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Crossing paths: cytokinin signalling and crosstalk

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Summary

Cytokinins are a major class of plant hormones that are involved in various aspects of plant development, ranging from organ formation and apical dominance to leaf senescence. Cytokinin and auxin have long been known to interact antagonistically, and more recent studies have shown that cytokinins also interact with other plant hormones to regulate plant development. A growing body of research has begun to elucidate the molecular and genetic underpinnings of this extensive crosstalk. The rich interconnections between the synthesis, perception and transport networks of these plant hormones provide a wide range of opportunities for them to modulate, amplify or buffer one another. Here, we review this exciting and rapidly growing area of cytokinin research.

Key words: Plant development, Cytokinin, Hormonal crosstalk, Auxin

Introduction

Over 100 years ago, several scientists discovered the existence of substances that were able to induce cell division in cultured or wounded plant tissue (Wiesner, 1892; Haberlandt, 1913; van Overbeek et al., 1941). In 1955, Miller and colleagues (Miller et al., 1955) isolated an active substance from herring sperm and named it kinetin; this was followed by the identification of trans-zeatin (Miller, 1961) and many other cytokinins (reviewed in Mok and Mok, 2001). Cytokinins, which were named for their ability to promote cytokinesis, are a class of phytohormone derived from adenine. Naturally occurring cytokinins can be divided into two groups based on their side chain (Fig. 1A): those with isoprene-derived side chains, which are predominant in plants; and those with aromatic side chains (Sakakibara, 2006).

Early work on cytokinins showed that they promote shoot growth, inhibit root growth, stimulate cell division and induce greening in calli (Miller et al., 1956). Since then, they have been discovered to be important in many developmental processes in plants, including organ formation, leaf senescence, shoot meristem formation and maintenance, apical dominance and seed germination (Mok and Mok, 2001). Cytokinin and auxin have been known to play antagonistic roles in many of these processes since cytokinin was first identified (Skoog and Miller, 1957), and this has remained an important theme in plant biology (Coenen and Lomax, 1997). Here, we provide an overview of cytokinin synthesis, transport and signalling pathways, focusing on recent major advances in our understanding of the crosstalk between cytokinin and auxin, and the interactions between cytokinin and other hormones; several recent reviews have addressed other aspects of cytokinin biology (Argueso et al., 2009; Argueso et al., 2010; Kudo et al., 2010; Frébort et al., 2011; Brenner et al., 2012; Hwang et al., 2012).

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Cytokinin synthesis and homeostasis

Cytokinin biosynthesis is a multi-step process requiring the activity of several different genes. In *Arabidopsis thaliana*, the model species for plant research, cytokinin biosynthesis is controlled by the ATP/ADP isopentenyltransferase (IPT) genes, which encode rate-limiting enzymes in cytokinin biosynthesis (Fig. 1B). Seven IPT genes (*AtIPT1* and *AtIPT3* through *AtIPT8*) are found in *Arabidopsis*, and they encode enzymes that synthesize the cytokinin precursor, isopentenyladenine (Kakimoto, 2001; Takei et al., 2001). Two cytochrome P450 monooxygenases (*CYP735A1* and *CYP735A2*) then catalyse the hydroxylation of isopentenyladenine-type cytokinins (Takei et al., 2004b). In addition, the LONELY GUY (LOG) gene family encodes enzymes that convert cytokinin from an inactive to an active form. Cytokinin degradation is mediated by the cytokinin oxidases, which were first described over 30 years ago (Pačes et al., 1971); these enzymes are encoded by the CYTOKININ OXIDASE (CKX) gene family (Houba-Hérin et al., 1999; Schmülling et al., 2003).

Cytokinin degradation and synthesis genes are both widely expressed, being active in tissues of both the shoot and the root (Nordström et al., 2004). Analyses of expression patterns using promoter-GUS fusions have demonstrated that cytokinin biosynthesis occurs in various tissues and cells in aerial organs as well as roots (Miyawaki et al., 2004). The IPT and CKX genes are widely expressed throughout the *Arabidopsis* root and have specific expression patterns (Werner et al., 2003; Miyawaki et al., 2004). Cytokinin biosynthesis and degradation pathways have recently been reviewed comprehensively by Frébort et al. (Frébort et al., 2011).

