Methodological advances in auxin and cytokinin biology.

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Introduction

The concept of plant hormones as chemical messengers that control plant growth and development is not a new one. Already in 1758, Duhamel du Monceau's experiments suggested communication between plant organs and showed that sap moving from the leaves controls root growth (du Monceau, 1758). More than a century later Julius von Sachs proposed that plants produce “organ-forming substances” - molecules moving to different parts of the plant where they control initiation and development of specific plant organs (von Sachs, 1880). Finally, Charles and Francis Darwin, with their experiments on phototropism of coleoptiles (described in "The Power of Movement in Plants" (Darwin, 1880)) that later led to the discovery of auxin by Went (1928), fully launched the modern research in plant growth substances.

The first note about cytokinin comes from 1913 when Gottlieb Haberlandt observed that compounds from phloem could stimulate cell division in potato parenchyma cells (Haberlandt, 1913). In the 1950s, kinetin, an active compound stimulating cell division, was isolated from herring sperm (Miller et al., 1956). The first naturally occurring cytokinin in plants named zeatin was isolated from immature maize endosperm (Letham, 1973).

Since these initial discoveries, a great number of studies have demonstrated an essential role of both auxin and cytokinin in the regulation of many aspects of plant growth and development including embryogenesis (Friml et al., 2003; Müller and Sheen, 2008), postembryonic organogenic processes such as root (Fukaki et al., 2002; Benková et al., 2003; De Smet et al., 2007; Laplaze et al., 2007; Bielach et al., 2012), and shoot branching (Leyser, 2009; Shimizu-Sato et al., 2009; Müller et al., 2015), root (Friml et al., 2002; Blilou et al., 2005; Dello Ioio et al., 2008; Růžička et al., 2009) and shoot apical meristem activity and phyllotaxis (Reinhardt et al., 2003; Zhao et al., 2010; Yoshida et al., 2011; Chickarmane et al., 2012) vasculature development (Mähönen et al., 2006a; Hejátko et al., 2009; Bishopp et al., 2011b) as

well as tropic responses (Rouse et al., 1998; Müller et al., 1998; Luschning et al., 1998). Importantly, a classic series of experiments by Skoog and Miller (1957) demonstrated that the ratio of cytokinin to auxin profoundly influences the morphogenesis of roots and shoots in plant tissue culture. This was one of the first studies revealing auxin and cytokinin interaction in the differentiation of plant organs and pointed at hormonal cross-talk as an important aspect of auxin and cytokinin regulatory functions (reviewed in Moubayidin et al., 2009; Depuydt and Hardtke 2011; Schaller et al., 2015).

Nevertheless, it has been primarily the recent boom of modern technologies and approaches including analytical chemistry, biochemistry, molecular biology, genetics, cell and developmental biology that have enabled rapid progress in deciphering the auxin and cytokinin activities at the molecular level. Due to ongoing improvements and development of new methods, we are gaining deeper insights into mechanisms that control auxin and cytokinin biosynthesis, distribution, perception and signal transduction as well as insights into their functions in the regulation of plant growth and development. In this review, we shall briefly discuss the major recent progress made in this area, and highlight the importance of continuous methodological improvements.

1. Discovery of auxin and cytokinin

Discovery of auxin is tightly linked with Darwin's early studies on coleoptiles. Based on the bending of coleoptiles toward unilateral light, the existence of a messenger molecule named auxein (from the Greek "auxein" meaning ''to grow'') was predicted, which was apparently transported from the site of light perception at the tip of coleoptile towards the site of response where bending occurs (Darwin 1880). Later, it was demonstrated that an asymmetric accumulation of auxin at the non-illuminated side compared to the illuminated side correlated with differential cell growth and organ bending (Boysen-Jensen, 1911). A model implementing a role for auxin and its asymmetric distribution in the regulation of plant tropic responses was proposed (Cholodny, 1927, 1928; Went, 1928). Although the existence of auxin as a molecule controlling plant growth had been predicted already by Darwin in 1880, its chemical identity remained unknown for a long time. In 1928 Went succeeded in capturing this growth substance from coleoptile tips into agar blocks and demonstrated its biological activity (Went, 1928).

However, due to insufficient analytical methods for detecting low amounts of the hormone, the first auxin (indole-3-acetic acid, IAA) was purified from human urine and culture filtrates of several fungi, both of which are rich sources of substances with auxin activity when tested in the bioassays (Kögl et al. 1934; Thimann and Koepfli 1935). A decade later IAA was eventually discovered in a plant (*Zea mays*) (Haagen-Smit et al. 1946).

The first experimental indication of the existence of cytokinins was reported by Gottlieb Haberlandt (1913), who observed that phloem sap can stimulate division of potato parenchyma cells. Further studies showed that compounds which trigger cell division are present in various other plant species (van Overbeek, 1941; Jablonski and Skoog, 1954). The first molecule with the ability to promote cell division was purified from autoclaved herring sperm DNA. The compound 6-(furfurylamino) purine was named kinetin, and although it is one of the most biologically active cytokinins, it is formed as a DNA degradation product and is not detected in plant tissues (Miller et al., 1955; Hall and de Ropp, 1955). The first naturally occurring cytokinin, zeatin, was almost simultaneously isolated from *Zea mays* by Miller (1961) and Letham (1963). Since then, many naturally occurring cytokinins have been isolated and found to be ubiquitous to all plant species (Salisbury and Ross, 1992).

The discovery and identification of auxin and cytokinins triggered the interest of researchers, who then diversified to explore pathways that underlie auxin and cytokinin biosynthesis and metabolism, their distribution, as well as perception and signal transduction of these two plant hormones. The establishment of *Arabidopsis thaliana* as a model organism for plant molecular biology was one of the important milestones in hormone molecular biology. The use of *Arabidopsis* for mutant screens based on sensitivities to auxin and cytokinin enabled the identification of genes and pathways controlling their metabolism, transport, perception and signaling. These in combination with novel technologies and approaches, such as large scale transcriptome profiling, proteomics, chemical genomics, and most recently mathematical modelling, resulted in major breakthroughs in our understanding of auxin and cytokinin biology.

2. Auxin and cytokinin: insights into biosynthesis.

Although IAA had been recognized as the main native auxin already in 1935 (Thimann, and Koepfli), the question as to how auxin is synthesized remained unanswered for more than 70 years afterwards. Using genetic and biochemical tools, it has been found that IAA is mainly

synthesized from L-tryptophan (Trp) via indole-3-pyruvate (IPA) in a two-step reaction catalysed by TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) and YUCCA (YUC) (Figure 1a). The TAA family of amino transferases which mediate the first step of the pathway was isolated from independent genetic screens for mutants affected in shade, ethylene, and responses to the auxin transport inhibitor NPA (Stepanova et al., 2008; Tao et al., 2008; Yamada et al., 2009). Severe auxin deficient phenotypes (in developmental processes such as embryogenesis, seedling growth, flower development, vascular patterning, root branching, tropisms, and shade avoidance) as well as reduced endogenous auxin levels were observed in mutants lacking activity of TAA1 and the homologous TAR1 and TAR2, which indicated their function in auxin homeostasis maintenance (Stepanova et al., 2008). The phenotypic defects observed in TAA1/TAR deficient mutants were partially rescued by auxin, whereas induction of TAA1 led to the accumulation of endogenous IPA. Importantly, the recombinant TAA1 protein has been found to catalyse the conversion of Trp into IPA *in vitro* thus providing evidence for its direct involvement in auxin biosynthesis (Stepanova et al., 2008; Tao et al., 2008).

Similarly to *TAA1, YUC* genes were originally identified by a genetic screen in *Arabidopsis*. Using an activation-tagged mutant library, a flavin-containing monooxygenase *YUC1* was isolated. The *YUC1* (*yuc1D*) gain-of-function mutant exhibits increase in endogenous IAA and phenotypic alterations mimicking high auxin activity. Disruption of several *YUC* genes in *Arabidopsis* leads to defects in embryogenesis, seedling growth, flower development, and vascular pattern formation (Cheng et al., 2006, 2007). The developmental defects of the loss-of-function *yuc* mutants are rescued by the bacterial auxin biosynthesis gene *iaaM*, supporting *YUC* genes function in auxin biosynthesis (Cheng et al., 2006).

Although previously proposed to act in two independent pathways, recent genetic and biochemical studies showed that the TAAs and YUCs catalyse two consecutive reactions in the same pathway that converts Trp to IAA. Multiple lines of evidence support this model including similarities of both *taa* and *yuc* mutants phenotypes (Won et al., 2011) and enhancement of the auxin related phenotypes when both *YUC* and *TAA* are overexpressed in the same plants (Mashiguchi et al., 2011). Additionally, the YUC auxin overproduction phenotypes are suppressed in the *taa* mutant backgrounds, indicating that TAA acts upstream of YUC-mediated auxin biosynthesis (Won et al., 2011). Direct measurement of IPA levels reveals that *yuc* mutants accumulate IPA whereas *taa* mutants are partially IPA deficient, suggesting thay TAAs

catalyses synthesis of IPA which is converted by YUCs to IAA (Mashiguchi et al., 2011; Won et al., 2011). Finally, *in vitro* biochemical assays have demonstrated that TAA can convert Trp to IPA and that YUCs produce IAA using IPA as a substrate (Mashiguchi et al., 2011).

Early physiological studies on auxin biosynthesis suggested that auxin is primarily synthesized in the young developing organs such as leaves, shoot apical meristems, and developing fruits and seeds (Bartel, 1997; Ljung et al., 2001). The expression pattern of *TAA* and *YUC* genes modifies this established view on auxin biosynthesis. Local auxin production seems to take place in very distinct cell types, including root and apical embryo meristems, the root cap, quiescent centre (QC), root proximal meristem, vasculature of hypocotyls, as well as apical hooks, thus hinting at the spatio-temporal control of the IAA biosynthesis throughout plant growth and development (Chent et al., 2006, 2007; Stepanova et al., 2008; Tao et al., 2008). Several transcription factors which control *TAA* and *YUC* genes expression have been identified and thus might determine spatio-temporal pattern of the IAA biosynthesis. *LEAFY COTYLEDON2* (*LEC2*) (Stone et al., 2008) *SHORT INTERNODES/ STYLISH* (*SHI/STY*) (Eklund et al., 2010), *PHYTOCHROME-INTERACTING FACTORs* (*PIFs*) (Franklin et al., 2011; Sun et al., 2012), *INDETERMINATE DOMAIN* (*IDD*) (Cui et al., 2012) and *PLETHORA* family members (Pinon et al., 2013) have been reported as transcriptional activators of *YUC* and *TAA1* genes. In contrast, the *SPOROCYTELESS/NOZZLE* (*SPL/NZZ*) transcription factor, has been shown to negatively regulate some of *YUC* genes (Li et al., 2008).

Chemical biology-based studies provided additional support for the central role of the IPA pathway in IAA production. Chemical screens for auxin inhibitors uncovered L-kynurenine and L-amino-oxyphenylpropionic acid (L-AOPP) as TAA inhibitors and yucasin as a YUC inhibitor. Application of these compounds reduces endogenous IAA levels and results in phenotype alterations mimicking mutants deficient in auxin biosynthesis (Soeno et al., 2010; He et al., 2011; Nishimura et al., 2014).

Overall, genetic and biochemical analyses support the YUCs/TAAs mediated auxin biosynthesis as the major pathway used to produce auxin during plant development, whereas other pathways catalysed by CYP79B2/B3, nitrilases, aldehyde oxidases, and pyruvate decarboxylases might not be the main pathways in auxin biosynthesis (Zhao, 2012).

The great progress in elucidation of the cytokinin biosynthesis pathway occurred almost 20 years after identification of the chemical nature of cytokinins by Miller (1961) and Letham

(1963). In 1978 Taya and co-workers reported biosynthesis of free cytokinins *in vitro* and demonstrated that cell-free extracts of the slime mold *Dictyostelium discoideum* converts adenosine monophosphate (AMP) and dimethylallyl pyrophosphate (DMAPP) to the active cytokinin iPMP (N6-(D2-isopentenyl)adenosine-5'-monophosphate (Taya et al., 1978). Subsequently, the *ISOPENTENYLTRANSFERASE* (*IPT*) gene from *Agrobacterium tumefaciens* was shown to encode an enzyme with similar activity (Akiyoshi et al., 1984). Later, nine *IPT*-homologues genes were identified by an *in silico* search in the *A.thaliana* genome. The expression of *IPT* genes (except *AtIPT2* and *AtIPT9*) in *E. coli* resulted in the secretion of the cytokinins isopentenyladenine (iP) and zeatin, confirming their function as cytokinin biosynthetic enzymes (Takei et al., 2001). *IPT* genes display distinct, tissue-specific patterns of expression, indicative of cytokinin production sites (Miyawaki et al., 2004; Takei et al., 2004a).

Free iP-riboside generated via the IPT pathway, as well as the corresponding base, are further stereospecifically hydroxylated to trans-zeatin forms. The *CYP735A1* and *CYP735A2* encoding cytochrome P450 monooxygenases with cytokinin trans-hydroxylase enzymatic activity were identified in *A.thaliana* by a screen employing an (*AtIPT4*)/*P450* co-expression system in *Saccharomyces cerevisiae* (Takei et al., 2004b).

The final step in cytokinin biosynthesis, conversion of the cytokinin ribotides to their active, free base forms is catalyzed by the cytokinin nucleoside 5´-monophosphate phosphoribohydrolase LONELY GUY (LOG). These were first identified in rice by a genetic screen for defects in the maintenance of shoot meristems (Kurakawa et al., 2007). In *A.thaliana*, seven homologous genes that encode active LOG enzymes were detected. The *LOG* genes are differentially expressed in various tissues during plant development. (Kuroha et al., 2009). In accordance with their predicted function the conditional overexpression of *LOG*s in *Arabidopsis* reduced the content of iP riboside 5´-phosphates and increased the levels of iP and the glucosides (Kuroha et al., 2009) Alternatively, the cytokinin ribotides are dephosphorylated to the ribosides and subsequently converted to free-base cytokinins (Chen and Kristopeit 1981a, 1981b), however the corresponding genes have not yet been identified (Figure 1b and c).

