, 2418-2432.
56. Larsen, P. B. & Cancel, J. D. (2004) *Plant J* **38**, 626-638.
57. Fu, H., Reis, N., Lee, Y., Glickman, M. H. & Vierstra, R. D. (2001) *EMBO J.* **20**, 7096-7107.
58. Schwechheimer, C., Serino, G., Callis, J., Crosby, W. L., Lyapina, S., Deshaies, R. J., Gray, W. M., Estelle, M. & Deng, X. W. (2001) *Science* **292**, 1379-1382.
59. Schwechheimer, C. (2004) *Biochim. Biophys. Acta* **1695**, 45-54.
60. Cope, G. A. & Deshaies, R. J. (2003) *Cell* **114**, 663-671.
61. Chamovitz, D. A., Wei, N., Osterlund, M. T., von Arnim, A. G., Staub, J. M., Matsui, M. & Deng, X. W. (1996) *Cell* **86**, 115-121.
62. von Arnim, A. G., Osterlund, M. T., Kwok, S. F. & Deng, X. W. (1997) *Plant Physiol* **114**, 779-788.
63. Seo, H. S., Yang, J. Y., Ishikawa, M., Bolle, C., Ballesteros, M. L. & Chua, N. H. (2003) *Nature* **423**, 995-999.
64. Osterlund, M. T., Wei, N. & Deng, X. W. (2000) *Plant Physiol* **124**, 1520-1524.
65. Osterlund, M. T., Hardtke, C. S., Wei, N. & Deng, X. W. (2000) *Nature* **405**, 462-466.
66. Schwechheimer, C. & Deng, X. W. (2000) *Semin. Cell Dev. Biol.* **11**, 495-503.
67. Cheng, Y., Dai, X. & Zhao, Y. (2004) *Plant Physiol* **135**, 1020-1026.
68. Colon-Carmona, A., Chen, D. L., Yeh, K. C. & Abel, S. (2000) *Plant Physiology* **Vol. 124**, 1728-1738.
69. Mockaitis, K. & Howell, S. H. (2000) *Plant J* **24**, 785-796.
70. Kovtun, Y., Chiu, W. L., Zeng, W. & Sheen, J. (1998) *Nature* **395**, 716-720.
71. DeLong, A., Mockaitis, K. & Christensen, S. (2002) *Plant Mol Biol* **49**, 285-303.

72. Morris, D. A. & Thomas (1978) *J. Exp. Bot.* **29**, 147-157.
73. Nowacki, J. & Bandurski, R. S. (1980) *Plant Physiol.* **65**, 422-427.
74. Baker, D. A. (2000) *Israel J. Plant Sci.* **48**, 199-203.
75. Cambridge, A. P. & Morris, D. A. (1996) *Planta* **199**, 583-588.
76. Lomax, T. L., Muday, G. K. & Rubery, P. H. (1995) in *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, ed. Davies, P. J. (Kluwer, Boston), pp. 509-530.
77. Rashotte, A. M., Brady, S. R., Reed, R. C., Ante, S. J. & Muday, G. K. (2000) *Plant Physiol* **122**, 481-490.
78. Rubery, P. H. & Sheldrake, A. R. (1974) *Planta* **118**, 101-121.
79. Raven, J. (1975) *New Phytologist* **74**, 163-172.
80. Goldsmith, M. H. M. (1977) *Annu. Rev. Plant. Physiol.* **28**, 439-478.
81. Lomax, T. L., Mehlhorn, R. J. & Briggs, W. R. (1985) *Proc Natl. Acad. Sci. U S. A* **82**, 6541-6545.
82. Benning, C. (1986) *Planta* **169**, 228-237.
83. Kramer, E. M. (2004) *Trends Plant Sci.* **9**, 578-582.
84. Parry, G., Marchant, A., May, S., Swarup, R., Swarup, K., James, N., Graham, N., Allen, T., Martucci, T., Yemm, A. *et al.* (2001) *J Plant Growth Regul* **20**, 217-225.