Cytokinin uptake and transport

Although the polar transport of auxin is relatively well understood and characterized, very little is known about how cytokinin is taken up by cells and transported in plants. Diffusion is likely to play an important role, yet evidence from *Chenopodium rubrum* cultures (Fußeder et al., 1989) and *Arabidopsis* cell cultures (Cedzich et al., 2008) suggests that cytokinin is also actively taken up by cells, although dedicated cytokinin transporters have yet to be identified. Furthermore, although grafting experiments have long suggested the importance of long-distance cytokinin transport, this phenomenon remains poorly understood, despite significant recent advances. Cytokinin uptake and transport have been reviewed by Kudo et al. (Kudo et al., 2010) but are summarised below.

Cellular uptake

The *PURINE PERMEASE* (*PUP*) gene family encodes broad-affinity transporters that are able to transport several cytokinins. *PUP1* was initially identified from suppression cloning in purine transport-deficient yeast and was shown to also recognize cytokinins (Gillissen et al., 2000). Bürkle et al. (Bürkle et al., 2003) showed direct, active uptake of adenine and various cytokinins by PUP1 and PUP2; the expression pattern of *PUP1* and *PUP2* suggest that they may play a role in the loading and unloading of cytokinins for long-distance transport. The

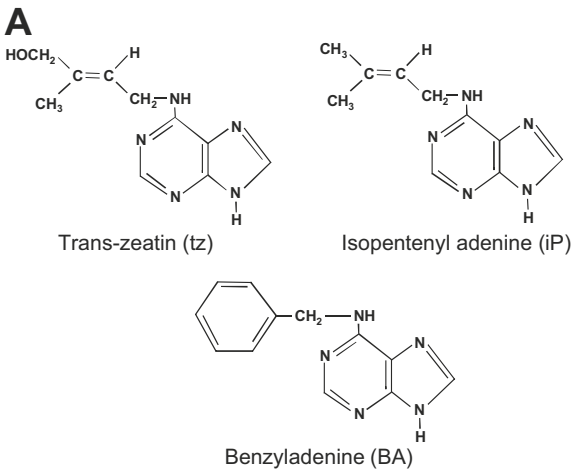

Fig. 1. Cytokinin and its biosynthesis. (**A**) Common cytokinins found in plants include the isoprenoid cytokinins trans-zeatin (tz) and isopentenyl-adenine (iP). Benzyladenine (BA) is a commonly used synthetic aromatic cytokinin. (**B**) The cytokinin biosynthesis pathway, highlighting the key enzymes involved. The initial step, in which dimethylallyl diphosphate (DMAPP) reacts with adenosine monophosphate (AMP) to form iP riboside 5′-monophosphate (iPRMP), is catalysed by the IPT gene family. Subsequently, cytochrome P450 mono-oxygenase (CYP735A) converts iPRMP into the tz nucleotide tz riboside 5′monophosphate (tzRMP). Finally, enzymes encoded by the LOG gene family catalyse the conversion of tzRMP into an active cytokinin form, in this case tz.

EQUILIBRATIVE NUCLEOSIDE TRANSPORT (ENT) gene family has also been implicated in cytokinin transport in *Arabidopsis*. Sun et al. (Sun et al., 2005) found a reduction in cytokinin uptake efficiency in *ent3* and *ent8* mutants. *OsENT2*, one of the four ENT genes in *Oryza sativa*, has also been shown to be a functional nucleoside transporter that may play a role in long-distance transport of nucleosides in growing plants (Hirose et al., 2005). In addition, *ENT6* has also recently been implicated as a transporter of nucleoside-type cytokinins in *Arabidopsis* (Hirose et al., 2008). Although these transporters may play a role in cytokinin loading for long-distance transport, their broad affinity and the lack of strong phenotypes in the mutants suggests that they do not contribute to major processes that regulate plant development.

Long-distance transport

Reciprocal grafting experiments between *ipt1;3;5;7* mutants and wild-type plants showed preferential transport of different cytokinin species; trans-zeatin (tz)-type cytokinins were transported from the root to the shoot, while isopentenyl adenine (iP)-type cytokinins moved from the shoot to the root (Matsumoto-Kitano et al., 2008). This is consistent with earlier grafting experiments in tobacco (Faiss et al., 1997) in which cytokinin overproduction in the root stock failed to release lateral branches from suppression or accelerate senescence, suggesting that the long-distance transport of cytokinin is somehow regulated. Further evidence came from the detection of cytokinin in sap and leaf exudates (Takei et al., 2002; Takei et al., 2004a). Long-distance transport of cytokinin through the phloem was recently directly demonstrated by Bishopp et al. (Bishopp et al., 2011b), who observed movement of radiolabelled cytokinins applied to the cotyledons to the root tip. Bishopp et al. were able to disrupt the rootwards flow of cytokinin by inducing callose synthesis in the phloem, demonstrating the importance of symplastic connections in the phloem to acropetal cytokinin transport.