Levels of active cytokinins in plant cells are tightly controlled. They might be either converted to storage forms through conjugation to glucose (Martine et al., 1998; Hou et al., 2004) or inactivated through irreversible cleavage by cytokinin oxidases (Werner et al., 2001; Werner et al., 2003), (Figure 1c). Development of highly sensitive analytical methods were instrumental in

the detection of numerous cytokinins metabolites and in deciphering complex cytokinin metabolism, followed by identification of the corresponding metabolic enzymes and genes (Letham and Palni 1983; Mok and Mok 2001; Tarkowski et al., 2009).

3. Transport of auxin and cytokinin

By definition, hormones are chemical messengers that are transported to distant tissues and organs to regulate their physiology and development. Darwin's early experiments on coleoptiles had already indicated that controlled transport of auxin from the tip of coleoptile to the bending region might be an essential part of the mechanism through which auxin executes its regulatory function. Later, based on the transport studies, it was proposed that cytokinins and auxin are synthesized only in root tips and shoot apices, respectively, and translocated to target tissues. Although the recent detailed investigations of expression patterns of auxin and cytokinin biosynthesis genes questions this over-simplified model, the tight control of hormone distribution through organs and tissues is considered to be the crucial component of their regulatory mechanisms. Nowadays, the broadly accepted concept is that both hormones are synthesized and act at various sites in a plant body and that they have coordinated functions as long-distance messengers as well as local signals.

The classical transport assays using radioactively labeled auxins outlined main routes of auxin movement in plants (Morris and Thomas, 1978). To transport auxin, plants use two distinct pathways: a non-polar passive distribution through phloem and an active cell-to-cell polar auxin transport (PAT). In the first pathway, most of the auxin and auxin derivatives are rapidly transported via unregulated flow in the mature phloem over long distances in both basipetal and acropetal directions (Nowacki and Bandurski, 1980). The second pathway is slower and acts over shorter distances, transporting auxin in a cell-to-cell manner from the shoot towards the root. In contrast to phloem transport, PAT is specific for active free auxins, occurs in a cell-to-cell manner and is strictly unidirectional. The main PAT stream from the apex towards the root occurs in the cambium and the adjacent partially differentiated xylem elements (Morris and Thomas, 1978; Lomax et al., 1995). In roots, the auxin stream continues acropetally towards the root tip, where part of the auxin is redirected backwards and transported through the root epidermis to the elongation zone (Rashotte et al., 2000).

Based on the chemical nature of auxin and the physiology of PAT, the model of cell-to-cell auxin of transport has been proposed, known as the chemiosmotic hypothesis (Rubery and Sheldrake, 1974; Raven, 1975). As a weak acid, a fraction of IAA exists in the acidic environment of the apoplast as the protonated, neutral form (IAAH), which may diffuse through the plasma membrane. In the more basic cytosol, auxin becomes deprotonated (IAA^-) and is unable to pass passively through the plasma membrane. The chemiosmotic hypothesis predicted that the exit of auxin anions from the cell is mediated by active efflux carriers and that the passive diffusion of auxin can be further facilitated by influx carriers. The polar membrane localization of the auxin efflux carriers in a file of adjacent cells would determine directionality of the auxin flow (Figure 2a).

It has been primarily genetic studies that led to discovery of genes required for auxin influx and efflux (Bennett et al., 1996; Gälweiler et al., 1998; Luschnig et al., 1998; Geisler et al., 2005; Cho et al., 2007). An auxin influx transporter *AUXIN RESISTANT1 (AUX1),* encoding an amino acid permease-like protein, was found in a screen for auxin resistant plants (Pickett et al., 1990). Strong insensitivity to membrane-impermeable auxin (2,4-D) suggested that the *aux1* mutation interferes with auxin uptake (Bennett et al., 1996), which was confined by the transport assays using a *Xenopus* oocyte expression system (Yang et al., 2006). The *A.thaliana* genome encodes four auxin influx transporters: *AUXIN RESISTANT1* (*AUX1)* and three *Like AUX1 (LAX1, LAX2, LAX3)* (Parry et al., 2001; Swarup et al., 2008; Péret et al., 2012). Thorough exploration of mutants lacking AUX1/LAX activity revealed the essential role of the auxin uptake in the regulation of gravitropism, phototropism, root branching, phyllotaxis, and root hair development (Bennett et al., 1996; Bainbridge et al., 2008; Stone et al., 2008; Swarup et al., 2008; Jones et al., 2009; Péret et al, 2012).

Genetic screens were also instrumental in identifying molecular components of auxin efflux. In the early nineties, the *A.thaliana* mutant, *pin-formed1* (*pin1*) with needle-like inflorescence was described. The characteristic phenotype similar to wild type plants treated with chemical inhibitors of auxin efflux indicated defects in auxin transport. Auxin transport assays in *pin1* stem segments confirmed severe reduction of the basipetal flow of auxin and pointed to a function for PIN1 in auxin efflux (Okada et al., 1991). Indeed, identification of the mutant locus revealed that *PIN1* encodes a putative transmembrane protein with a predicted topology of transporter proteins (Gälweiler et al., 1998). Auxin transport assays in *Arabidopsis* and tobacco

cell suspension culture as well as in heterologous non-plant systems including yeast, mammalian HeLa cells and *Xenopus* oocytes have provided evidence for an auxin efflux capacity of PIN proteins (Petrášek et al., 2006; Yang and Murphy, 2009; Barbez et al., 2013; Zourelidou et al., 2014). The *Arabidopsis PIN* gene family consists of eight members (Zažímalová et al., 2007; Adamovski and Friml 2015). Based on the localization and domain organization, these were divided into two groups. The first group consists of PIN1, PIN2, PIN3, PIN4 and PIN7 and are located at the plasma membrane. The second group comprising PIN5, PIN6, and PIN8 have a reduced middle hydrophilic loop and are located at the endoplasmic reticulum (ER), where they presumably control auxin flow between the cytosol and ER lumen, thus possibly affecting subcellular auxin homeostasis (Mravec et al., 2009; Ding et al., 2012). Similarly, PIN-LIKES proteins (PILS) are located in the ER and might play a role in regulation of intracellular auxin homeostasis (Barbez et al., 2012) (Figure 2).

In addition to the PIN family of plant-specific auxin transporters, plant orthologs of the mammalian ATP-binding cassette subfamily B (ABCB)-type transporters of the multidrug resistance/phosphoglycoprotein (ABCB/MDR/PGP) protein family (Noh et al., 2001; Verrier et al., 2008) have been implicated in auxin transport. Biochemical evidence for the ABCB proteins auxin transport activity has been demonstrated both in plant and non-plant systems. In contrast to polar localization of PINs, which corresponds with known direction of auxin flow, the ABCBs presumably act in nondirectional long-distance auxin transport controlling amount of auxin in these streams (Noh et al., 2001; Verrier et al., 2008; Peer et al., 2011).

The chemiosmotic hypothesis predicted that the polar membrane localization of auxin transporters determines the directionality of the auxin flow. This concept was supported by observations of a polar subcellular localization for PIN proteins (Gälweiler et al., 1998; Luschnig et al., 1998) and a tight correlation between PIN polarity and directions of auxin flow (Wisniewska et al., 2006). Phosphorylation of PINs controlled by a set of kinases and phosphatases (Benjamins et al., 2001; Friml et al., 2004; Michniewicz et al., 2007; Zhang et al., 2010; Huang et al., 2010; Zourelidou et al., 2014), Ca^{2+} signaling (Zhang et al., 2011), cell wall (Feraru et al., 2011) or mechanical signals orienting the plant microtubule network (Heisler et al., 2010) were found to determine PIN protein activity and polarity. Cell-biological studies revealed that PIN auxin efflux transporters may not solely reside at the plasma membrane since they undergo constitutive cycles of endocytosis and recycling back to the plasma membrane (Geldner

et al., 2001; Dhonukshe et al., 2007) (Figure 2c). The constitutive endocytosis and recycling of PIN proteins depends on complex subcellular trafficking machinery including the coat protein clathrin (Dhonukshe et al., 2007; Kitakura et al., 2011; Wang et al., 2013), ADP-ribosylation factor guanine-nucleotide exchange factors ARF - GEFs (Geldner et al., 2001, 2003; Kleine-Vehn et al., 2008a,b; Naramoto et al., 2014); ARF-GTPase-activating protein VASCULAR NETWORK DEFECTIVE3 (Naramoto et al., 2010), the related ARF-GEF GNOM-LIKE1 (Teh and Moore, 2007) and small GTPase Rab1b (Feraru et al., 2012). Downstream of endocytosis, the early endosomal trafficking of PINs is controlled by another ARF-GEF, BFA-visualized endocytic trafficking defective1, and the Sec1/Munc18 family protein BEN2 (Tanaka et al., 2009, 2013). The endocytosis and constitutive recycling of PIN proteins has been implicated in the maintenance of PIN polar localization and as a mechanism for rapid modifications of PIN polarity during various developmental processes including embryogenesis (Friml et al., 2003; Robert et al., 2013), lateral root organogenesis (Benkova et al., 2003; Dubrovsky et al., 2008) or tropic responses (Friml et al., 2002, Kleine Vehn et al., 2010; Ding et al., 2011; Rakusova et al., 2011).

Like auxin, cytokinins are highly mobile molecules. However, in contrast to the well characterized transport machinery of auxin, the nature of cytokinin transport is less clear. Long-distance transport of cytokinin is supported by the discovery of cytokinins in xylem and phloem sap (Gillissen et al., 2000; Burkle et al., 2003; Bishopp et al., 2011a). In xylem sap, the major form of cytokinin is tZ-riboside (tZR) (Beveridge et al. 1997; Takei et al. 2001; Hirose et al. 2008), while in phloem sap iP-type cytokinins, such as iP-ribosides and iP-ribotides are detected (Corbesier et al. 2003; Hirose et al. 2008). Accordingly, grafting experiments between wild-type plants and cytokinin biosynthesis mutants showed preferential transport of different cytokinins; trans-zeatin tZ-type cytokinins were transported from the root to the shoot, while iP-type cytokinins moved from the shoot to the root (Matsumoto-Kitano et al., 2008). Thus, plants might use tZ- type as an acropetal messenger and iP-type cytokinins as basipetal messengers (Kudo et al., 2010). Recently, transport assays using radiolabeled cytokinins confirmed basipetal movement of cytokinin through the phloem and revealed that basipetal transport of cytokinin occurs through symplastic connections in the phloem (Bishopp et al. 2011b). Reverse genetics approaches applied to systematically characterize the ATP-binding cassette transporter proteins in *A. thaliana* yielded the identification of ABCG14 as a transporter involved in the long-

distance acropetal (root to shoot) translocation of the root-synthesized cytokinin. Plasma membrane-located ABCG14 is expressed primarily in the central cylinder of roots and loss of ABCG14 activity interferes with the translocation of tZ-type cytokinins from roots to shoots. *In planta* feeding of radiolabeled tZ suggests that ABCG14 acts as an efflux pump (Zhang et al., 2014).

Mechanisms of cytokinin uptake into cells have been studied using radiolabeled cytokinins in *Arabidopsis* cell cultures. Experiments predicted the presence of proton-coupled high-, medium-, and low affinity cytokinin transport systems (Burkle et al., 2003; Cedzich et al., 2008). So far, the equilibrative nucleoside transporter (ENT) family and the purine permease (PUP) family have been found to facilitate cytokinin transport (Burkle et al. 2003, Li et al., 2003; Hirose et al. 2005). Among *Arabidopsis* PUP family proteins (Gillissen et al., 2000), active uptake of free cytokinin bases and several adenine derivatives by PUP1 and PUP2 was demonstrated using a yeast system (Burkle et al., 2003). Expression of PUP2 in the phloem of *Arabidopsis* leaves suggested a role for PUP2 in phloem loading and unloading for long-distance transport of adenine and possibly cytokinins (Burkle et al., 2003). Among the plant ENT transporters, competitive uptake studies in yeast cells showed that *Arabidopsis* ENT3, ENT6, ENT7 and rice ENT2 can facilitate uptake of iP-riboside and tZ-riboside (Li et al., 2003; Hirose et al., 2005). Furthermore, mutants lacking either ENT3 or ENT8 exhibit reduced cytokinin uptake efficiency (Sun et al., 2005). Distinct expression patterns of *ENT* genes detected in root, leaf, and flower vasculature suggest that they may act differently during plant growth and development (Li et al., 2003; Sun at al., 2005; Hirose et al., 2008), however their function as cytokinin transporters *in planta* needs to be experimentally supported. In summary, in contrast to high substrate specificity of the auxin transport system, translocation of cytokinins *in planta* seems to be mediated through transporters with affinities to a broader spectrum of molecules such as purine derivatives and nucleosides.

4. Perception and signal transduction of auxin and cytokinin.

Solving the puzzle of auxin and cytokinin perception mechanism has been undoubtedly one of the biggest challenges of the last years. Establishment of the *Arabidopsis* genetic model has provided excellent tools to address this long standing question and it has been forward

genetic screens in *Arabidopsis* that have led to the identification of backbone elements of both auxin and cytokinin signal transduction cascades. Genetics in combination with advanced molecular and biochemical approaches enabled the achievement of a comprehensive view on the molecular principles of auxin and cytokinin perception and signal transduction.

Several independent forward genetic screens for mutants insensitive to auxin (Rouse et al., 1998; Ruegger et al., 1997, 1998) and expression profiling to isolate auxin inducible genes (Abel et al., 1995; Hagen and Guilfoyle 2002; Abel and Theologis 1996; Ulmasov et al., 1997) led to identification of all key molecular components required for auxin response such as TIR1 (encoding for F-box component of the E3 ubiquitin ligase $SCF^{TIR1/AFBs}$), the auxin early inducible *Aux/IAA* genes as well as the ARF transcription factors that recognise auxin response elements in the promoters of the *Aux/IAAs* (Gray et al., 1999; Abel and Theologis 2010). However, how these genes might constitute the pathway sensing and transducing hormonal signal was not obvious. Using advanced genetic and biochemical approaches the auxin signalling circuit has been resolved and TIR1 identified as the auxin receptor. It has been shown that auxin mediates interaction between TIR1/AFBs and Aux/IAA proteins which stimulates Aux/IAAs ubiquitination by $SCF^{TIR1/AFBs}$ E3-ubiquitin ligases for subsequent degradation by the proteasome. This leads to de-repression of ARFs, and transcriptional regulation of downstream response genes. At low auxin concentration, Aux/IAAs form a complex with ARF transcription factors and the transcriptional corepressor TOPLESS (TPL), thus preventing the ARFs from regulating target genes (Gray et al., 2001; Dharmasiri et al., 2005a,b; Kepinski and Leyser 2005; Tan et al., 2007; Szemenyei et al., 2008) (Figue 2b).

