Plant evolution: AGC kinases tell the auxin tale

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The signaling molecule auxin is a central regulator of plant development, which instructs tissue and organ patterning, and couples environmental stimuli to developmental responses. Here, we discuss the function of PINOID (PID) and the phototropins, members of the plant specific AGCVIII protein kinases, and their role in triggering and regulating development by controlling PIN-FORMED (PIN) auxin transporter-generated auxin gradients and maxima. We propose that the AGCVIII kinase gene family evolved from an ancestral phototropin gene, and that the co-evolution of PID-like and PIN gene families marks the transition of plants from water to land. We hypothesize that the PID-like kinases function in parallel to, or downstream of, the phototropins to orient plant development by establishing the direction of polar auxin transport.

Plant development directed by the hormone auxin

The development of flowering plants is dualistic: on the one hand, it follows strict programs producing uniform flowers and embryos; on the other hand, it can be flexible, particularly during vegetative development. In view of the predominantly sessile nature of plants, this flexible development is crucial to enable adaptation to changes in the environment. The plant hormone auxin is well recognized as a central regulator of both flexible growth responses, such as tropisms, and strict developmental programs, such as organ formation and patterning [1–5]. A characteristic of this signaling molecule is that it is actively transported in a directional manner, for example from young developing aerial organs to the root system. This polar auxin transport (PAT) generates auxin maxima and gradients, which are instrumental in directing growth and in positioning the formation of new organs. The PIN-FORMED (PIN) auxin efflux carriers have been characterized as central rate-limiting components that determine the direction of auxin transport through their asymmetric subcellular localization (Box 1 and reviewed in Refs [6–8]) [6–10].

The only component in the polar targeting of PIN proteins that has so far been identified is the PINOID (PID) protein kinase [11]. The PID gene was identified through Arabidopsis pid loss-of-function mutants, which phenocopy a mutant in the PIN-FORMED1 (PIN1) auxin efflux carrier gene. The phenotype of the pid mutant already suggested a role for PID as a regulator of PAT [12,13]. Recently, it was shown that PID is necessary for proper apical localization of PIN1 proteins in epidermal cells of the inflorescence meristem, which is required to generate auxin maxima in the meristem that are initiation points for lateral organ formation. In pid loss-of-function mutants, PIN1 localizes at the basal membrane, which deprives the meristem of auxin, and prevents the initiation and positioning of new lateral organs, thus resulting in the pin-shaped inflorescences that are characteristic of the pid mutant [11]. The action of PID does not seem to be restricted to the polar targeting of PIN1, because overexpression of PID results in a basal-to-apical (bottom-to-top) switch of PIN1, as well as PIN2 and PIN4, in root meristem cells. The fact that different PIN proteins are apicalized in response to PID and that PID expression is upregulated by auxin, suggests that PID is involved in feedback control of PAT [11,12,14–16].

A previous comparison of the PID protein kinase with known kinases indicated that it belongs to the plant-specific AGCVIII protein serine-threonine kinase family [12,17]. AGC kinases are named after protein kinase A (PKA), cyclic GMP-dependent protein kinases (PKG) and protein kinase C (PKC), three classes of animal protein kinases that are involved in receptor-mediated growth factor signal transduction. We have performed a detailed bioinformatic analysis of the Arabidopsis AGCVIII kinase family, which, besides PID, also comprises the well-known phototropins [18], and have studied their evolutionary origin and function in plant development. We hypothesize that AGCVIII kinases evolved from an ancestral phototropin, and that the acquisition of PID and the PIN transporters in plant evolution marks the transition of plants from water to land. Below we summarize the data that support our hypothesis.

The plant-specific characteristics of the AGCVIII kinases

The Arabidopsis genome encodes 37 AGC kinases, of which 23 classify to the AGCVIII group (see Figure S1 in the online supplementary material). Flowering plants do not have the typical animal PKA, PKC and PKG kinases. The AGCVIII kinases might therefore represent plant orthologs of these animal kinases.

One characteristic of members of the AGCVIII subfamily is the substitution of the conserved DFG amino acid motif in subdomain VII of the catalytic domain for the DFD amino acid triplet (Figure 1a). The DFD triplet is not plant-specific
Box 1. Fine tuning PID-dependent polar localization of PIN proteins

The chemiosmotic hypothesis proposed in the 1970s for auxin transport predicted that asymmetrically distributed auxin efflux carriers are the drivers of PAT. Two types of proteins have now been acknowledged as auxin efflux carriers. First, the PIN family of transporters was identified through the Arabidopsis pin-formed and ethylene-insensitive root 1 mutants that phenocopied wild-type plants grown on PAT inhibitors. These mutants led to the cloning of PIN1 and PIN2 respectively, and the subsequent identification of six other PIN genes in the Arabidopsis genome. PIN1, 2, 3, 4 and 7 show different tissue- and cell-type specific asymmetric subcellular localizations and have crucial roles in phyllotaxis, tropic growth and embryo patterning [8]. The localization and function of PIN5, 6 and 8 is still unknown [8]. In addition, several multi-drug-resistant/P-glycoprotein (MDR/Pgp)-type ATP-binding cassette (ABC) proteins were shown to act as auxin-efflux carriers [37]. In contrast to PIN proteins, MDR/Pgp proteins in most cases do not show a pronounced asymmetric subcellular localization, and it is as yet unclear whether they are part of the PIN-dependent, or another parallel, auxin transport pathway [8,37].