Cytokinin signalling

The major components of the cytokinin signalling pathway were identified in a series of seminal papers around the turn of the century. Cytokinin signalling is mediated by a two-component signalling pathway (Fig. 2) similar to the two-component signalling systems (TCSs) found in bacteria. In brief, cytokinin induces autophosphorylation of a histidine kinase (HK) protein, which results in the transfer of a phosphoryl group from a phospho-accepting histidine residue in the kinase domain to an aspartate residue. The phosphoryl is then transferred to a conserved histidine on a histidine phosphotransferase (HP) protein. From there, it is finally transferred to an aspartate in the receiver domain of a response regulator (RR). For a thorough overview of cytokinin signalling, the reader is referred to the recent review by Hwang et al. (Hwang et al., 2012).

Histidine kinases: the cytokinin receptors

The first indication of a link between cytokinin and the bacterial TCS was the discovery that overexpression of *CYTOKININ INDEPENDENT* (*CKI*), a gene homologous to the HKs in the bacterial TCS, induced a cytokinin response in plants (Kakimoto, 1996). A few years later, the research of several groups independently converged on the identification of the first cytokinin receptor. Mähönen et al. (Mähönen et al., 2000) identified the gene responsible for the *wooden leg* (*wol*) mutation (Scheres et al., 1995) as a histidine kinase involved in two-component signalling in plants. The *WOL* gene was discovered to be allelic with *CYTOKININ RESPONSE 1* (*CRE1*), which Inoue et al. (Inoue et al., 2001) simultaneously discovered to be a cytokinin receptor, as loss-of-function mutants showed a reduced cytokinin response. Yamada et al. (Yamada et al., 2001) demonstrated the cytokinin-binding activity of WOL/CRE1 *in vitro* and showed that the *wol* mutation abolished this activity. Meanwhile, labs working with *WOL/CRE1* under the name *ARABIDOPSIS HISTIDINE KINASE 4* (*AHK4*) showed that it could act as a cytokinin sensor in bacteria (Suzuki et al., 2001) and that its histidine kinase activity was cytokinin dependent (Ueguchi et al., 2001). *AHK2* and *AHK3* were identified as cytokinin receptors shortly afterwards (Hwang and Sheen, 2001).

Arabidopsis mutants lacking all three receptors (*AHK2*, *AHK3* and *AHK4*) show no cytokinin response in a variety of assays and produce small infertile plants (Higuchi et al., 2004; Nishimura et al., 2004; Riefler et al., 2006). The receptors show differing sensitivity to different cytokinin species, suggesting there may be some specificity in the signalling pathway (Spíchal et al., 2004; Romanov et al., 2006). Recent research has found that a large majority of cytokinin receptors are present on the ER membrane, suggesting that this compartment may play an important role in cytokinin signal transduction (Caesar et al., 2011; Wulfetange et al., 2011). For an overview of our current understanding of cytokinin receptors, the reader is referred to a recent review by Heyl et al. (Heyl et al., 2012).