85. Marchant, A., Bhalerao, R., Casimiro, I., Eklof, J., Casero, P. J., Bennett, M. & Sandberg, G. (2002) *Plant Cell* **14**, 589-597.
86. Marchant, A., Kargul, J., May, S. T., Muller, P., Delbarre, A., Perrot-Rechenmann, C. & Bennett, M. J. (1999) *EMBO J* **18**, 2066-2073.
87. Parry, G., Delbarre, A., Marchant, A., Swarup, R., Napier, R., Perrot-Rechenmann, C. & Bennett, M. J. (2001) *Plant J* **25**, 399-406.
88. Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K. & Bennett, M. (2001) *Genes Dev.* **15**, 2648-2653.
89. Reinhardt, D., Pesce, E. R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J. & Kuhlemeier, C. (2003) *Nature* **426**, 255-260.
90. Friml, J. & Palme, K. (2002) *Plant Mol. Biol.* **49**, 273-284.
91. Friml, J. (2003) *Curr. Opin. Plant Biol.* **6**, 7-12.
92. Geisler, M. & Murphy, A. S. (2006) *FEBS Lett.* **580**, 1094-1102.
93. Muller, A., Guan, C., Galweiler, L., Tanzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E. & Palme, K. (1998) *EMBO J.* **17**, 6903-6911.

94. Galweiler, L., Guan, C., Muller, A., Wisman, E., Mendgen, K., Yephremov, A. & Palme, K. (1998) *Science* **282**, 2226-2230.
95. Wisniewska, J., Xu, J., Seifertova, D., Brewer, P. B., Ruzicka, K., Blilou, I., Roquie, D., Benkova, E., Scheres, B. & Friml, J. (2006) *Science*.
96. Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G. & Friml, J. (2003) *Cell* **115**, 591-602.
97. Blakeslee, J. J., Peer, W. A. & Murphy, A. S. (2005) *Curr. Opin. Plant Biol.* **8**, 494-500.
98. Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K. & Scheres, B. (2005) *Nature* **433**, 39-44.
99. Geldner, N., Friml, J., Stierhof, Y. D., Jurgens, G. & Palme, K. (2001) *Nature* **413**, 425-428.
100. Okada, K., Ueda, J., Komaki, M. K., Bell, C. J. & Shimura, Y. (1991) *Plant Cell* **3**, 677-684.
101. Ottenschlager, I., Wolff, P., Wolverson, C., Bhalerao, R. P., Sandberg, G., Ishikawa, H., Evans, M. & Palme, K. (2003) *Pnas* vol. **100**, 2987-2991.
102. Shin, H., Shin, H. S., Guo, Z., Blancaflor, E. B., Masson, P. H. & Chen, R. (2005) *Plant J.* **42**, 188-200.
103. Friml, J., Benkova, E., Blilou, I., Wisniewska, J., Hamann, T., Ljung, K., Woody, S., Sandberg, G., Scheres, B., Jurgens, G. *et al.* (2002) *Cell* **108**, 661-673.
104. Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R. & Jurgens, G. (2003) *Nature* **426**, 147-153.
105. Vieten, A., Vanneste, S., Wisniewska, J., Benkova, E., Benjamins, R., Beeckman, T., Luschig, C. & Friml, J. (2005) *Development* **132**, 4521-4531.
106. Paponov, I. A., Teale, W. D., Trebar, M., Blilou, I. & Palme, K. (2005) *Trends Plant Sci.* **10**, 170-177.
107. Chen, R., Hilson, P., Sedbrook, J., Rosen, E., Caspar, T. & Masson, P. H. (1998) *Proc Natl. Acad. Sci. U S A* **95**, 15112-15117.