The PID protein kinase is the first, and for now only, identified determinant of the polar targeting of PIN proteins [11]. PID kinase activity is regulated by three factors: (i) by phosphorylation of the catalytic activation loop by 3-phosphoinositide-dependent kinase 1 (PDK1), which enhances PID kinase activity [19,41]; (ii) by phosphorylated phosphatidylinositol (PtdIns) and phosphatidic acid (PA), phospholipids that bind PID most strongly and most probably enable its association with the plasma membrane [19]; and (iii) by Ca2+-binding proteins, which bind to PID in a calcium-dependent manner and regulate its kinase activity [40]. At the plasma membrane, PID partially co-localizes with PIN proteins [15,35]. Overexpression of a functional PID:GFP fusion in Arabidopsis indicates that the subcellular localization of PID in the root meristem is cell-type specific (Figure 3d–f). In columella cells, PID shows random non-polar localization (Figure 3d), similar to PIN2 [39], whereas in the epidermal cell layer, PID shows apico-basal polarity (Figure 3d and e) that partially overlaps with PIN2 [11,35]. Recently, evidence was found for direct phosphorylation of PIN proteins by PID [35]. How exactly this phosphorylation affects the polar subcellular localization of PIN proteins is still unknown. It is probable, however, that PID-dependent polar targeting of PINs is tightly regulated by a combined action of PDK1, phospholipids and Ca2+-binding proteins.

and defines a class of AGC protein kinases that can be found in all eukaryotes. Another characteristic is the presence of an amino acid insertion between the conserved subdomains VII and VIII of the catalytic domain (VII–VIII insertion), which ranges from 36 to 90 residues in the Arabidopsis family members (Figure 1a and b). The insertion in combination with the DFD triplet is specific for the plant AGC group of protein kinases. Recent data suggest a role for the VII–VIII insertion in the subcellular localization of these protein kinases [19] (Box 1).

Phylogeny of the Arabidopsis AGCVIII kinases

To determine the evolutionary relationships between the members of the Arabidopsis AGCVIII subfamily, the sequences corresponding to the catalytic domain were used to construct an AGCVIII-specific phylogenetic tree (Figure 1b). In a previous phylogenetic analysis using the full length amino acid sequences, the plant AGC kinases were classified into two groups [20]. However, in our analysis, we found that AGCVIII kinases classify into four distinct groups, which we named AGC1–AGC4 (Figure 1). The largest group (AGC1) is formed by 13 putative protein kinases and comprises orthologs of the first protein kinases to be identified in plants; for example, Phaseolus vulgaris protein kinase 1 (PvPK1) [21]. The AGC4 group is formed by the phototropins PHOT1 and PHOT2, which are characterized by an N-terminal photoreceptor domain with two chromophore-binding LOV (light, oxygen, voltage) domains and a C-terminal protein kinase domain [22]. PID, AGC3–4, WAG1 and WAG2 [ wag after the phenotype of the corresponding mutants, which have an enhanced sinuosoidal growth of the root, also known as root waving (reviewed in Ref. [23]) form the third group (AGC3). The kinases within these three subgroups share conserved residues within the VII–VIII insertion, including a conserved AEP amino acid triplet that is located close to subdomain VIII (Figure 1a). Interestingly, this AEP triplet is not found in the four remaining kinases that form the AGC2 group, which demonstrates that they are more distantly related to phototropins than was previously thought [20] (Figure 1).

The genes encoding AGCVIII members are not clustered or do not show a clear organization in the Arabidopsis genome [19] (Figure 1b). Five of the genes do not contain an intron, whereas the other 18 genes contain one or more introns. Interestingly, one intron is found in members of all four groups at a conserved position in the region encoding kinase subdomain VIa (Figure 1b), indicating that the AGCVIII genes originate from a single ancestral kinase. Arabidopsis PHOT1 and PHOT2 carry multiple introns at identical positions, corroborating their relatedness.

In conclusion, our analysis of the Arabidopsis AGCVIII kinases shows that they classify into four groups, and indicates that the encoding gene family originated from a single ancestral kinase gene through multiple independent duplication steps. Interestingly, each group seems to perform a different function in plants, with AGC1 kinases having a role in cell organization [24], AGC2 kinases in stress responses [25,26], AGC3 kinases in the regulation of PAT [12,27], and AGC4 kinases in chloroplast avoidance and phototropism [28,29]. The last two subgroups have clear links with PAT and will therefore be discussed in more detail.