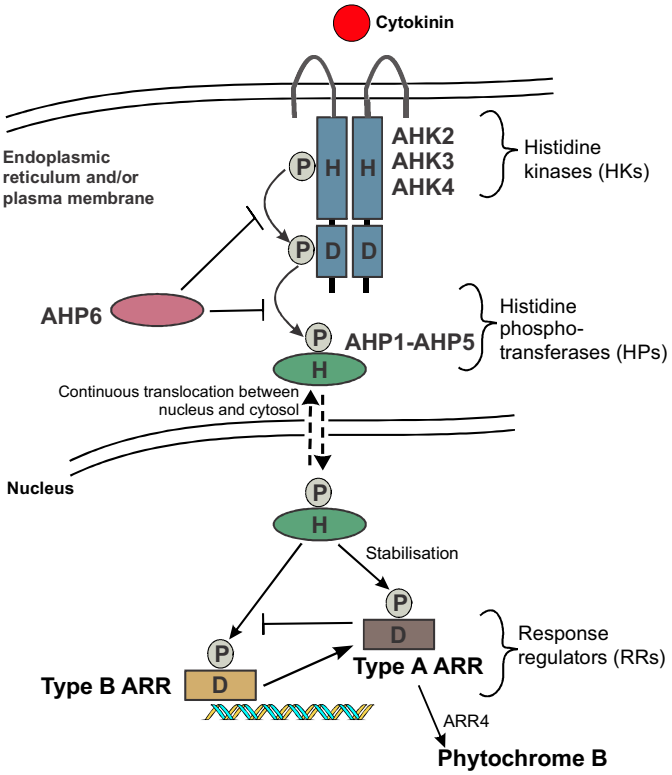

Fig. 2. Cytokinin signalling occurs via a two-component system. The cytokinin receptors *Arabidopsis* histidine kinases (AHKs) are primarily localized on the endoplasmic reticulum, as well as on the plasma membrane. Cytokinin binds to AHK proteins, inducing conformational changes that trigger a phosphorelay. A phosphoryl group (P) is first transferred from a conserved His (H) to an Asp (D) residue within the receptor and is then relayed to five *Arabidopsis* histidine phosphotransferase proteins (AHP1-AHP5). The pseudo-HP AHP6 inhibits cytokinin signalling by competing with AHP1-5 for phosphotransfer. The AHPs continuously translocate between the cytosol and the nucleus, where the *Arabidopsis* response regulators (ARRs) are in turn phosphorylated. Phosphorylation of the type A ARRs stabilizes them. The phosphorylated type B ARRs can bind DNA and initiate transcription of cytokinin-responsive genes, including the type A ARRs, which act as inhibitors of cytokinin signalling. Although type A ARRs are generally considered negative regulators, ARR4 has been shown to upregulate phytochrome B.

Histidine phosphotransferase proteins

The *Arabidopsis* genome encodes five HP proteins (AHP1-AHP5) (Suzuki et al., 1998) that have been shown to have phosphorelay activity *in vitro* (Imamura et al., 2001; Suzuki et al., 2002; Tanaka et al., 2004). A sixth HP protein, AHP6, lacks the conserved histidine residue and so acts as a pseudo-phosphotransferase; by competing with the true HPs, AHP6 acts as an inhibitor of cytokinin signalling (Mähönen et al., 2006). The AHP proteins are known to shuffle between the cytosol and the nucleus. Although earlier studies in protoplasts (Hwang and Sheen, 2001) reported that this translocation was cytokinin dependent, more recent work by Punwani et al. (Punwani et al., 2010) has shown that the translocation occurs continuously in a cytokinin-independent manner. The quintuple AHP mutant created by Hutchison et al. (Hutchison et al., 2006) has a reduced cytokinin phenotype, though not as severe as the triple receptor mutant. However, Deng et al. (Deng et al., 2010) noted that the *ahp2* allele in this mutant was not a null allele; replacing it with a null allele produced a quintuple AHP mutant that was seedling lethal.

Response regulators

The 23 functional response regulators (RRs) in *Arabidopsis* are divided into three groups, two of which (type A and type B) are involved in cytokinin signalling. Phosphorylation of the type A ARRs acts to stabilize them (To et al., 2007), whereas phosphorylation of the type B ARRs enables them to bind to DNA and initiate transcription of downstream targets, including the type A ARRs. D'Agostino et al. (D'Agostino et al., 2000) showed that the type A ARRs are transcriptionally upregulated by cytokinin. Type A ARRs only have a receiver domain and are generally thought to act as inhibitors of cytokinin signalling. Although genetic analysis has confirmed the negative activity of several of the type A ARRs (To et al., 2004), *ARR4* has also been shown to interact positively with phytochrome B (Sweere et al., 2001). However, type B ARRs have additional domains and are therefore able to activate the transcription of cytokinin responsive genes (Sakai et al., 1998; Sakai et al., 2000; Sakai et al., 2001). Work with high-order mutants (To et al., 2004; Mason et al., 2005; Argyros et al., 2008) and overexpression lines (Kiba et al., 2004; Tajima et al., 2004; Ren et al., 2009) has confirmed this general picture.

A novel class of response regulators was identified by Rashotte et al. (Rashotte et al., 2006). The CYTOKININ RESPONSE FACTOR (CRF) family consists of six closely related *APETALA2* (*AP2*) transcription factors, which were identified by a phylogenetic analysis of the ethylene response factor family. Although only *CRF2*, *CRF5* and *CRF6* were found to be upregulated by cytokinin, all six CRFs rapidly translocated to the nucleus upon cytokinin application. This upregulation required functional type B ARRs, whereas the migration of the CRFs to the nucleus was found to depend only on HK and HP proteins, thus representing a branching point in the two-component signalling pathway. Microarray analysis showed that the CRFs overlap with the type B ARRs in activating many cytokinin target genes; single and multiple loss-of-function mutations in the CRFs led to defects in cotyledon and leaf development, as well as embryo lethality in the case of *crf5 crf6*. Recently, published work has shown that the CRFs can form both homo- and heterodimers with one another, and has identified the protein domain required for CRF-CRF interaction and interactions with other cytokinin signalling components (Cutcliffe et al., 2011).