Although the framework which outlines the core molecular mechanism of auxin perception and signal transduction has been recognised, the question as to how *TIR1/AFB*, *Aux/IAAs* and *ARF* families, each comprising many homologous members, mediate specific developmental output remains to be answered. As indicated by recent studies, multiple levels of control appear to exist, including spatio-temporal specific expression of individual auxin signalling pathway components (Overvoorde et al., 2005; Okushima et al., 2005), as well as differences in affinities of the TIR1/AFB auxin receptors for the Aux/IAA repressors (Calderón-Villalobos et al., 2012; Moss et al., 2015), of Aux/IAA repressors for the ARFs transcription factors (Vernoux et al., 2011; Lee et al., 2014; Korasick et al., 2014; Nanao et al., 2014;

Shimizu-Mitao and Kakimoto, 2014), and of ARFs for their binding motifs in promoters of the target genes (Boer et al., 2014), which may allow fine-tuning of auxin responses.

After a period of biochemical attempts in the early 1970s to identify the cytokinin receptors, the forward genetic screens turned out to be successful strategies. In a screen of the activation tagged *Arabidopsis* mutants for cytokinin independent growth, the sensor histidine kinase CKI1 was recovered. This finding suggested that the multi-step phosphorelay similar to bacterial two-component signalling system might underlie the cytokinin signal transduction (Kakimoto, 1996). Another screen for cytokinin insensitive mutants led to identification of the *CRE1* (*CYTOKININ RESISTANT 1*) encoding a sensor histidine kinase related to CKI1 (Inoue et al., 2001). At about the same time, the *WOODEN LEG (WOL)* mutant allele of the AHK4/CRE1 gene (exhibiting severe defects in the vasculature differentiation; Mähönen et al., 2000) was identified, along with the AHK2 and AHK3 homologues required for cytokinin response (Hwang and Sheen et al., 2001; Ueguchi et al 2001; Higuchi et al., 2004; Nishimura et al., 2004). Elegant experiments in yeast and bacteria provided first evidence that CRE1/AHK4 functions as a cytokinin receptor (Inoue et al., 2001; Ueguchi et al., 2001, Suzuki et al., 2001); later corroborated by direct binding assays with radiolabeled cytokinins (Romanov et al., 2005, 2006; Stolz et al., 2011).

Subsequent studies focusing on the downstream signaling cascade revealed that genes with high similarity to molecular elements of the multi-step phosphorelay pathway including sensor histidine kinases (AHKs), histidine phosphotransfer proteins (AHPs) and response regulators (ARRs) are present in the *Arabidopsis* genome (Mizuno, 2005; Schaller et al., 2008). Genetic and biochemical characterization of their functions in the cytokinin response yielded the current model of the cytokinin signalling pathway. In brief, a cascade of auto- and transphosphorylation events triggered by cytokinin leads to activation of AHK receptors and transduction of the signal to downstream components. Downstream of the AHK receptors, the AHPs continuously translocate between cytosol and nucleus to mediate signalling by activating type-B ARABIDOPSIS RESPONSE REGULATORS (ARRs), transcription factors which then trigger the transcription of specific genes. A negative feed-back loop is provided by type-A ARRs, which inhibit the activity of type-B ARRs by an unknown mechanism (Hwang and Sheen, 2001; Sakai et al., 2001; Mason et al., 2005; Hutchison et al., 2006; To et al., 2007, Argyros et al., 2008; Kieber and Schaller, 2014). Furthermore, a family of F-box proteins, called

the KISS ME DEADLY (KMD) family, targets type-B ARR proteins for degradation and attenuates cytokinin pathway activity (Kim et al., 2013) (Figure 3). The large majority of cytokinin receptors localize to the ER, suggesting a central role of this compartment in cytokinin signaling (Caesar et al., 2011; Wulfetange et al., 2011); nevertheless, a small part of the cytokinin receptors might perceive a signal from the plasma membrane (Wulfetange et al., 2011).

Recently, a set of cytokinin-regulated transcription factors named cytokinin response factors (CRFs) have been described as a potential branch emerging from the classical multi-step phosphorelay parallel to that of type-B ARRs (Rashotte et al., 2006). CRFs are members of the AP2/EREBP family of transcription factors, containing a single AP2–DNA binding domain, distinct from both DREB and AP2 proteins. There are eight members of CRF family in *Arabidopsis* (CRF1-CRF8) with CRF7 and CRF8 being atypical as they lack C-terminal extensions (Sakuma et al., 2002; Nakano et al., 2006; Rashotte and Goertzen, 2010). The transcript abundance of certain *CRFs* (*CRF2*, CRF5 and *CRF6*) is rapidly upregulated by cytokinin (Rashotte et al., 2006). Protein-protein interaction analysis indicated that CRFs are able to interact with each other to form homo- and/or heterodimers as well as with components of the classical cytokinin signaling pathway. Transcriptome analysis has revealed a large overlap in CRFs and type B ARR targets, pointing at a close link between both branches of the cytokinin signaling pathway.

However, how the specificity of cytokinin response is achieved by the signalling cascade, where each step is supported by a gene family comprising several members, awaits further investigation.

Importantly, elucidation of the molecular elements and mechanistic principles of auxin and cytokinin transduction pathways has enabled the development of specific sensors for monitoring auxin and cytokinin *in planta*. Nowadays, highly sensitive reporters such as *DR5* (Ulmasov et al., 1997); DII-VENUS (Band et al., 2012; Brunoud et al., 2012), and *TCS* (Müller and Sheen; 2008) are extensively used for mapping auxin and cytokinin activities, respectively, and demonstrate a great potential of these tools for better understanding of the roles of auxin and cytokinin in plant development.

5. Auxin and cytokinin interaction in regulation of plant development.

Since the initial discovery of auxin and cytokinin, the number of reports supporting their regulatory role in various aspects of plant development has accumulated. Moreover, studies of auxin and cytokinin function in plant cell suspension growth provided the first evidence of hormonal interaction and its role in directing plant development. The experiments of Skoog and Miller (1957) demonstrated that both auxin and cytokinin are not only required to induce and maintain cell division and growth in plant tissue culture, but that the auxin:cytokinin ratio determines distinct organogenic pathways. A high ratio of cytokinin to auxin stimulated formation of shoots, whereas a low ratio induced root regeneration. Tight communication between auxin and cytokinin is crucial for proper establishment of meristems in early embryogenesis (Muller and Sheen, 2008; Su et al., 2011), ovule development (Bencivenga et al., 2012), shoot apical meristem activity and phylotaxis (Reinhardt et al., 2003; Werner et al., 2003; Leibfried et al., 2005; Zhao et al., 2010), shoot and root branching (Domagalska and Leyser, 2011; Laskowski et al., 1995, 2008; Laplaze et al., 2007; Bielach, et al., 2012; Marhavý et al., 2011; 2014), root growth and meristem maintenance (Dello Ioio et al., 2008). Hence the deciphering of molecular and mechanistic bases of auxin and cytokinin interaction became one of the major themes in plant biology. Over the years, research on developmental processes in plants has uncovered genes and networks, giving first insights into molecular mechanisms of auxin and cytokinin cross-talk in the context of these complex developmental programs. Here, a few examples of auxin-cytokinin crosstalk mechanisms and their relevance in coordination of specific developmental processes are discussed.

It has been shown that specification of the root pole during the early phases of embryogenesis is dependent on the tightly balanced activity of auxin and cytokinin. Auxin was found to stimulate expression of the cytokinin signaling repressors *ARR7* and *ARR15* and thus to attenuate the output of the cytokinin pathway. Lack of this auxin-driven negative feedback loop resulted in the up-regulation of the cytokinin response and severe patterning defects at the embryonic root pole (Müller & Sheen, 2008). Interestingly, recent observations hint at another auxin-cytokinin regulatory module acting in the early embryogenesis. Among the transcriptional targets of AUXIN RESPONSE FACTOR (ARF5/MP), previously linked with embryonic root specification (Hardtke and Berleth, 1998; Hamann et al., 2002), TARGET OF MONOPTEROS (TMO3), coding for the CRF2 was identified (Schlereth et al., 2010). Expression of CRF2 and homologous genes is cytokinin responsive and interference with their functions leads to severe

embryonic defects (Rashotte et al., 2006). Furthermore, two auxin efflux transporters (*PIN1* and *PIN7*), both shown to control distribution of auxin during early embryogenesis (Friml et al., 2003), were identified as CRF2 transcriptional targets (Šimášková et al., 2015). However, how these two regulatory circuits jointly coordinate early embryogenesis requires further investigation.

Auxin and cytokinin act in an antagonistic manner to define the root apical meristem size by promoting cell division and differentiation, respectively (Dello Ioio et al., 2007, Růžička et al., 2009). A complex network of auxin and cytokinin interactions has been implicated in the root meristem activity control. Cytokinin modulates the auxin pathway by affecting the expression of its signaling components. Cytokinin (through the AHK3 receptor and ARR1 and ARR12 response regulators) was shown to directly activate transcription of the auxin repressor *IAA3/SHORT HYPOCOTYL 2* (*SHY2*). This leads to the attenuation of auxin responses and reduced expression of *PIN* auxin efflux transporters (Vieten et al., 2005; Dello Ioio et al., 2008, Pernisová et al., 2009, Růžička et al., 2009). Consequently, a decreased abundance of PINs limits the auxin supply to the root apical meristem, thereby restricting its meristematic activity (Dello Ioio et al., 2008; Růžička et al., 2009). Besides this transcription-based regulation of auxin activity and distribution, cytokinin was also found to modulate the endocytic trafficking of PIN1 by redirecting this membrane protein for lytic degradation in the vacuoles. (Zhang et al., 2011; Marhavý et al., 2011). This alternative mode of cytokinin action provides a mechanism for rapid control of auxin fluxes; and as recently suggested, the enhanced depletion of PIN1 at specific polar domains by cytokinin might also modulate direction of the auxin flow (Marhavý et al., 2014).

Another mechanism through which auxin and cytokinin balance each other's activities occurs by a crosstalk between their metabolic pathways. High cytokinin levels promote auxin biosynthesis (Jones et al., 2010) and auxin, in turn, gives feedback on the cytokinin metabolism by inducing CYTOKININ OXIDASE (CKX) thereby decreasing cytokinin levels (Eklöf et al., 1997, Nordström et al., 2004; Carabelli et al., 2007). On the other hand, in the root apical meristem, auxin enhances (in an IAA3/SHY2 dependent manner) the expression of ISOPENTENYL TRANSFERASE5 (IPT5), which encodes a rate limiting enzyme in the cytokinin biosynthesis, eventually resulting in the local up-regulation of cytokinin levels (Dello Ioio et al., 2008, Miyawaki et al., 2004).

Both auxin and cytokinin exhibit specific functions in the shoot apical meristem. High cytokinin promotes proliferation of undifferentiated cells, whereas auxin coordinates organogenesis in the peripheral zone (Schaller et al., 2015). Cytokinin participates in the WUSCHEL/WUS-CLAVATA/CLV, the core regulatory loop controlling shoot apical meristem activity, by stimulating WUS expression (Gordon et al., 2009). By direct repression of the ARR7 and ARR15 cytokinin signaling repressors, WUS further reinforces the cytokinin promoting effect on the WUS-mediated pathway (Leibfried et al., 2005). An important additional input in this cytokinin-driven regulation is provided by auxin. In mutants defective in auxin biosynthesis, transport and signaling, expression of *ARR7* and *ARR15* was found to be enhanced, and the *ARF5/MP* transcription factor was identified as a direct repressor of their transcription (Zhao et al., 2010). This constitutes a regulatory circuit in which auxin enhances cytokinin response by attenuating the expression of the cytokinin signaling repressors, and consequently promoting WUS activity in the WUS-CLV loop.

At the peripheral zone of the short apical meristem, new organ formation is triggered by auxin (Reinhardt et al., 2003). Studies following pathways regulated by auxin transport and response revealed that initiation of the lateral organs is accompanied by modulations in the polarity of PIN1 and redirection of the auxin towards incipient primordia (Heisler et al., 2005). The accumulation of auxin correlates with a decrease in *SHOOT MERISTEMLESS* (*STM*) expression, which eventually results in lower cytokinin at the peripheral zone (Hamant et al., 2002). How PIN1 polarization throughout the shoot apical meristem is coordinated and whether cytokinin contributes to the regulation of polar auxin transport through mechanisms analogous to these detected in root is unknown. Nevertheless, a reduced level of PIN1 in the maize *ARR* repressor ortholog mutant *abphyl 1* supports such a scenario (Lee et al., 2009). Recently, Besnard et al. (2014) provide further evidence for cytokinin function in the peripheral zone and coordination of lateral organ initiation. Analysis of *AHP6* expression patterns along with monitoring of auxin and cytokinin sensitive reporters indicates that AHP6, which acts as a repressor of cytokinin signalling (Mähönen et al., 2006), regulates the spatiotemporal pattern of cytokinin activity at the shoot apical meristem periphery. The cytokinin inhibitory fields generated downstream of auxin by AHP6 might stabilize auxin fields, thereby increasing robustness of the phyllotactic patterning (Besnard et al., 2014).

Studies of auxin-cytokinin cross-talk directing other developmental process (including initiation and organogenesis of ovules; vasculature differentiation, shoot and root meristem activity and lateral branching (reviewed in Moubayidin et al., 2009; Depuydt and Hardtke 2011; Schaller et al., 2015) point towards specific as well as common aspects of mechanisms mediating mutual communication between these two hormonal pathways.