Phototropins – remnants of the ancestral plant AGCVIII kinase

Phototropins, which constitute the AGC4 group, were discovered through a screen for Arabidopsis mutants that lack directive growth of the hypocotyl of dark-grown seedlings towards a blue light source [29]. This screen identified several non-phototropic mutants, one of which is mutated in the gene encoding the blue light receptor PHOT1 [18,29]. The Arabidopsis genome also encodes a homolog of PHOT1 known as PHOT2. PHOT1 is the major player in the phototropic growth of seedling hypocotyls and roots at low intensity light conditions, whereas PHOT2 functions in triggering the auxin-mediated phototropic response under high intensity light conditions. Moreover, the PHOTs have been found to function redundantly in blue-light-induced chloroplast movement and stomatal opening [22]. Phototropins are the only members of the AGCVIII family so far identified in unicellular green algae. Therefore, they might represent the first descendants of the
Figure 1. The Arabidopsis AGCVIII protein kinase family. (a) Schematic representation of the catalytic kinase domain of the Arabidopsis AGCVIII protein kinases. The eleven conserved subdomains of the catalytic kinase domain are represented as blue boxes labeled with Roman numbers. The (length of the) amino acid insertion between subdomains VII and VIII (red), the typical DFD (Asp-Phe-Asp) signature in sub-domain VII (green), the conserved basic pocket (purple), and the AEP (Ala-Glu-Pro) triplet close to sub-domain VIII (blue) are indicated. (b) Phylogenetic tree of the 23 Arabidopsis AGCVIII protein kinases based on an alignment of their catalytic kinase domains subdivides the Arabidopsis AGCVIII kinases into four distinct groups. A comparison of the total protein size, the length of the VII–VIII insertion and the intron positions within the region coding for the catalytic domain is indicated. The positions of the highly conserved intron (red arrowheads) and the more variable introns (orange, white and yellow arrowheads) are indicated.
ancstral AGCVIII protein kinase. The PHOT gene of the green alga *Chlamydomonas* can partially restore phototropism, chloroplast positioning and stomatal opening in response to blue light when expressed in the Arabidopsis phot1 phot2 double mutant [30], indicating that phototropin function and signaling is conserved in plants and algae. A comparison of catalytic kinase domains of 31 selected phototropin-related proteins from 14 representative plant species revealed two major groups (see Figure S2 in the online supplementary material): (i) the PHOT1-like proteins that are characterized by the conserved CLTSCKPQ amino acid signature in the VII–VIII insertion; and (ii) the PHOT2-like proteins. Interestingly, genes encoding PHOT2-like photoreceptors are found in all plant groups, whereas the PHOT1-like genes are restricted to seed plants (Table 1, Figure 2; see Figure S2 in the online supplementary material).

Taking all these observations into consideration, we speculate that optimization of light perception mediated by PIN and AGC3 kinases evolved later in evolution in land plants. Therefore, the PHOT2-like proteins probably represent the most ancient AGCVIII protein kinases from which the other AGCVIII kinases evolved. A duplication of the ancestral PHOT2 gene gave rise to the low-fluence phototropin-encoding PHOT1 gene, which is found only in spermatophytes.
by PHOT2-like proteins, for example the high intensity light avoidance of chloroplasts, is one of the ancient traits in plant evolution. A more detailed analysis and functional characterization of phototropins throughout the plant kingdom should provide further evidence for our hypothesis.

**AGC3 kinases direct auxin transport**

The PID-containing subgroup (AGC3) is composed of four genes in both Arabidopsis and the monocot Oryza sativa. Apart from PID, two other AGC3 members in Arabidopsis have been characterized in more detail: WAG1 and WAG2. Homologs of WAG1 were initially discovered in Cucumis sativus and Pisum sativum as auxin-induced and light-repressed genes [31–34]. Also the Arabidopsis WAG1 and WAG2 transcript levels were found to be negatively regulated by light [27,34], and it is therefore probable that one or both of the WAG genes are auxin-responsive, similar to the Cucumis sativus homolog CsPK3 or the PID gene in Arabidopsis [12,31]. Loss-of-function mutations in WAG1 or WAG2 result in weak root waving phenotypes, double mutants show a constitutive root waving phenotype, and root curling is more resistant to the PAT inhibitor 1-naphthylphthalamic acid (NPA). Because root waving is linked to PAT [23], and the PAT-inhibitor resistant root-curling phenotype is characteristic for mutants in PAT [35], it is probable that the WAG kinases, similar to PID, are involved in the regulation of auxin transport [23,27].