Cytokinin signalling targets

Numerous transcriptomics experiments using microarrays and, more recently, RNA-sequencing have been used to explore the downstream targets of cytokinin signalling (Rashotte et al., 2003; Brenner et al., 2005; Brenner and Schmülling, 2012). Argueso et al. (Argueso et al., 2010) have reviewed some of the transcriptional networks identified by these approaches, but one prominent class of genes that has received special attention includes those that are involved in crosstalk with other hormonal pathways; these genes, which include signalling genes responsive to auxin (*SHY2*/*IAA3*, *AXR3*/*IAA17*) and gibberellins (*GNL*/*CGA1*/*GATA22*, *GNC*/*GATA21*), have recently been reviewed by Brenner et al. (Brenner et al., 2012).

Cytokinin crosstalk with other hormones

Just as the turn of the century was a fruitful period for our understanding of cytokinin signalling, the new decade has seen an explosion in our understanding of the crosstalk between cytokinin and other hormone pathways, particularly auxin. For over half a century, interactions between auxin and cytokinin have been known

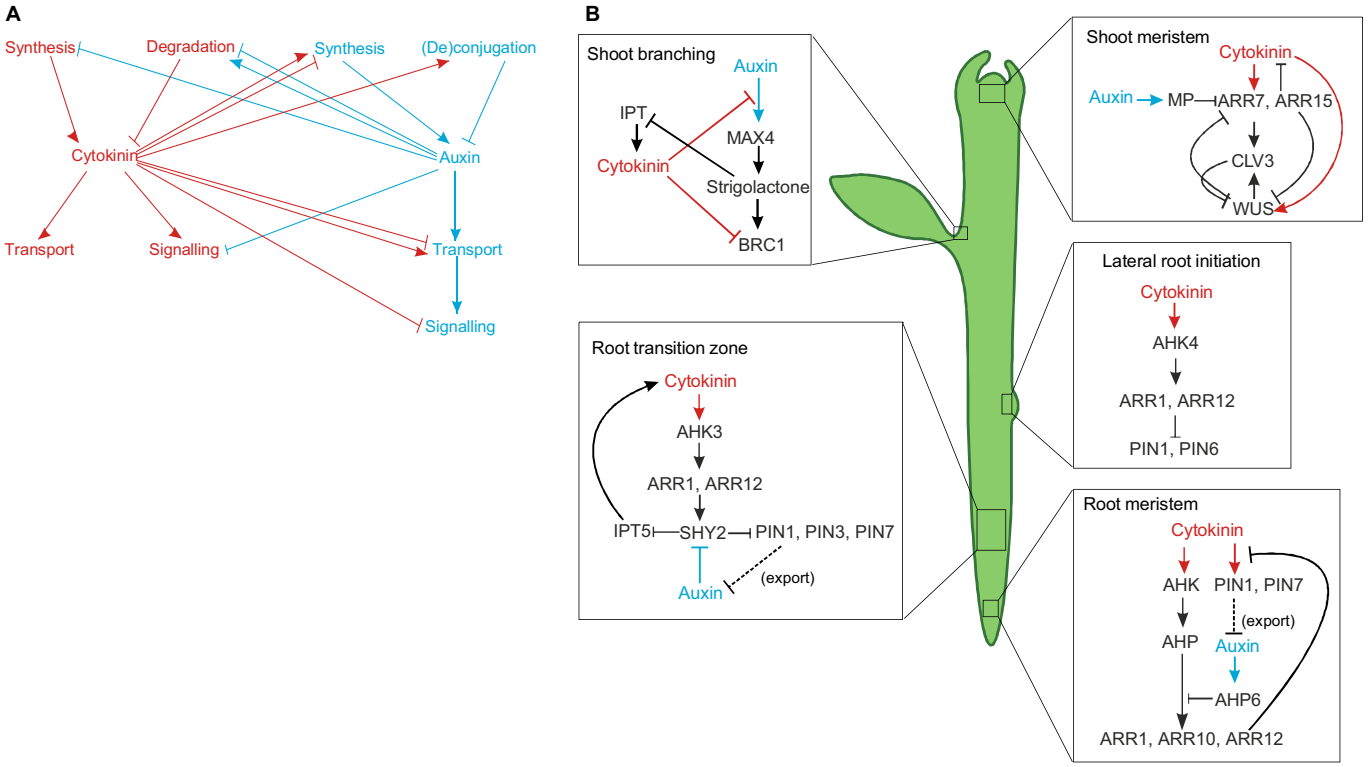

Fig. 3. Cytokinin interacts with auxin in several developmental contexts. (**A**) There are multiple points of interaction between the auxin (blue) and cytokinin (red) pathways. In addition to the inter-hormonal interactions shown, both hormones regulate their own metabolism and perception, adding further complexity to the pathways. (**B**) Interactions between auxin and cytokinin are crucial for many important aspects of plant development (e.g. in shoot branching, the shoot meristem, the root transition zone, lateral root initiation and in the root meristem; see main text for details) and most of these interactions involve mutual feedback loops. Auxin and auxin activity are depicted in blue; cytokinin and cytokinin activity are depicted in red. AHK, *Arabidopsis* histidine kinase 4; AHP, *Arabidopsis* histidine phosphotransferase; ARR, *Arabidopsis* response regulator ; BRC1, branched 1; CLV3, clavata 3; IPT, isopentenyltransferase; MAX4, more axillary branching 4; MP, monopteros; PIN, pin-formed; SHY, short hypocotyl; WUS, wuschel.

to be play a crucial role in many aspects of plant development, often acting antagonistically (Coenen and Lomax, 1997), but we are now beginning to assemble an understanding of this antagonism on a molecular and mechanistic level (Fig. 3; Tables 1, 2). Crosstalk between cytokinin and other hormones is also being unravelled at the same time, although the interactions with auxin have received most of the attention.