108. Petrasek, J., Mravec, J., Bouchard, R., Blakeslee, J. J., Abas, M., Seifertova, D., Wisniewska, J., Tadele, Z., Kubes, M., Covanova, M. *et al.* (2006) *Science*.
109. Noh, B., Murphy, A. S. & Spalding, E. P. (2001) *Plant Cell* **13**, 2441-2454.
110. Murphy, A. S., Hoogner, K. R., Peer, W. A. & Taiz, L. (2002) *Plant Physiol* **128**, 935-950.
111. Geisler, M., Blakeslee, J. J., Bouchard, R., Lee, O. R., Vincenzetti, V., Bandyopadhyay, A., Titapiwatanakun, B., Peer, W. A., Bailly, A., Richards, E. L. *et al.* (2005) *Plant J* **44**, 179-194.
112. Terasaka, K., Blakeslee, J. J., Titapiwatanakun, B., Peer, W. A., Bandyopadhyay, A., Makam, S. N., Lee, O. R., Richards, E. L., Murphy, A. S., Sato, F. *et al.* (2005) *Plant Cell* **17**, 2922-2939.
113. Butler, J. H., Hu, S., Brady, S. R., Dixon, M. W. & Muday, G. K. (1998) *Plant J* **13**, 291-301.

114. Steinmann, T., Geldner, N., Grebe, M., Mangold, S., Jackson, C. L., Paris, S., Galweiler, L., Palme, K. & Jurgens, G. (1999) *Science* **286**, 316-318.
115. Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Muller, P., Delbarre, A., Ueda, T., Nakano, A. & Jurgens, G. (2003) *Cell* **112**, 219-230.
116. Paciorek, T., Zazimalova, E., Ruthardt, N., Petrasek, J., Stierhof, Y. D., Kleine-Vehn, J., Morris, D. A., Emans, N., Jurgens, G., Geldner, N. *et al.* (2005) *Nature* **435**, 1251-1256.
117. Bennett, S., Alvarez, J., Bossinger, G. & Smyth, D. (1995) *Plant J.* **8**, 505.
118. Christensen, S. K., Dagenais, N., Chory, J. & Weigel, D. (2000) *Cell* **100**, 469-478.
119. Benjamins, R., Quint, A., Weijers, D., Hooykaas, P. & Offringa, R. (2001) *Development* **128**, 4057-4067.
120. Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P. B., Ljung, K., Sandberg, G. *et al.* (2004) *Science* **306**, 862-865.
121. Garbers, C., DeLong, A., Deruere, J., Bernasconi, P. & Soll, D. (1996) *EMBO J* **15**, 2115-2124.
122. Dai, Y., Wang, H., Li, B., Huang, J., Liu, X., Zhou, Y., Mou, Z. & Li, J. (2006) *Plant Cell* **18**, 308-320.
123. Benjamins, R., Ampudia, C. S., Hooykaas, P. J. & Offringa, R. (2003) *Plant Physiol* **132**, 1623-1630.
124. Du, L. & Poovaiah, B. W. (2004) *Plant Mol. Biol.* **54**, 549-569.
125. Dieterle, M., Thomann, A., Renou, J. P., Parmentier, Y., Cognat, V., Lemonnier, G., Muller, R., Shen, W. H., Kretsch, T. & Genschik, P. (2005) *Plant J.* **41**, 386-399.
126. Figueroa, P., Gusmaroli, G., Serino, G., Habashi, J., Ma, L., Shen, Y., Feng, S., Bostick, M., Callis, J., Hellmann, H. *et al.* (2005) *Plant Cell*.
127. Benjamins, R. Functional analysis of the PINOID protein kinase in *Arabidopsis thaliana*. 2004. Leiden University.
128. Abas, L., Benjamins, R., Malenica, N., Paciorek, T., Wirniewska, J., Moulinier-Anzola, J. C., Sieberer, T., Friml, J. & Luschig, C. (2006) *Nat. Cell Biol.* **8**, 249-256.