With increasing amounts of confirmed molecular interactions and circuits that determine hormone activity at the level of metabolism, transport, perception, and signaling, the prediction of hormone regulatory network behavior and output becomes unfeasible Modelling and mathematical simulations provide a novel means to address these issues and help to achieve better understanding of the complexity and dynamics of hormone action (Voß et al., 2014). .

For example, studies of the transcription factor *PHABULOSA* (*PHB*) and cytokinin in controlling the root meristem size showed that cytokinin regulates *microRNA165/166* and that both cytokinin and *microRNA165/166* jointly regulate *PHB*. In return, PHB promotes cytokinin biosynthesis by stimulation *IPT7* expression (Dello Ioio et al., 2012). One-dimensional model and mathematical simulations provided insights into the functioning of such a complicated molecular network, showing that this regulatory loop restrains the reduction and accelerates the recovery of PHB levels thus providing robustness against cytokinin fluctuations (Dello Ioio et al., 2012).

A combination of experimental and modelling approaches has also been applied to integrate auxin and cytokinin pathways in the specification of vascular patterning. A two-dimensional multicellular model of Muraro et al., 2014 incorporated previous findings of a mutually inhibitory interaction between auxin and cytokinin, mediated through the auxin inducible repressor of the cytokinin signaling AHP6; cytokinin feedback on the PIN auxin efflux carriers and SHORT ROOT (SHR) promoted expression of the mobile *microRNA165/166* which silences *PHB* to form a gradient of *PHB* mRNA that controls the specification of xylem and inhibits *AHP6* expression (Bishopp et al., 2011b; Carlsbecker et al., 2010). Mathematical simulations revealed that this gene regulatory network is not sufficient to establish proper expression patterns of key marker genes as observed experimentally, and predicted additional negative regulators of cytokinin signaling and the mutual degradation of both *microRNA165*/6 and *PHB* mRNA (Muraro et al., 2014).

A genetic network tested in the model simulation of De Rybel et al., 2014 integrated two incoherent feed-forward loops and evaluated their impact on the patterning of vascular tissues. One of the feed-forward loops implements auxin-cytokinin antagonistic regulations of PIN mediated auxin efflux (Bishopp et al., 2011b; Mähönen et al., 2006). A second loop is based on the experimental identification of interaction between MONOPTEROS/ARF5 and TARGET OF MONOPTEROS5 /LONESOME HIGHWAY (TMO5)/LHW) and LONELY GUY4 (LOG4) which mediates auxin-dependent control of the cytokinin biosynthesis (De Rybel et al., 2013). The authors show that the individual subnetworks provide specific regulatory inputs, one generating a high-auxin domain whereas a second defines sharp boundaries between the high auxin domain and the neighboring cytokinin response domain. Integration of both regulatory circuits is sufficient to generate distinct hormonal zones and establishment of stable patterns within a vascular tissue (De Rybel et al., 2014).

Conclusion

History of auxin and cytokinin from the initial discoveries by brothers Darwin's (1880) and Gottlieb Haberlandt (1919) is a beautiful demonstration of unceasing continuity of research. Novel findings are integrated into existing hypotheses and models and deepen our understanding of biological principles. At the same time new questions are triggered and hand to hand with this new methodologies are developed to address these new challenges.

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References

Abel, S., Nguyen, M.D., Theologis, A. (1995) The PS-IAA4/5-like family of early auxin-inducible mRNAs in *Arabidopsis thaliana*. *J Mol Biol* 251, 533-49.

Abel, S., and Theologis, A. (1996) Early genes and auxin action. *Plant Physiol* 111, 9-17.

Abel, S., and Theologis, A. (2010) Odyssey of auxin. *Cold Spring Harb Perspect Biol* 2, a004572.

Adamowski, M., Friml, J. (2015) PIN-dependent auxin transport, action, regulation, and evolution. *Plant Cell* 27, 20-32.

Akiyoshi, D.E., Klee, H., Amasino, R.M., Nester, E.W., Gordon, M.P. (1984) T-DNA of *Agrobacterium tumefaciens* encodes an enzyme of cytokinin biosynthesis. *Proc Natl Acad Sci USA* 81, 5994-98.

Argyros, R.D., Mathews, D.E., Chiang, Y.H., Palmer, C.M., Thibault, D.M., Etheridge, N., Argyros, D.A., Mason, M.G., Kieber, J.J., and Schaller, G.E. (2008) Type B response regulators of *Arabidopsis* play key roles in cytokinin signaling and plant development. *Plant Cell* 20, 2102-2116.

Bainbridge, K., Guyomarc'h, S., Bayer, E., Swarup, R., Bennett, M., Mandel, T., and Kuhlemeier, C. (2008) Auxin influx carriers stabilize phyllotactic patterning. *Genes Dev* 22, 810-823.

Band, L.R., Wells, D.M., Larrieu, A., Sun, J., Middleton, A.M., French, A.P., Brunoud, G., Sato, E.M., Wilson, M.H., Péret, 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., Bennett, M.J. (2012). Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. *Proc Natl Acad Sci USA* 109, 4668-4673.

Barbez, E., Kubeš M, Rolčík J, Béziat C, Pěnčík A, Wang B, Rosquete MR, Zhu J, Dobrev PI, Lee Y, Zažímalovà E, Petrášek J, Geisler M, Friml J, Kleine-Vehn J. (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* 485, 119–122.

Barbez, E., Lanková, M., Parezová, M., Maizel, A., Zazímalová, E., Petrášek, J., Friml, J., and Kleine-Vehn, J. (2013). Single-cellbased system to monitor carrier driven cellular auxin homeostasis. *BMC Plant Biol* 13, 20.

Bartel, B. (1997) Auxin Biosynthesis. Annu Rev Plant Physiol Plant Mol Biol. 48, 51–66.

Bencivenga, S., Simonini, S., Benková, E., Colombo, L. (2012) The transcription factors BEL1 and SPL are required for cytokinin and auxin signaling during ovule development in *Arabidopsis*. *Plant Cell* 24, 2886-97.

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, M.J., Marchant, A., Green, H.G., May, S.T., Ward, S.P., Millner, P.A., Walker, A.R., Schulz, B., and Feldmann, K.A. (1996) *Arabidopsis AUX1* gene, a permease-like regulator of root gravitropism. *Science* 273, 948-950.

Beveridge, C.A., Murfet, I.C., Kerhoas, L., Sotta, B., Miginiac, E., and Rameau, C. (1997) The shoot controls zeatin riboside export from pea roots. Evidence from the branching mutant *rms4*. *Plant J* 11, 339-345.

Besnard, F., Refahi, Y., Morin, V., Marteaux, B., Brunoud, G., Chambrier, P., Rozier, F., Mirabet, V., Legrand, J., Lainé, S., Thévenon, E., Farcot, E., Cellier, C., Das, P., Bishopp, A., Dumas, R., Parcy, F., Helariutta, Y., Boudaoud, A., Godin, C., Traas, J., Guédon, Y., Vernoux, T. (2014) Cytokinin signalling inhibitory fields provide robustness to phyllotaxis. *Nature* 16, 417-421.

Bielach, A., Podlešáková, K., Marhavý, P., Duclercq, J., Cuesta, C., Müller, B., Grunewald, W., Tarkowski, P., and Benková, E. (2012). Spatiotemporal regulation of lateral root organogenesis in *Arabidopsis* by cytokinin. *Plant Cell* 24, 3967-3981.

Bishopp, A., Benkova, E., and Helariutta, Y. (2011a) Sending mixed messages, auxin-cytokinin crosstalk in roots. *Curr Opin Plant Biol* 14, 10-16.

Bishopp, A., Lehesranta, S., Vaten, A., Help, H., El-Showk, S., Scheres, B., Helariutta, K., Mahonen, A.P., Sakakibara, H., and Helariutta, Y. (2011b) Phloem-transported cytokinin regulates polar auxin transport and maintains vascular pattern in the root meristem. *Curr Biol* 21, 927-932.

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., López-Vidrieo, I., Franco-Zorrilla, J.M., de Vries, S.C., Solano, R., Weijers, D., Coll, M. (2014) Structural basis for DNA binding specificity by the auxin-dependent ARF transcription factors. *Cell* 156, 577-89.

Boysen-Jensen, P. (1911) La transmission de l'irritation phototropique dans l'avena. *Bulletin Academie Des Sciences et Lettres de Montpellier* 3, 1–24.

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

Burkle, L., Cedzich, A., Dopke, C., Stransky, H., Okumoto, S., Gillissen, B., Kuhn, C., and Frommer, W.B. (2003) Transport of cytokinins mediated by purine transporters of the PUP family expressed in phloem, hydathodes, and pollen of Arabidopsis. *Plant J* 34, 13-26.

Caesar, K., Thamm, A.M., Witthoft, J., Elgass, K., Huppenberger, P., Grefen, C., Horak, J., and Harter, K. (2011) Evidence for the localization of the *Arabidopsis* cytokinin receptors AHK3 and AHK4 in the endoplasmic reticulum. *J Exp Bot* 62, 5571-5580.

Calderón Villalobos, L.I., Lee, S., De Oliveira, C., Ivetac, A., Brandt, W., Armitage, L., Sheard, L.B., Tan, X., Parry, G., Mao, H., Zheng, N., Napier, R., Kepinski, S., Estelle, M. (2012) A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. *Nature Chemical Biology* 8, 477-485.

Carabelli, M., Possenti, M., Sessa, G., Ciolfi, A., Sassi, M., Morelli, G., Ruberti, I. (2007) Canopy shade causes a rapid and transient arrest in leaf development through auxin-induced cytokinin oxidase activity. *Genes & Development* 21, 1863-1868.

Carlsbecker, A., Lee, J.Y., Robertsm, C.J., Dettmer, J., Lehesranta, S., Zhou, J., Lindgren, O., Moreno-Risueno, M.A., Vatén, A., Thitamadee, S., Campilho, A., Sebastian, J., Bowman, J.L., Helariutta, Y., Benfey, P.N. (2010) Cell signalling by *microRNA165/6* directs gene dose-dependent root cell fate. *Nature* 465, 316-321.

Cedzich, A., Stransky, H., Schulz, B., Frommer, W.B. (2008) Characterization of cytokinin and adenine transport in *Arabidopsis* cell cultures. *Plant Physiol* 148, 1857-1867.

Chen CM, Kristopeit SM (1981a) Metabolism of cytokinin, dephosphorylation of cytokinin ribonucleotide by 5'-nucleotidases from wheat germ cytosol. *Plant Physiol* 67, 494-498.

Chen CM, Kristopeit SM (1981b) Metabolism of cytokinin, deribosylation of cytokinin ribonucleoside by adenine nucleosidase from wheat germ cells. *Plant Physiol* 68, 1020-1023.

Cheng, Y., Dai, X., and Zhao, Y. (2006) Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. *Genes Dev* 20, 1790-1799.

Cheng, Y., Dai, X., and Zhao, Y. (2007) Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in *Arabidopsis*. *Plant Cell* 19, 2430-2439.

Chickarmane, V.S., Gordon, S.P., Tarr, P.T., Heisler, M.G., and Meyerowitz, E.M. (2012) Cytokinin signaling as a positional cue for patterning the apical–basal axis of the growing *Arabidopsis* shoot meristem. *Proc Natl Acad Sci USA* 109, 4002-4007.

Cho, M., Lee, S.H., and Cho, H.T. (2007) P-glycoprotein4 displays auxin efflux transporter-like action in *Arabidopsis* root hair cells and tobacco cells. *Plant Cell* 19, 3930-3943.

Cholodny, N. (1927) Wuchshormone und Tropismen bei den Pflanzen. *Biologisches Zentralblatt* 47, 604-626.

Cholodny, N. (1928) Beiträge zur hormonalen Theorie von Tropismen. *Planta* 6, 118-134.

Corbesier, L., Prinsen, E., Jacqmard, A., Lejeune, P., Van Onckelen, H., Périlleux, C., and Bernier, G. (2003) Cytokinin levels in leaves, leaf exudate and shoot apical meristem of *Arabidopsis thaliana* during floral transition. *J Exp Bot* 54, 2511-2517.

Cui, D., Zhao, J., Jing, Y., Fan, M., Liu, J., Wang, Z., Xin, W., Hu, Y. (2013) The *Arabidopsis* IDD14, IDD15, and IDD16 cooperatively regulate lateral organ morphogenesis and gravitropism by promoting auxin biosynthesis and transport. *PLoS Genet* 9, e1003759.

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

Dello Ioio, R., Galinha, C., Fletcher, A.G., Grigg, S.P., Molnar, A., Willemsen, V., Scheres, B., Sabatini, S., Baulcombe, D., Maini, P.K., Tsiantis, M. (2012) A PHABULOSA/cytokinin feedback loop controls root growth in *Arabidopsis*. *Curr Biol* 22, 1699-1704.

Dello Ioio, R., Linhares, F.S., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Costantino, P., and Sabatini, S. (2007) Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Curr Biol* 17, 678-682.

Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M.T., Aoyama, T., Costantino, P., and Sabatini, S. (2008) A genetic framework for the control of cell division and differentiation in the root meristem. *Science* 322, 1380-1384.

De Rybel, B., Adibi, M., Breda, A.S., Wendrich, J.R., Smit, M.E., Novák, O., Yamaguchi, N., Yoshida, S., Van Isterdael, G., Palovaara, J., Nijsse, B., Boekschoten, M.V., Hooiveld, G., Beeckman, T., Wagner, D., Ljung, K., Fleck, C., Weijers, D. (2014) Plant development. Integration of growth and patterning during vascular tissue formation in Arabidopsis. *Science* 345, 1255215.

De Rybel, B., Möller, B., Yoshida, S., Grabowicz, I., Barbier de Reuille, P., Boeren, S., Smith, R.S., Borst, J.W., Weijers, D. (2013) A bHLH complex controls embryonic vascular tissue establishment and indeterminate growth in *Arabidopsis*. *Dev Cell* 24, 426-437.

Depuydt, S., and Hardtke, C.S. (2011) Hormone signalling crosstalk in plant growth regulation. *Curr Biol* 21, R365-373.

De Smet, I., Tetsumura, T., De Rybel, B., Frey, N.F., Laplaze, L., Casimiro, I., Swarup, R., Naudts, M., Vanneste, S., Audenaert, D., Inze, D., Bennett, M.J., and Beeckman, T. (2007) Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* 134, 681-690.