Over half a century ago, Skoog and Miller (Skoog and Miller, 1957) demonstrated that *in vitro* organogenesis could be controlled by varying the relative quantities of auxin and cytokinin in the growth medium. The antagonism between these two phytohormones has since been a continuing theme in plant biology, although the molecular and mechanistic bases for this antagonism have only recently begun to be unravelled. We now know that there is extensive crosstalk between the two hormones at all levels – synthesis, perception and transport – and we are beginning to understand how these networks interact (Fig. 3A) to control a wide variety of plant responses.

Auxin-mediated regulation of cytokinin synthesis and signalling

The expression of the IPT genes has been shown to respond to levels of auxin. An RT-PCR analysis of the IPT genes by Miyawaki et al. (Miyawaki et al., 2004) found that *IPT5* and *IPT7* were upregulated within 4 hours of auxin application; Dello Ioio et al. (Dello Ioio et al., 2008) later demonstrated that the upregulation of *IPT5* by auxin is mediated by *SHY2/IAA3*. A few years later, Tanaka et al. (Tanaka et al., 2006) confirmed the effect of auxin on *IPT* expression while investigating apical dominance in pea, *Pisum sativum*. Guided by the amino acid sequence of the *Arabidopsis* IPT proteins, they identified two IPT homologs in pea (*PsIPT1* and *PsIPT2*) that showed increased expression in the second nodal stem following decapitation, suggesting that auxin from the shoot apex normally represses these genes. This was confirmed by incubating excised stem segments with and without auxin; IPT expression only persisted in segments incubated without auxin. Finally, application of the auxin transport inhibitor 2,3,5-tri-iodobenzoic acid (TIBA) around the internode also led to increased IPT expression levels, demonstrating that these genes are normally repressed by auxin transported from the shoot apex.

However, other studies have found that treatment with auxin leads to a reduction in cytokinin biosynthesis. Using quantitative RT-PCR, Takei et al. (Takei et al., 2004b) observed a significant decrease in the expression of the cytokinin biosynthesis gene *CYP735A* when *Arabidopsis* seedlings were treated with auxin for 1 hour. Nordström et al. (Nordström et al., 2004) used deuterium labelling to directly measure the synthesis rates of the auxin indole-3-acetic-acid (IAA) and cytokinins; they found that treatment of *Arabidopsis* seedlings with auxin led to a significant reduction in cytokinin biosynthesis, while increasing cytokinin synthesis using an inducible version of the bacterial *ipt* gene did not affect IAA pool size or synthesis rate until 36 hours later. Based on this, they concluded that auxin can regulate the pool of available cytokinins but that the effect of cytokinin on auxin is