To corroborate this possible functional relatedness, we analyzed the subcellular localization of WAG1, WAG2 and PID in Arabidopsis thaliana protoplasts (Figure 3a–c), and found that all three kinases localize predominantly to the plasma membrane; however, WAGs can also be found in the nucleus. Our observations are partially in contrast to those of Zegzouti and co-workers, who concluded that the WAG kinases are localized in the nucleus [19]. Because their conclusion was based on the expression of fusions with the green fluorescent protein (GFP) in yeast cells, our own observations on functional yellow fluorescent protein (YFP) fusions expressed in Arabidopsis protoplasts are more likely to reflect the subcellular localization of the WAG kinases in planta. The plasma membrane localization, together with the phenotypes observed on the loss-of-function mutants and the similarity in amino acid sequence between PID and the WAG kinases, suggest that these kinases act in the same or in a parallel pathway to regulate the PAT machinery.

**The evolution of auxin-dependent plant development: AGC kinases tell the tale**

From an evolutionary perspective, the phototropins and AGC3 kinases seem to recapitulate plant evolution (Figure 2). It is unsurprising that PHOT2-like genes represent the most ancient AGCVIII protein kinases, because the optimization of photosynthesis in response to light intensities in the first eukaryotic photosynthetic cells was a crucial trait in plant evolution. The essential function of PHOT2-like genes has been well conserved and our analysis indicates that PHOT2-like genes are found in representative species throughout the plant kingdom (Figure 2). By contrast, PHOT1-like genes are only found in spermatophytes, indicating that PHOT1-mediated tropic growth in response to low intensity light evolved later as an important determinant of proper development of soil-born germinating seedlings (Figure 2).

As for auxin-dependent processes, a tryptophan-dependent biosynthesis pathway has been found in green and brown algae (Chlorophytes and Charophytes) [36]. By contrast, no genes encoding PID, PID-like kinases or PIN auxin efflux carriers have been identified in the green algal genomes of Chlamydomonas or Ostreococcus (Table 1) and, although auxin transport seems to regulate directional growth and patterning in the brown algae [36], there is no clear evidence for PIN-dependent auxin efflux in these early plant forms (reviewed in Ref. [36]). In fact, auxin transport in Charophytes might well be mediated by the multi-drug-resistant/P-glycoprotein (MDR/PGP) type of transporters (Box 1), which are found throughout the entire plant kingdom, and, like PIN proteins, exhibit auxin efflux activity and sensitivity to PAT inhibitors [8,36,37].

Genes encoding candidate homologs of the AGC3 kinases and PIN auxin efflux carriers have been identified in the moss Physcomitrella [38] (www.cosmoss.org), and in many other land plants (Figure 2, Table 1). This, together with the demonstrated functional relationship between PID and PINs [11], suggests that these two gene families co-evolved, and that AGC3 kinase-regulated PIN-dependent PAT might have had an important role in the adaptation of plants during the transition from water to land (Figure 2, Table 1).

In conclusion, there is a strong correlation between the known functions of the AGC3 and AGC4 kinases in plant development, and their distribution throughout the plant kingdom, which suggests that new AGC kinases were acquired during most crucial steps in plant evolution.
Concluding remarks and future perspectives

Although there is much information about how differential auxin distribution couples environmental stimuli to developmental responses, such as directional growth, it is still unclear how the different components in the PAT pathway work coordinately to orient this auxin-directed plant development. Two subgroups of the AGC-VIII protein kinases are directly involved in this process. On the one hand, light-activated phototropins induce rapid Ca²⁺ release into the cytosol and initiate differential auxin transport leading to auxin accumulation in the cell layers at the dark side of the hypocotyl [39]. On the other hand, PID, and possibly other AGC3 kinases, directs PAT by determining the correct polar localization of PIN proteins during embryo development and organ formation in the shoot apical meristem.

Although none of the AGC3 kinases has been directly connected to phototropic growth, the observation that the activity of PID is regulated by interacting calcium-binding proteins [40] suggests that these kinases are downstream components of the phototropin signal transduction pathway. Whether the other AGC3 kinases similar to PID direct PAT through direct phosphorylation of PIN proteins, whether one or more AGC3 kinases affect the subcellular PIN localization during phototropism and whether there is a link between PHOT-induced calcium release and regulation on the activity of AGC3 kinases through calcium-binding proteins are key questions to be addressed by future research.

In conclusion, based on the data presented here, we propose that those AGCVIII kinases that have an essential role in plant development, recapitulate plant evolution. Phototropins represent the most ancient AGCVIII kinase forms that regulate highly conserved processes in plants, such as optimization of light perception, and AGC3 kinases co-evolved with PIN auxin transporters in multicellular plants during their colonization of land, and act together, possibly downstream of the phototropins, to orient plant development by establishing the directionality of auxin transport.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2007.10.004.

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