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.

Ding, Z., Galván-Ampudia, C.S., Demarsy, E., Łangowski, Ł., 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.

Ding, Z., Wang, B., Moreno, I., Dupláková, N., Simon, S., Carraro, N., Reemmer, J., Pěnčík, A., Chen, X., Tejos, R., Skůpa, P., Pollmann, S., Mravec, J., Petrášek, J., Zažímalová, E., Honys, D., Rolčík, J., Murphy, A., Orellana, A., Geisler, M., Friml, J. (2012) ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in *Arabidopsis*. *Nat Commun* 3, 941.

Domagalska, M.A., and Leyser, O. (2011) Signal integration in the control of shoot branching. *Nat Rev Mol Cell Biol* 12, 211-221.

Dubrovsky, J.G., Sauer, M., Napsucialy-Mendivil, S., Ivanchenko, M.G., Friml, J., Shishkova. S., Celenza, J., Benková, E. (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proc Natl Acad Sci USA* 105, 8790-8794.

Du Monceau, D.,1758. La physique des arbres, Vol. I. Paris, HL Guérin & LF Delatour.

Eklöf, S., Åstot, C., Blackwell, J., Moritz, T., Olsson, O., Sandberg, G. 1997. Auxin-cytokinin interactions in wild-type and transgenic tobacco. *Plant and Cell Physiology* 38, 225-235.

Eklund, D.M., Ståldal, V., Valsecchi, I., Cierlik, I., Eriksson, C., Hiratsu, K., Ohme-Takagi, M., Sundström, J.F., Thelander, M., Ezcurra, I., Sundberg, E. (2010) The *Arabidopsis thaliana* STYLISH1 protein acts as a transcriptional activator regulating auxin biosynthesis. *Plant Cell* 22, 349-363.

Feraru, E., Feraru, M.I., Kleine-Vehn, J., Martinière, A., Mouille, G., Vanneste, S., Vernhettes, S., Runions, J., Friml J. (2011) PIN polarity maintenance by the cell wall in *Arabidopsis*. *Curr Biol* 21, 338-343.

Feraru, E., Paciorek, T., Feraru, M.I., Zwiewka, M., De Groodt, R., De Rycke, R., Kleine-Vehn, J., and Friml, J. (2010) The AP-3 b adaptin mediates the biogenesis and function of lytic vacuoles in *Arabidopsis*. *Plant Cell* 22, 2812–2824.

Franklin, K.A., Lee, S.H., Patel, D., Kumar, S.V., Spartz, A.K., Gu, C., Ye, S., Yu, P., Breen, G., Cohen, J.D., Wigge, P.A., Gray, W.M. (2011) Phytochrome-interacting factor 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proc Nat Acad Sci USA* 108, 20231-20235.

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

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., 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.

Fukaki, H., Tameda, S., Masuda, H., and Tasaka, M. (2002) Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of *Arabidopsis*. *Plant J* 29, 153-168.

Gälweiler, L., Guan, C., Muller, A., Wisman, E., Mendgen, K., Yephremov, A., and Palme, K. (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* 282, 2226-2230.

Geisler, M., Blakeslee, J.J., Bouchard, R., Lee, O.R., Vincenzetti, V., Bandyopadhyay, A., Titapiwatanakun, B., Peer, W.A., Bailly, A., Richards, E.L., Ejendal, K.F., Smith, A.P., Baroux, C., Grossniklaus, U., Muller, A., Hrycyna, C.A., Dudler, R., Murphy, A.S., and Martinoia, E. (2005) Cellular efflux of auxin catalyzed by the *Arabidopsis* MDR/PGP transporter AtPGP1. *Plant J* 44, 179-194.

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.

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.

Gillissen, B., Burkle, L., Andre, B., Kuhn, C., Rentsch, D., Brandl, B., and Frommer, W.B. (2000) A new family of high-affinity transporters for adenine, cytosine, and purine derivatives in *Arabidopsis*. *Plant Cell* 12, 291-300.

Gordon, S.P., Chickarmane, V.S., Ohno, C., Meyerowitz, E.M. (2009) Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. *Proc Natl Acad Sci USA* 106, 16529-16534.

Gray, W.M., del Pozo, J.C., Walker, L., Hobbie, L., Risseeuw, E., Banks, T., Crosby, W.L., Yang, M., Ma, H., and Estelle, M. (1999) Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes Dev* 13, 1678-1691.

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.

Haagen-Smit, A.J., Dandliker, W.B., Wittwer, S.H., Murneek, A.E. (1946) Isolation of 3-indoleacetic acid from immature corn kernels. *Am J Bot* 33, 118–120.

Haberlandt, G. (1913) Zur physiologie der zellteilung. *Sitzungsber Akad Wiss Berlin Phys Math C* 1, 318-345.

Hagen, G., and Guilfoyle, T. (2002) Auxin-responsive gene expression, genes, promoters and regulatory factors. *Plant Mol Biol* 49, 373-385.

Hall, R.H., De Ropp, R.S. (1955) Formation of 6-furfurylaminopurine from DNA breakdown products. *J Am Chem Soc* 77, 6400.

Hamann, T., Benkova, E., Baurle, I., Kientz, M., and Jurgens, G. (2002) The *Arabidopsis* BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes Dev* 16, 1610-1615.

Hamant, O., Nogué, F., Belles-Boix, E., Jublot, D., Grandjean, O., Traas, J., Pautot, V. (2002) The KNAT2 homeodomain protein interacts with ethylene and cytokinin signaling. *Plant Physiology* 130, 657-665.

Hardtke, C.S., Berleth, T. (1998) The *Arabidopsis* gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J* 17, 1405-1411.

He, W., Brumos, J., Li, H., Ji, Y., Ke, M., Gong, X., Zeng, Q., Li, W., Zhang, X., An, F., Wen, X., Li, P., Chu, J., Sun, X., Yan, C., Yan, N., Xie, D.Y., Raikhel, N., Yang, Z., Stepanova, A.N., Alonso, J.M., Guo, H. (2011) A small-molecule screen identifies L-kynurenine as a competitive inhibitor of TAA1/TAR activity in ethylene-directed auxin biosynthesis and root growth in *Arabidopsis*. *Plant Cell* 23, 3944–3960.

Heisler, M.G., Hamant, O., Krupinski, P., Uyttewaal, M., Ohno, C., Jönsson, H., Traas, J., 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.

Heisler, M.G., Ohno, C., Das, P., Sieber, P., Reddy, G.V., Long, J.A., and Meyerowitz, E.M. (2005) Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the *Arabidopsis* inflorescence meristem. *Curr Biol* 15, 1899-1911.

Hejátko, J., Ryu, H., Kim, G.T., Dobesová, R., Choi, S., Choi, S.M., Soucek, P., Horák, J., Pekárová, B., Palme, K., Brzobohaty, B., and Hwang, I. (2009) The histidine kinases CYTOKININ-INDEPENDENT1 and ARABIDOPSIS HISTIDINE KINASE2 and 3 regulate vascular tissue development in *Arabidopsis* shoots. *Plant Cell* 21, 2008-2021.

Higuchi, M., Pischke, M.S., Mahonen, A.P., Miyawaki, K., Hashimoto, Y., Seki, M., Kobayashi, M., Shinozaki, K., Kato, T., Tabata, S., Helariutta, Y., Sussman, M.R., and Kakimoto, T. (2004) In planta functions of the *Arabidopsis* cytokinin receptor family. *Proc Natl Acad Sci USA* 101, 8821-8826.

Hirose, N., Makita, N., Yamaya, T., Sakakibara, H. (2005) Functional characterization and expression analysis of a gene, OsENT2, encoding an equilibrative nucleoside transporter in rice suggest a function in cytokinin transport. *Plant Physiol* 138, 196-206.

Hirose, N., Takei, K., Kuroha, T., Kamada-Nobusada, T., Hayashi, H., and Sakakibara, H. (2008) Regulation of cytokinin biosynthesis, compartmentalization and translocation. *J Exp Bot* 59, 75-83.

Hou, B., Lim, E.K., Higgins, G., and Bowles, D.J. (2004) N-Glucosylation of cytokinins by glycosyltransferases of *Arabidopsis thaliana*. *J Biol Chem* 279, 47822-47832.

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.

Hutchison, C.E., Li, J., Argueso, C., Gonzalez, M., Lee, E., Lewis, M.W., Maxwell, B.B., Perdue, T.D., Schaller, G.E., Alonso, J.M., Ecker, J.R., and Kieber, J.J. (2006) The Arabidopsis histidine phosphotransfer proteins are redundant positive regulators of cytokinin signaling. *Plant Cell* 18, 3073-3087.

Hwang, I., and Sheen, J. (2001) Two-component circuitry in *Arabidopsis* cytokinin signal transduction. *Nature* 413, 383-389.

Inoue, T., Higuchi, M., Hashimoto, Y., Seki, M., Kobayashi, M., Kato, T., Tabata, S., Shinozaki, K., and Kakimoto, T. (2001) Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. *Nature* 409, 1060-1063.

Jablonski, J.R., and Skoog, F. (1954) Cell enlargement and cell division in excised tobacco pith tissue. *Physiol Plant* 7,16-24.

Jones, A.R., Kramer, E.M., Knox, K., Swarup, R., Bennett, M.J., Lazarus, C.M., Leyser, H.M., and Grierson, C.S. (2009) Auxin transport through non-hair cells sustains root-hair development. *Nat Cell Biol* 11, 78-84.

Jones, B., Gunneras, S.A., Petersson, S.V., Tarkowski, P., Graham, N., May, S., Dolezal, K., Sandberg, G., and Ljung, K. (2010) Cytokinin regulation of auxin synthesis in *Arabidopsis* involves a homeostatic feedback loop regulated via auxin and cytokinin signal transduction. *Plant Cell* 22, 2956-2969.

Kakimoto, T. (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. *Science* 274, 982-985.

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

Kieber, J.J. and Schaller G.E.(2014) Cytokinins. The Arabidopsis Book, e0168.

Kim, H.J., Chiang, Y.H., Kieber, J.J., and Schaller, G.E. (2013) SCF(KMD) controls cytokinin signaling by regulating the degradation of type-B response regulators. *Proc Natl Acad Sci USA* 110, 10028-10033.

Kitakura, S., Vanneste, S., Robert, S., Löfke, C., Teichmann, T., Tanaka, H., and Friml, J. (2011) Clathrin mediates endocytosis and polar distribution of PIN auxin transporters in *Arabidopsis*. *Plant Cell* 23, 1920-1931.

Kleine-Vehn, J., Dhonukshe, P., Sauer, M., Brewer, P.B., Wisniewska, J., Paciorek, T., Benkova, E., and Friml, J. (2008) ARF GEF-dependent transcytosis and polar delivery of PIN auxin carriers in *Arabidopsis*. *Curr Biol* 18, 526-531.

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 USA* 107, 22344-22349.

Kleine-Vehn, J., and Friml, J. (2008) Polar targeting and endocytic recycling in auxin-dependent plant development. *Annu Rev Cell Dev Biol* 24, 447-473.

Korasick, D.A., Westfall, C.S., Lee, S.G., Nanao, M.H., Dumas, R., Hagen, G., Guilfoyle, T.J., Jez, J.M., Strader, L.C. (2014) Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression. *Proc Natl Acad Sci USA* 111, 5427-5432.

Kögl, F. , Erxleben, H., and Haagen-Smit, A. J. (1934) Über die Isolierung der Auxine a und b aus pfl anzlichen Materialien. 9. Mitteilung über pfl anzliche Wachstumsstoffe. *Hoppe-Seyler's Zeitschrift fur Physiologische Chemie* 225, 215-229.

Kudo, T., Kiba, T., and Sakakibara, H. (2010) Metabolism and long-distance translocation of cytokinins. *J Integr Plant Biol* 52, 53-60.

Kurakawa, T., Ueda, N., Maekawa, M., Kobayashi, K., Kojima, M., Nagato, Y., Sakakibara, H., and Kyozuka, J. (2007) Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* 445, 652-655.

Kuroha, T., Tokunaga, H., Kojima, M., Ueda, N., Ishida, T., Nagawa, S., Fukuda, H., Sugimoto, K., and Sakakibara, H. (2009) Functional analyses of LONELY GUY cytokinin-activating enzymes reveal the importance of the direct activation pathway in Arabidopsis. *Plant Cell* 21, 3152-3169.

Laplaze, L., Benkova, E., Casimiro, I., Maes, L., Vanneste, S., Swarup, R., Weijers, D., Calvo, V., Parizot, B., Herrera-Rodriguez, M.B., Offringa, R., Graham, N., Doumas, P., Friml, J., Bogusz, D., Beeckman, T., and Bennett, M. (2007) Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* 19, 3889-3900.

Laskowski, M., Grieneisen, V.A., Hofhuis, H., Hove, C.A., Hogeweg, P., Maree, A.F., and Scheres, B. (2008) Root system architecture from coupling cell shape to auxin transport. *PLoS Biol* 6, e307.

Laskowski, M.J., Williams, M.E., Nusbaum, H.C., and Sussex, I.M. (1995) Formation of lateral root meristems is a two-stage process. *Development* 121, 3303-3310.

Lee, B.H., Johnston, R., Yang, Y., Gallavotti, A., Kojima, M., Travençolo, B.A., Costa Lda F., Sakakibara, H., Jackson, D. (2009) Studies of aberrant *phyllotaxy1* mutants of maize indicate complex interactions between auxin and cytokinin signaling in the shoot apical meristem. *Plant Physiology* 150, 205-216.

Lee, S., Sundaram, S., Armitage, L., Evans, J.P., Hawkes, T., Kepinski, S., Ferro, N., Napier, R.M. (2014) Defining binding effi ciency and specifi city of auxins for SCF(TIR1/AFB)-Aux/IAA co-receptor complex formation. *ACS Chemical Biology* 9, 673-682.

Leibfried, A., To, J.P., Busch, W., Stehling, S., Kehle, A., Demar, M., Kieber, J.J., and Lohmann, J.U. (2005) WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* 438, 1172-1175.

Letham, D.S., Palni, L.M.S. (1983) The biosynthesis and metabolism of cytokinins. *Annu Rev Plant Physiol* 34, 163-197.