Table 1. Auxin regulates cytokinin activity

Component	Function	Effect of auxin	Reference
IPT5 and IPT7	Cytokinin biosynthesis	Upregulated	(Miyawaki et al., 2004)
IPT5	Cytokinin biosynthesis	Upregulated (via SHY2/IAA3)	(Dello Ioio et al., 2008)
CYP735A	Cytokinin biosynthesis	Downregulated	(Takei et al., 2004b)
PsIPT1 and PsIPT2	Cytokinin biosynthesis	Downregulated	(Tanaka et al., 2006)
CKX2, CKX4 and CKX7	Cytokinin degradation	Downregulated	(Werner et al., 2006)
CKX1 and CKX6	Cytokinin degradation	Upregulated, transport dependent	(Werner et al., 2006)
ARR7 and ARR15	Cytokinin signalling (type A ARR)	Upregulated in embryo	(Muller and Sheen, 2008)
ARR7 and ARR15	Cytokinin signalling (type A ARR)	Downregulated in shoot (via MP/ARF5)	(Zhao et al., 2010)
CRF2	Cytokinin signalling (response regulator)	Upregulated (via MP/ARF5)	(Schlereth et al., 2010)
AHP6	Cytokinin signalling repressor (pseudo-HP)	Upregulated	(Bishopp et al., 2011a)

AHP, *Arabidopsis* histidine phosphotransferase; ARF, auxin response factor; ARR, *Arabidopsis* response regulator; CKX, cytokinin oxidase; CRF, cytokinin response factor; CYP735A, cytochrome P450, family 735, subfamily A; IAA, indole-3-acetic acid inducible; IPT, isopentyl transferase; MP, monopteros; SHY, short hypocotyl.

indirect and therefore probably not an effective mechanism to control development.

In addition to regulating cytokinin biosynthesis, auxin has also been shown to affect cytokinin degradation by regulating the CKX gene family. Using semi-quantitative RT-PCR, Werner et al. (Werner et al., 2006) measured the response of the seven *Arabidopsis* CKX genes to auxin and found that *CKX2*, *CKX4* and *CKX7* were weakly downregulated in response to auxin. By contrast, *CKX1* and *CKX6* were reported to be upregulated by auxin, although they were also strongly downregulated by the application of the polar transport inhibitor 1-naphthylphthalamic acid (NPA), suggesting that their expression depends on polar auxin transport.

Auxin has also been discovered to regulate cytokinin signalling directly. In an elegant experiment, Müller and Sheen (Müller and Sheen, 2008) showed that auxin directly activates two type A ARRs, *ARR7* and *ARR15,* thereby inhibiting cytokinin signalling (Fig. 3B). They noted that the domain of *ARR7* and *ARR15* expression in *Arabidopsis* embryos corresponded more with the auxin signalling domain than the cytokinin signalling domain, as reported by a synthetic reporter of two-component signalling, TCS::GFP. Furthermore, treating embryos with the synthetic auxin 2,4-D led to an expansion of the ARR domain. Following this, the researchers identified auxin-responsive elements in the promoters of these two genes and were able to abolish the auxin response by generating point mutations in these response elements. Zhang et al. (Zhang et al., 2011) showed that, contrary to earlier results, the *arr7 arr15* double mutant is not embryo lethal. Earlier reports of gametophytic or embryo lethality resulted from reduced pollen production in the *arr7* or *arr15* heterozygous mutants; the homozygous mutants of either gene produce viable pollen.

Zhao et al. (Zhao et al., 2010) found contrasting results in the shoot apical meristem (SAM), where they reported that auxin represses *ARR7* and *ARR15*; several hours after treatment of the inflorescence apex with the auxin naphthaleneacetic acid (NAA), the transcripts of both genes were reduced by about half. Expression of *ARR7* and *ARR15* drastically increased following treatments with the protein synthesis inhibitor cycloheximide; this is characteristic of auxin-regulated genes, as they are normally repressed by a bound auxin response factor (ARF). Expression also increased in the *arf5/mp* mutant, suggesting that *ARF5/MP* (*MONOPTEROS*) might mediate the auxin-dependent regulation of these ARRs; chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assay confirmed that MP binds directly to the auxin-responsive elements (REs) in the *ARR15* promoter. Another response regulator, *CRF2*, was simultaneously identified as a target of *MP/ARF5* in a microarray and was confirmed by ChIP (Schlereth et al., 2010). By mutating the auxin REs in the *ARR15* promoter, Zhao et al. (Zhao et al., 2010) showed that these elements repress *ARR15* in the SAM but activate it in other domains, explaining the discrepancy between their observations and those of Muller and Sheen. These results demonstrate the complexity of the crosstalk between hormones: various components can be up- or downregulated in a context-dependent manner, providing an elegant mechanism for interaction between the two hormone pathways. Owing to the differing response of these type A ARRs, auxin and cytokinin are able to act antagonistically in the embryonic root meristem but synergistically in the SAM.