Letham, D.S. (1963) Zeatin, a factor inducing cell division isolated from Zea mays. *Life Sci* 8, 569-573.

Leyser, O. (2009) The control of shoot branching, an example of plant information processing. *Plant Cell Environ* 32, 694-703.

Li, G., Liu, K., Baldwin, S.A., Wang, D. (2003) Equilibrative nucleoside transporters of *Arabidopsis thaliana*. cDNA cloning, expression pattern, and analysis of transport activities. *J Biol Chem* 278, 35732-35742.

Li, L.C., Qin, G.J., Tsuge, T., Hou, X.H., Ding, M.Y., Aoyama, T., Oka, A., Chen, Z., Gu, H., Zhao, Y., Qu, L.J. (2008) SPOROCYTELESS modulates YUCCA expression to regulate the development of lateral organs in *Arabidopsis*. *New Phytol* 179, 751-764.

Ljung, K., Bhalerao, R.P., and Sandberg, G. (2001) Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. *Plant J* 28, 465-474.

Lomax, T.L., Muday, G.K. and Rubery, P.H. (1995) Auxin transport. In, P.J. Davies (Ed.) Plant Hormones, Physiology, Biochemistry and Molecular Biology, Kluwer, Dordrecht, Netherlands, pp. 509-530.

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.

Marhavý, P., Duclercq, J., Weller, B., Feraru, E., Bielach, A., Offringa, R., Friml, J. Schwechheimer, C., Murphy, A., and Benková, E. (2014) Cytokinin controls polarity of PIN1-dependent auxin transport during lateral root organogenesis. *Curr Biol* 24, 1031-1037.

Marhavý, P., Bielach, A., Abas, L., Abuzeineh, A., Duclercq, J., Tanaka, H., Pařezová, M., Petrášek, J., Friml, J., Kleine-Vehn, J., and Benková, E. (2011) Cytokinin modulates endocytic trafficking of PIN1 auxin efflux carrier to control plant organogenesis. *Dev Cell* 21, 796-804.

Martin R.C. , Mok M.C., and Mok D.W.S. (1999) Isolation of a cytokinin gene, ZOG1, encoding zeatin O-glucosyltransferase from *Phaseolus lunatus Proc Natl Acad Sci USA* 96, 284-289.

Mashiguchi, K,, Tanaka, K., Sakai, T., Sugawara, S., Kawaide, H., Natsume, M., Hanada, A., Yaeno, T., Shirasu, K., Yao, H., McSteen, P., Zhao, Y., Hayashi, K., Kamiya, Y., Kasahara, H.. (2011) The main auxin biosynthesis pathway in Arabidopsis. *Proc Natl Acad Sci USA* 108, 18512-18517.

Mason, M.G., Mathews, D.E., Argyros, D.A., Maxwell, B.B., Kieber, J.J., Alonso, J.M., Ecker, J.R., and Schaller, G.E. (2005) Multiple type-B response regulators mediate cytokinin signal transduction in Arabidopsis. *Plant Cell* 17, 3007-3018.

Matsumoto-Kitano, M., Kusumoto, T., Tarkowski, P., Kinoshita-Tsujimura, K., Václavíková, K., Miyawaki, K., and Kakimoto, T. (2008) Cytokinins are central regulators of cambial activity. *Proc Natl Acad Sci USA* 105, 20027-20031.

Mähönen, A.P., Bonke, M., Kauppinen, L., Riikonen, M., Benfey, P.N., and Helariutta, Y. (2000) A novel two-component hybrid molecule regulates vascular morphogenesis of the *Arabidopsis* root. *Genes Dev* 14, 2938-2943.

Mähönen, A.P., Bishopp, A., Higuchi, M., Nieminen, K.M., Kinoshita, K., Tormakangas, K., Ikeda, Y., Oka, A., Kakimoto, T., and Helariutta, Y. (2006a) Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* 311, 94-98.

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.

Miller, C.O. (1961) Kinetin-like compound in maize. *Proc Natl Acad Sci USA* 47, 170-174.

Miller, C.O., Skoog, F., von Saltza, M., Strong, F.M. (1955) Kinetin, a cell division factor from deoxyribonucleic acid. *J Am Chem Soc* 77, 1392.

Miyawaki, K., Matsumoto-Kitano, M., Kakimoto, T. (2004) Expression of cytokinin biosynthetic isopentenyltransferase genes in *Arabidopsis*, tissue specificity and regulation by auxin, cytokinin, and nitrate. *Plant J* 37, 128-138.

Mok, D.W., and Mok, M.C. (2001) Cytokinin Metabolism and Action. *Annu Rev Plant Physiol Plant Mol Biol* 52, 89-118.

Morris, D.A. and Thomas, (1978) A microautoradiographic study of auxin transport in the stem of intact pea seedlings (*Pisum sativum L.*). *J Exp Bot* 29, 147-157.

Moss, B.L., Mao, H., Guseman, J.M., Hinds, T.R., Hellmuth, A., Kovenock, M., Noorassa, A., Lanctot, A., Villalobos, L.I., Zheng, N., Nemhauser, J.L. (2015) Rate Motifs Tune Auxin/Indole-3-Acetic Acid Degradation Dynamics. *Plant Physiol* 169, 803-813.

Moubayidin, L., Di Mambro, R., and Sabatini, S. (2009) Cytokinin-auxin crosstalk. *Trends Plant Sci* 14, 557-562.

Mravec, J., Skůpa, P., Bailly, A, Hoyerová, K., Krecek, P., Bielach, A., Petrásek, J., Zhang, J., Gaykova, V., Stierhof, Y.D., Dobrev, P.I., Schwarzerová, K., Rolcík, J., Seifertová, D., Luschnig, C., Benková, E., Zazímalová, E., Geisler, M., Friml, J., (2009) Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* 459, 1136-1140.

Muraro, D., Mellor, N., Pound, M.P., Help, H., Lucas, M., Chopard, J., Byrne, H.M., Godin, C., Hodgman, T.C., King, J.R., Pridmore, T.P., Helariutta, Y., Bennett, M.J., Bishopp, A. (2014) Integration of hormonal signaling networks and mobile microRNAs is required for vascular patterning in *Arabidopsis* roots. *Proc Natl Acad Sci USA* 111, 857-862.

Müller, D., Waldie, T., Miyawaki, K., To, J.P., Melnyk, C.W., Kieber, J.J., Kakimoto, T., Leyser, O. (2015) Cytokinin is required for escape but not release from auxin mediated apical dominance. *Plant J* 82, 874-886.

Müller, A., Guan, C., Gälweiler, L., Tanzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E., and Palme, K. (1998) AtPIN2 defines a locus of *Arabidopsis* for root gravitropism control. *EMBO J* 17, 6903-6911.

Müller, B., and Sheen, J. (2008) Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. *Nature* 453, 1094-1097.

Nakano, T., K. Suzuki, T. Fujimura and H. Shinshi (2006) Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiol* 140, 411-432.

Nanao, M.H., Vinos-Poyo, T., Brunoud, G., Thévenon, E., Mazzoleni, M., Mast, D., Lainé, S., Wang, S., Hagen, G., Li, H., Guilfoyle, T.J., Parcy F, Vernoux T, Dumas R. (2014) Structural basis for oligomerization of auxin transcriptional regulators. *Nat Commun* 7, 3617.

Naramoto, S., Kleine-Vehn, J., Robert, S., Fujimoto, M., Dainobu, T., Paciorek, T., Ueda, T., Nakano, A., Van Montagu, M.C.E., Fukuda, H., and Friml, J. (2010) ADP-ribosylation factor machinery mediates endocytosis in plant cells. *Proc Natl Acad Sci USA* 107, 21890-21895.

Naramoto, S., Otegui, M.S., Kutsuna, N., de Rycke, R., Dainobu, T., Karampelias, M., Fujimoto, M., Feraru, E., Miki, D., Fukuda, H., Nakano, A., Friml J. (2014) Insights into the localization and function of the membrane trafficking regulator GNOM ARF-GEF at the Golgi apparatus in *Arabidopsis. Plant Cell* 26, 3062-3076.

Nishimura, T., Hayashi, K., Suzuki, H., Gyohda, A., Takaoka, C., Sakaguchi, Y., Matsumoto, S., Kasahara, H., Sakai, T., Kato, J., Kamiya, Y., Koshiba, T. (2014) Yucasin is a potent inhibitor of YUCCA, a key enzyme in auxin biosynthesis. *Plant J* 77, 352-366.

Nishimura, C., Ohashi, Y., Sato, S., Kato, T., Tabata, S., and Ueguchi, C. (2004) Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of shoot and root growth in *Arabidopsis*. *Plant Cell* 16, 1365-1377.

Noh, B., Murphy, A.S., and Spalding, E.P. (2001) Multidrug resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development. *Plant Cell* 13, 2441-2454.

Nordström, A., Tarkowski, P., Tarkowska, D., Norbaek, R., Astot, C., Dolezal, K., Sandberg, G. (2004) Auxin regulation of cytokinin biosynthesis in Arabidopsis thaliana, A factor of potential importance for auxin-cytokinin-regulated development. *Proc Natl Acad Sci USA* 101, 8039-8044.

Nowacki, J. and Bandurski, R.S. (1980) Myo-inositol esters of indole-3-acetic acid as seed auxin precursors of Zea mays L. *Plant Physiol* 65, 422-427.

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.

Okushima, Y., Overvoorde, P.J., Arima, K., Alonso, J.M., Chan, A., Chang, C., Ecker, J.R., Hughes, B., Lui, A., Nguyen, D., Onodera, C., Quach, H., Smith, A., Yu, G., and Theologis, A. (2005) Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*, unique and overlapping functions of ARF7 and ARF19. *Plant Cell* 17, 444-463.

Overvoorde, P.J., Okushima, Y., Alonso, J.M., Chan, A., Chang, C., Ecker, J.R., Hughes, B., Liu, A., Onodera, C., Quach, H., Smith, A., Yu, G., Theologis, A. (2005) Functional

genomic analysis of the *AUXIN/INDOLE-3-ACETIC ACID* gene family members in *Arabidopsis thaliana*. *Plant Cell* 17, 3282-3300.

Parry, G., Delbarre, A., Marchant, A., Swarup, R., Napier, R., Perrot-Rechenmann, C., and Bennett, M.J. (2001) Novel auxin transport inhibitors phenocopy the auxin influx carrier mutation *aux1*. *Plant J* 25, 399-406.

Peer, W.A., Blakeslee, J.J., Yang, H., Murphy, A.S. (2011) Seven things we think we know about auxin transport. *Mol Plant* 4, 487-504.

Péret, B., Swarup, K., Ferguson, A., Seth, M., Yang, Y., Dhondt, S., James, N., Casimiro, I., Perry, P., Syed, A., Yang, H., Reemmer, J., Venison, E., Howells, C., Perez-Amador, M.A., Yun, J., Alonso, J., Beemster, G.T., Laplaze, L., Murphy, A., Bennett, M.J., Nielsen, E., Swarup, R. (2012) *AUX/LAX* genes encode a family of auxin influx transporters that perform distinct functions during *Arabidopsis* development. *Plant Cell* 24, 2874-2885.

Pernisová, M., Klima, P., Horak, J., Valkova, M., Malbeck, J., Soucek, P., Reichman, P., Hoyerova, K., Dubova, J., Friml, J., Zazimalova, E., and Hejatko, J. (2009) Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux. *Proc Natl Acad Sci USA* 106, 3609-3614.

Petrášek, 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.

Pickett, F.B., Wilson, A.K., and Estelle, M. (1990) The *aux1* mutation of *Arabidopsis* confers both auxin and ethylene resistance. *Plant Physiol* 94, 1462-1466.

Pinon, V., Prasad, K., Grigg, S.P., Sanchez-Perez, G.F., Scheres, B. (2013) Local auxin biosynthesis regulation by PLETHORA transcription factors controls phyllotaxis in *Arabidopsis*. *Proc Natl Acad Sci USA* 110, 1107-1112.

Rakusová, H., Gallego-Bartolomé, J., Vanstraelen, M., Robert, H.S., Alabadí, D., Blázquez, M.A., Benková, E., and Friml, J. (2011) Polarization of PIN3-dependent auxin transport for hypocotyl gravitropic response in *Arabidopsis thaliana*. *Plant J* 67, 817-826.

Raven, J.A. (1975) Transport of indoleacetic acid in plant cells in relation to ph and electrical potential gradients, and its significance for polar iaa transport. *New Phytologist* 74, 163-172.

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

Rashotte, A.M., Mason, M.G., Hutchison, C.E., Ferreira, F.J., Schaller, G.E., and Kieber, J.J. (2006) A subset of *Arabidopsis* AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. *Proc Natl Acad Sci USA* 103, 11081-11085.

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, H.S., Grones, P., Stepanova, A.N., Robles, L.M., Lokerse, A.S., Alonso, J.M., Weijers, D., Friml, J. (2013) Local auxin sources orient the apical-basal axis in *Arabidopsis* embryos. *Curr Biol* 23, 2506-2512.

Romanov, G.A., Lomin, S.N., and Schmülling, T. (2006) Biochemical characteristics and ligand-binding properties of *Arabidopsis* cytokinin receptor AHK3 compared to CRE1/AHK4 as revealed by a direct binding assay. *J Exp Bot* 57, 4051-4058.

Romanov, G.A., Spíchal, L., Lomin, S.N., Strnad, M., and Schmülling, T. (2005) A live cell hormone-binding assay on transgenic bacteria expressing a eukaryotic receptor protein. *Anal Biochem* 347, 129-134.

Rouse, D., Mackay, P., Stirnberg, P., Estelle, M., Leyser, O. (1998) Changes in auxin response from mutations in an *AUX/IAA* gene. *Science* 279, 1371-1373.

Rubery, P.H., and Sheldrake, A.R. (1974) Carrier-mediated auxin transport. *Planta* 118, 101-121.

Ruegger, M., Dewey, E., Gray, W.M., Hobbie, L., Turner, J., Estelle, M. (1998) The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes Dev* 12, 198-207.

Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G., and Estelle, M. (1997) Reduced naphthylphthalamic acid binding in the *tir3* mutant of *Arabidopsis* is associated with a reduction in polar auxin transport and diverse morphological defects. *Plant Cell* 9, 745-757.

Růžička, K., Simaskova, M., Duclercq, J., Petrasek, J., Zazimalova, E., Simon, S., Friml, J., Van Montagu, M.C., and Benkova, E. (2009) Cytokinin regulates root meristem activity via modulation of the polar auxin transport. *Proc Natl Acad Sci USA* 106, 4284-4289.

Sachs, J. (1880) *Arb Bot Inst Würzburg* 3, 452-488.

Sakai, H., Honma, T., Aoyama, T., Sato, S., Kato, T., Tabata, S., and Oka, A. (2001) ARR1, a transcription factor for genes immediately responsive to cytokinins. *Science* 294, 1519-1521.

Sakuma, Y., Q. Liu, J. G. Dubouzet, H. Abe, K. Shinozaki and K. Yamaguchi-Shinozaki (2002) "DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression." *Biochem Biophys Res Commun* 290, 998-1009.

Schaller, G.E., Bishopp, A., Kieber, J.J. (2015) The yin-yang of hormones, cytokinin and auxin interactions in plant development. *Plant Cell*. 27, 44-63.

Schaller, G.E., Kieber, J.J., and Shiu, S.-H. (2008) Two-component signaling elements and histidyl-aspartyl phosphorelays. in The Arabidopsis Book, C. Somerville and E. Meyerowitz, eds (Rockville, MD, American Society of Plant Biologists).

Schlereth A, Möller B, Liu W, Kientz M, Flipse J, Rademacher EH, Schmid M, Jürgens G, Weijers D. (2010) MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* 464, 913-916.

Shimizu-Mitao, Y., and T. Kakimoto. (2014) Auxin sensitivities of all *Arabidopsis* Aux/IAAs for degradation in the presence of every TIR1/AFB. *Plant & Cell Physiology* 55, 1405-1459.

Šimášková, M., O'Brien, J.A., Khan, M., Van Noorden, G., Ötvös, K., Vieten, A., De Clercq, I., Van Haperen, J.M., Cuesta, C., Hoyerová, K., Vanneste, S., Marhavý, P., Wabnik, K., Van Breusegem, F., Nowack, M., Murphy, A., Friml, J., Weijers, D., Beeckman, T., Benková, E. (2015) Cytokinin response factors regulate PIN-FORMED auxin transporters. *Nat Commun* 6, 8717.

Shimizu-Sato, S., Tanaka, M., and Mori, H. (2009) Auxin-cytokinin interactions in the control of shoot branching. *Plant Mol Biol* 69, 429-435.

Skoog, F., and Miller, C. (1957) Chemical regulation of growth and organ formation in plant tissue cultured in vitro. *Symp Soc Exp Biol* 11, 118-131.

Soeno, K., Goda, H., Ishii, T., Ogura T, Tachikawa T, Sasaki E, Yoshida S, Fujioka S, Asami T, Shimada Y. (2010) Auxin biosynthesis inhibitors, identified by a genomics-based approach, provide insights into auxin biosynthesis. *Plant Cell Physiol* 51, 524-536.

Stepanova, A.N., Robertson-Hoyt, J., Yun, J., Benavente, L.M., Xie, D.Y., Dolezal, K., Schlereth, A., Jurgens, G., and Alonso, J.M. (2008) TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* 133, 177-191.

Stolz, A., Riefler, M., Lomin, S.N., Achazi, K., Romanov, G.A., and Schmülling, T. (2011) The specificity of cytokinin signalling in *Arabidopsis thaliana* is mediated by differing ligand affinities and expression profiles of the receptors. *Plant J* 67, 157-168.

Stone, S.L., Braybrook, S.A., Paula, S.L., Kwong, L.W., Meuser, J., Pelletier, J., Hsieh, T.F., Fischer, R.L., Goldberg, R.B., Harada, J. (2008) *Arabidopsis* LEAFY COTYLEDON2 induces maturation traits and auxin activity, implications for somatic embryogenesis. *Proc Natl Acad Sci USA* 105, 3151-3156.

Stone, B.B., Stowe-Evans, E.L., Harper, R.M., Celaya, R.B., Ljung, K., Sandberg, G., and Liscum, E. (2008) Disruptions in AUX1-dependent auxin influx alter hypocotyl phototropism in *Arabidopsis*. *Mol Plant* 1, 129-144.

Sun, J., Hirose, N., Wang, X., Wen, P., Xue, L., Sakakibara, H., and Zuo, J. (2005) *Arabidopsis SOI33/AtENT8* gene encodes a putative equilibrative nucleoside transporter that is involved in cytokinin transport in planta. *J Integr Plant Biol* 47, 588-603.

Sun, J., Qi, L., Li, Y., Chu, J., Li, C. (2012) PIF4-mediated activation of *YUCCA8* expression integrates temperature into the auxin pathway in regulating *Arabidopsis* hypocotyl growth. *PLoS Genet* 8, e1002594.

Su, Y.H., Liu, Y.B., and Zhang, X.S. (2011) Auxin-cytokinin interaction regulates meristem development. *Mol Plant* 4, 616-625.

Suzuki, T., Miwa, K., Ishikawa, K., Yamada, H., Aiba, H., and Mizuno, T. (2001) The Arabidopsis sensor His-kinase, AHK4, can respond to cytokinins. *Plant Cell Physiol* 42, 107-113.

Swarup, K., Benkova, E., Swarup, R., Casimiro, I., Peret, B., Yang, Y., Parry, G., Nielsen, E., De Smet, I., Vanneste, S., Levesque, M.P., Carrier, D., James, N., Calvo, V., Ljung, K., Kramer, E., Roberts, R., Graham, N., Marillonnet, S., Patel, K., Jones, J.D., Taylor, C.G., Schachtman, D.P., May, S., Sandberg, G., Benfey, P., Friml, J., Kerr, I., Beeckman, T., Laplaze, L., and Bennett, M.J. (2008) The auxin influx carrier LAX3 promotes lateral root emergence. *Nat Cell Biol* 10, 946-954.

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

Takei, K., Sakakibara, H., Sugiyama, T. (2001) Identification of genes encoding adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme, in *Arabidopsis thaliana*. *J Biol Chem* 276, 26405-26410.

Takei, K., Ueda, N., Aoki, K., Kuromori, T., Hirayama, T., Shinozaki, K., Yamaya, T., Sakakibara, H. (2004a) *AtIPT*3, an Arabidopsis isopentenyltransferase gene, is a key determinant of macronutrientresponsive cytokinin biosynthesis. *Plant Cell Physiol* 45, 1053-1062.

Takei, K., Yamaya, T., Sakakibara, H. (2004b) *Arabidopsis CYP735A1* and *CYP735A2* encode cytokinin hydroxylases that catalyze the biosynthesis of trans-zeatin. *J Biol Chem* 279, 41866-41872.

Tanaka, H., Kitakura, S., De Rycke, R., De Groodt, R., and Friml, J. (2009) Fluorescence imaging-based screen identifies ARF GEF component of early endosomal trafficking. *Curr Biol* 19, 391-397.

Tanaka, H., Kitakura, S., Rakusová, H., Uemura, T., Feraru, M.I., De Rycke, R., Robert, S., Kakimoto, T., and Friml, J. (2013) Cell polarity and patterning by PIN trafficking through early endosomal compartments in *Arabidopsis thaliana*. *PLoS Genet* 9, e1003540.

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.

Tao, Y., Ferrer, J.L., Ljung, K., Pojer, F., Hong, F., Long, J.A., Li, L., Moreno, J.E., Bowman, M.E., Ivans, L.J., Cheng, Y., Lim, J., Zhao, Y., Ballaré, C.L., Sandberg, G., Noel, J.P., Chory, J. (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* 133, 164-176.

Taya, Y., Tanaka, Y., Nishimura S. (1978) 5'-AMP is a direct precursor of cytokinin in *Dictyostelium discoideum*. *Nature* 271, 545-547.

Tarkowski, P., Ge, L., Wan Hong Yong, J., Swee Ngin Tan. (2009) Analytical methods for cytokinins. *Trends in Analytical Chemistry*, Vol. 28, No. 3.

Teh, O.K., and Moore, I. (2007) An ARF-GEF acting at the Golgi and in selective endocytosis in polarized plant cells. *Nature* 448, 493-496.

Thimann, K.V., Koepfli, J.B. (1935) Identity of the growth-promoting and root-forming substances of plants. *Nature* 135, 101.

To, J.P., Deruere, J., Maxwell, B.B., Morris, V.F., Hutchison, C.E., Ferreira, F.J., Schaller, G.E., and Kieber, J.J. (2007) Cytokinin regulates type-A Arabidopsis Response Regulator activity and protein stability via two-component phosphorelay. *Plant Cell* 19, 3901-3914.

Ueguchi, C., Sato, S., Kato, T., and Tabata, S. (2001) The AHK4 gene involved in the cytokinin-signaling pathway as a direct receptor molecule in *Arabidopsis thaliana*. *Plant Cell Physiol* 42, 751-755.

Ulmasov, T., Hagen, G., Guilfoyle T.J. (1997) ARF1, a transcription factor that binds auxin response elements. *Science* 276, 1865-1868.

Ulmasov, T., Murfett, J., Hagen, G., Guilfoyle, T.J. (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* 9, 1963-1971.

Van Overbeek, J., Conklin, M.E., Blakeslee, A.F. (1941) Factors in coconut milk essential for growth and development of very young datura embryos. *Science* 94, 350-351.

Vernoux, T., Brunoud, G., Farcot, E., Morin, V., Van den Daele, H., Legrand, J., Oliva, M., Das, P., Larrieu, A., Wells, D., Guédon, Y., Armitage, L., Picard, F., Guyomarch, S., Cellier, C., Parry, G., Koumproglou, R., Doonan, J.H., Estelle, M., Godin, C., Kepinski, S., Bennett, M., De Veylder, L., Traas, J. (2011) The auxin signalling network translates dynamic input into robust patterning at the shoot apex. *Mol Syst Biol* 7, 508

Verrier, P.J., Bird, D., Burla, B., Dassa, E., Forestier, C., Geisler, M., Klein, M., Kolukisaoglu, U., Lee, Y., Martinoia, E., Murphy, A., Rea, P.A., Samuels, L., Schulz, B., Spalding, E.J.,

Yazaki, K., and Theodoulou, F.L. (2008) Plant ABC proteins--a unified nomenclature and updated inventory. *Trends Plant Sci* 13, 151-159.

Vieten, A., Vanneste, S., Wisniewska, J., Benkova, E., Benjamins, R., Beeckman, T., Luschnig, C., and Friml, J. (2005) Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. *Development* 132, 4521-4531.

Voß, U., Bishopp, A., Farcot, E., Bennett, M.J. (2014) Modelling hormonal response and development. *Trends Plant Sci* 19, 311-319.

Wang, C., Yan, X., Chen, Q., Jiang, N., Fu, W., Ma, B., Liu, J., Li, C.,Bednarek, S.Y., and Pan, J. (2013) Clathrin light chains regulate clathrin-mediated trafficking, auxin signaling, and development in *Arabidopsis*. *Plant Cell* 25, 499–516.

Went, F. (1928) Wuchsstoff und Wachstum. *Receuil Des Travaux Botaniques Neerlandais* 25, 1–116.

Werner, T., Motyka, V., Strnad, M., and Schmulling, T. (2001) Regulation of plant growth by cytokinin. *Proc Natl Acad Sci USA* 98, 10487-10492.

Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., and Schmulling, T. (2003) Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell* 15, 2532-2550.

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.

Won, C., Shen, X., Mashiguchi, K., Zheng, Z., Dai, X., Cheng, Y., Kasahara, H., Kamiya, Y., Chory, J., Zhao, Y. (2011) Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in *Arabidopsis*. *Proc Natl Acad Sci USA* 108, 18518-18523.

Wulfetange, K., Lomin, S.N., Romanov, G.A., Stolz, A., Heyl, A., and Schmulling, T. (2011) The cytokinin receptors of *Arabidopsis* are located mainly to the endoplasmic reticulum. *Plant Physiol* 156, 1808-1818.

Yamada, M., Greenham, K., Prigge, M.J., Jensen, P.J., and Estelle, M. (2009) The *TRANSPORT INHIBITOR RESPONSE2* gene is required for auxin synthesis and diverse aspects of plant development. *Plant Physiol* 151, 168–179.

Yang, H., and Murphy, A.S. (2009) Functional expression and characterization of *Arabidopsis* ABCB, AUX 1 and PIN auxin transporters in *Schizosaccharomyces pombe*. *Plant J* 59, 179–191.

Yang, Y., Hammes, U.Z., Taylor, C.G., Schachtman, D.P., and Nielsen, E. (2006) High-affinity auxin transport by the AUX1 influx carrier protein. *Curr Biol* 16, 1123-1127.

Yoshida, S., Mandel, T., and Kuhlemeier, C. (2011) Stem cell activation by light guides plant organogenesis. *Genes Dev 25*, 1439-1450.

Zažímalová, E., Krecek, P., Skupa, P., Hoyerova, K., and Petrasek, J. (2007) Polar transport of the plant hormone auxin - the role of PIN-FORMED (PIN) proteins. *Cell Mol Life Sci* 64, 1621-1637.

Zhang, J., Nodzynski, T., Pencik, A., Rolcik, J., and Friml, J. (2010) PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. *Proc Natl Acad Sci USA* 107, 918-922.

Zhang, J., Vanneste, S., Brewer, P.B., Michniewicz, M., Grones, P., Kleine-Vehn, J., Löfke, C., Teichmann, T., Bielach, A., Cannoot, B., Hoyerová, K., Chen, X., Xue, H.W., Benková,

E., Zažímalová, E., Friml, J. (2011) Inositol trisphosphate-induced Ca^{2+} signaling modulates auxin transport and PIN polarity. *Dev Cell* 20, 855–866.

Zhang, K., Novak, O., Wei, Z., Gou, M., Zhang, X., Yu, Y., Yang, H., Cai, Y., Strnad, M., Liu, C.J. (2014) *Arabidopsis* ABCG14 protein controls the acropetal translocation of root-synthesized cytokinins. *Nat Commun* 5, 3274.

Zhang, W., To, J.P., Cheng, C.Y., Eric Schaller, G., and Kieber, J.J. (2011) Type-A response regulators are required for proper root apical meristem function through the post-transcriptional regulation of PIN auxin efflux carriers. *Plant J* 68, 1-10

Zhao, Z., Andersen, S.U., Ljung, K., Dolezal, K., Miotk, A., Schultheiss, S.J., and Lohmann, J.U. (2010) Hormonal control of the shoot stem-cell niche. *Nature* 465, 1089-1092.

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., Schwechheimer, C. (2014) Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. *Elife*, 3.

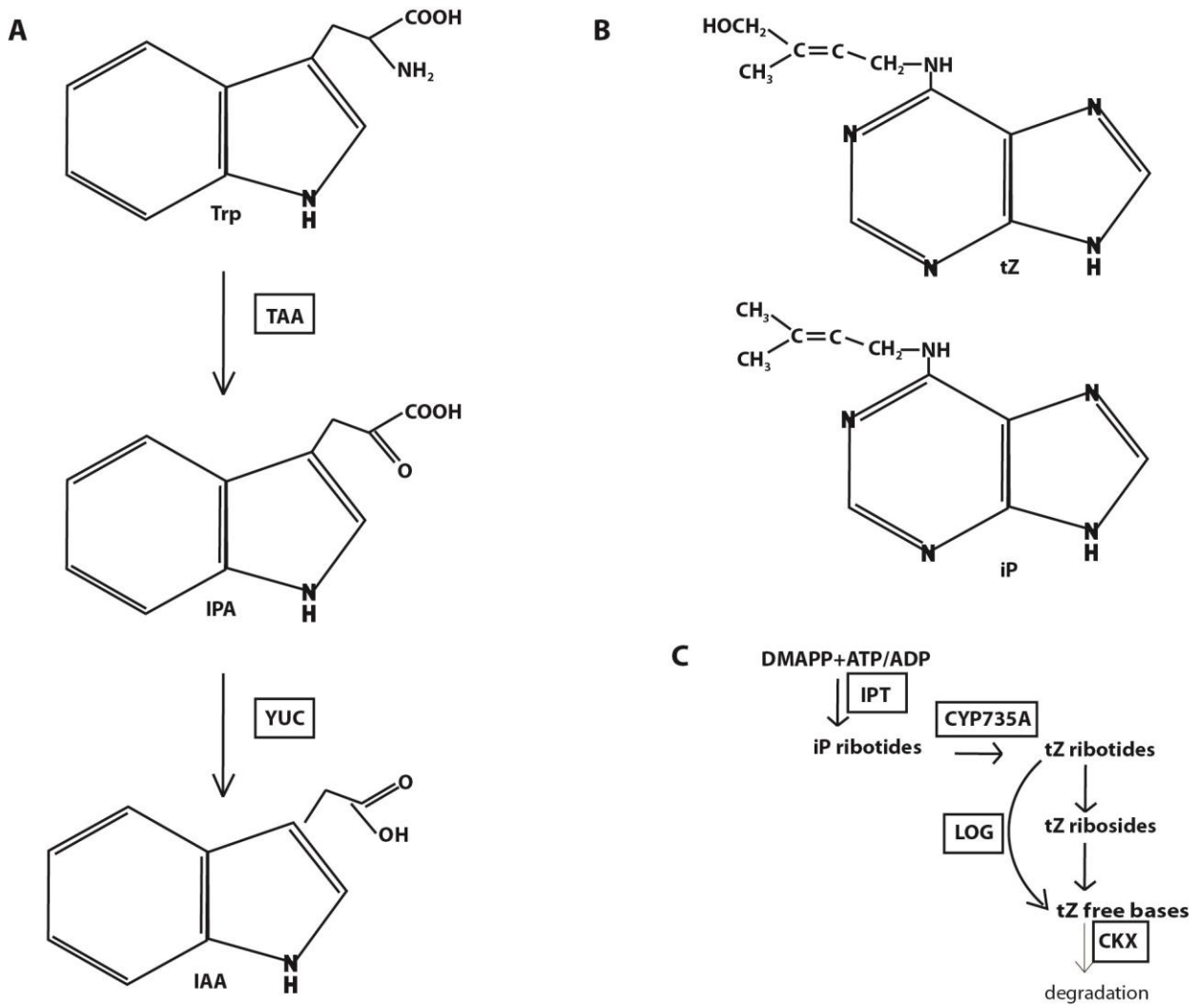

Figure 1. Biosynthesis of auxin and cytokinins. (**A**) Auxin (IAA) is synthesized from tryptophan (Trp) precursor in two step pathway catalyzed by TAA and YUCCA. (**B**) Common plant cytokinins trans-zeatin (tZ) and isopentenyl-adenine (iP). (**C**) Core steps of cytokinin metabolism. Biosynthesis of tZ cytokinin is initiated by adenosine phosphate-isopentenyltransferase (IPT) using dimethylallyl diphosphate (DMAPP) and adenosine 5´-diphosphate (ADP), or adenosine 5´-triphosphate (ATP) to form iP-ribotides which are converted to the corresponding tZ-ribotides by cytochrome P450 monooxygenases (CYP735As). tZ-ribotides can be dephosphorylated to tZ-ribosides or directly converted to active free bases by cytokinin nucleoside 5'-monophosphate phosphoribohydrolase (LOG).

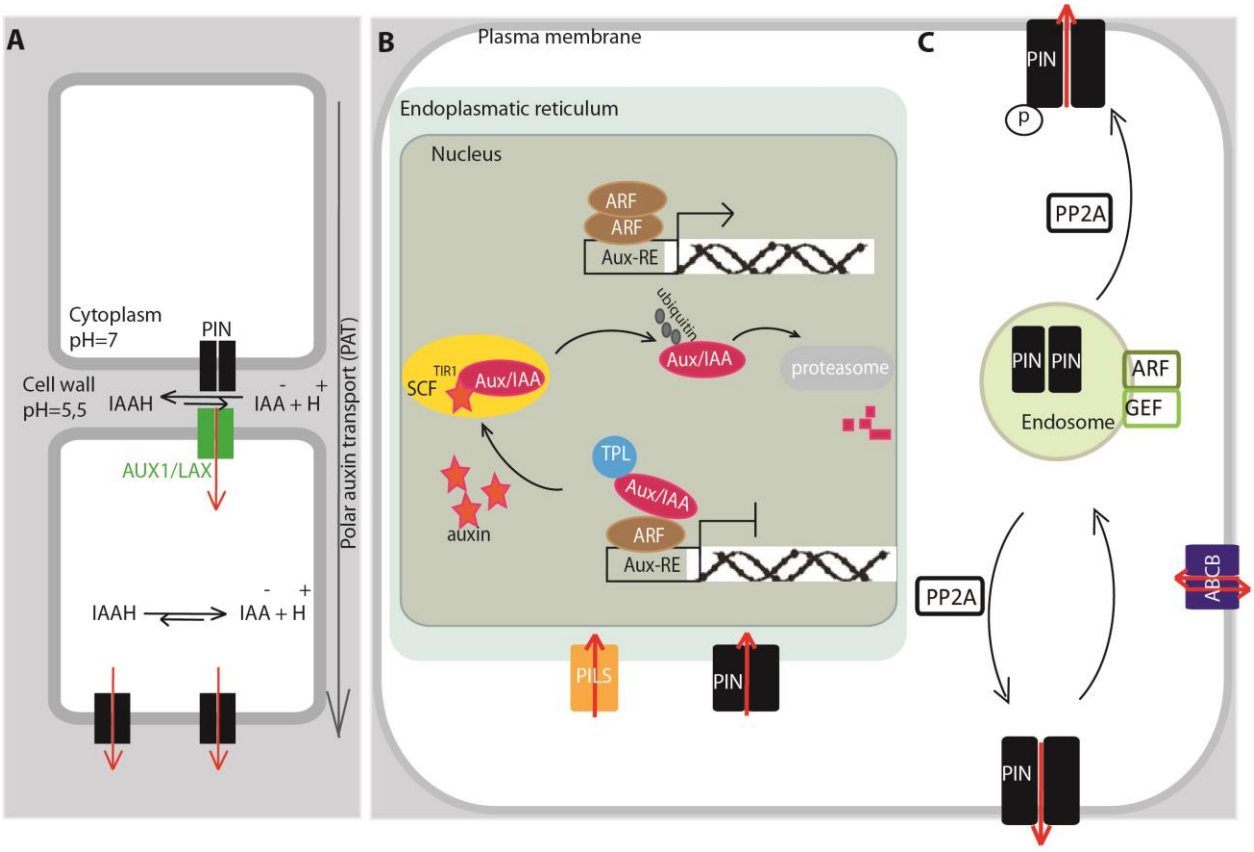

Figure 2

Figure 2. Model of auxin transport and signaling. (**A**) Chemiosmotic hypothesis for polar auxin transport. In the acidic apoplast auxin is protonated. The protonated auxin either passively diffuses through the plasma membrane or is actively transported by AUX1/LAX influx carriers into the cell. In the neutral cytosol auxin becomes deprotonated and can leave the cell only by auxin efflux carriers such as PIN proteins and PGP transporters. (**B**) Under low auxin conditions, Aux/IAAs form a complex with ARF transcription factors and the TPL corepressor, thus thereby inhibiting AuxRE-mediated gene transcription. At higher concentrations, auxin stimulates ubiquitin-mediated proteolysis of Aux/IAA catalysed by an SCF^{TIR1} E3 ubiquitin ligase. Degradation of Aux/IAAs relieves the ARF repression and allows transcription. (**C**) Outside the nucleus PIN auxin efflux transporters cycle between endosomes and the plasma membrane. The exocytosis requires the activity of GNOM, an ADP-ribosylation factor GTPase guanine nucleotide exchange factor (ARF-GEF), whereas endocytosis occurs in a clathrin-dependent manner. The PIN phosphorylation status, controlled by PINOID kinase (PID) and protein phosphatase 2A (PP2A), determines PINs recruitment to apical or basal targeting pathways. The short PIN proteins and PILS located in the ER might regulate of intracellular auxin homeostasis.

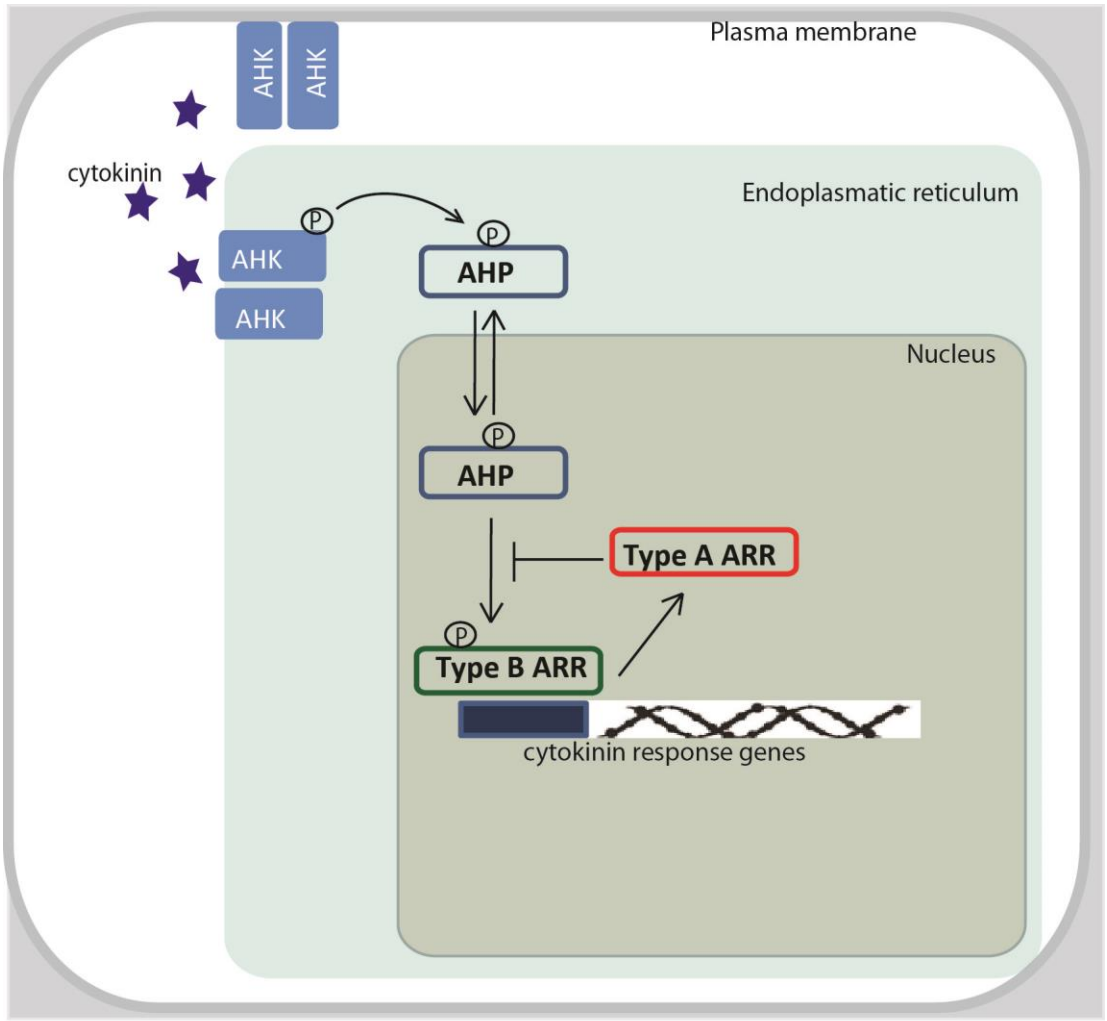

Figure 3

Figure 3. Model of cytokinin signaling pathway. Cytokinin binds to cytokinin receptor (AHKs) and initiates the phosphorelay signal transduction cascade. The phosphate is transferred from receptor to histidine phosphotransfer proteins (AHPs) followed by the phosphorylation and activation of the type B response regulator (ARR) proteins in the nucleus. A negative feed-back loop is provided by type-A ARRs, which inhibits the activity of type-B ARRs.