Cytokinin-mediated modulation of auxin metabolism and transport

Conversely, cytokinin regulates auxin biosynthesis. By measuring the incorporation of deuterium into IAA by gas chromatography – multiple reaction monitoring-mass spectrometry (GC-MRM-MS), Jones et al. (Jones et al., 2010) observed that treatment with various cytokinins led to increased auxin synthesis in young leaves, the shoot apex and the root system; this effect was amplified in the cytokinin hypersensitive ARR mutant *arr3 arr4 arr5 arr6*. By contrast, treatment of the cytokinin-insensitive quadruple AHP mutant *ahp1 ahp2 ahp3 ahp4* resulted in a decrease in IAA biosynthesis, suggesting that the relationship between cytokinin signalling and auxin synthesis may not be straightforward; roots of this mutant also had a higher baseline rate of IAA biosynthesis than wild type, further hinting at the complexity of this interaction. Cytokinin treatments did not increase IAA biosynthesis in the *axr3-1* mutant, which is hypersensitive to auxin because the increased stability of the AXR3/IAA17 protein; this mutant also has increased steady state levels of IAA. Transcript profiling following cytokinin induction identified many auxin biosynthesis genes as upregulated; genes responsible for IAA storage and degradation via conjugation and deconjugation also responded positively to cytokinin (Table 2). Taken together, these data suggest that degradation of IAA17 is important in maintaining auxin levels and that cytokinin regulates auxin metabolism by increasing the production or stability of this protein.

Several research groups independently discovered that cytokinin also regulates auxin signalling and, in particular, auxin transport. Laplaze et al. (Laplaze et al., 2007) showed that the repression of lateral root primordium initiation by cytokinin happens via changes to the PIN class of auxin efflux transporters (Fig. 3B). Based on observations of transcriptional reporter lines, they reported that the expression of *PIN1* and *PIN6* in lateral root primordia is reduced and diffuse when plants are treated with cytokinin; cytokinin treatments also led to reduced expression of *PIN1*, *PIN2*, *PIN3*,

Table 2. Cytokinin regulates auxin activity

Component	Function	Effect of cytokinin	Reference
ASA1/WEI2, PAT/TRP1 and IGPS	Biosynthesis of auxin precursor (Trp)	Upregulated	(Jones et al., 2010)
CYP79B2, YUCCA6 and NIT3	Trp-dependent auxin biosynthesis	Upregulated	(Jones et al., 2010)
AMI1, YUCCA5 and YUCCA5-like	Auxin biosynthesis	Downregulated	(Jones et al., 2010)
GH3.17 and GH3.9	Auxin conjugation (inactivation)	Upregulated	(Jones et al., 2010)
ILL6	Auxin de-conjugation (activation)	Upregulated	(Jones et al., 2010)
AXR1	Regulation of auxin signalling (involved in degradation of Aux/IAA)	Upregulated	(Brenner and Schmulling, 2012)
IAA7, IAA13 and others	Regulation of auxin signalling (AUX/IAA transcriptional repressors)	Downregulated	(Brenner and Schmulling, 2012)
AXR3/IAA17	Regulation of auxin signalling (Aux/IAA transcriptional repressor)	Increased production/stability	(Jones et al., 2010)
SHY2/IAA3	Regulation of auxin signalling (Aux/IAA transcriptional repressor)	Upregulated	(Taniguchi et al., 2007; Dello Ioio et al., 2008)
AUX1	Auxin influx transporter	Upregulated	(Brenner et al., 2005)
PsAUX1	Auxin influx transporter	Upregulated	(Kalousek et al., 2010)
PsPIN1	Auxin efflux transporter	Upregulated, basalized	(Kalousek et al., 2010)
PIN1	Auxin efflux transporter	Lateralized	(Bishopp et al., 2011a)
PIN1	Auxin efflux transporter	Endocytosed (via AHK4, ARR2 and ARR12)	(Marhavy et al., 2011)
PIN2	Auxin efflux transporter	Upregulated	(Brenner and Schmulling, 2012)
PIN3	Auxin efflux transporter	Downregulated, but perhaps via auxin?	(Bishopp et al., 2011a)
PIN7	Auxin efflux transporter	Upregulated	(Bishopp et al., 2011a; Růžička et al., 2009; Dello Ioio et al., 2008)
PIN1, PIN3 and PIN4	Auxin efflux transporter	Downregulated post-transcriptionally	(Zhang et al., 2011)
PIN1 and PIN6	Auxin efflux transporter	Downregulated in lateral root initiation	(Laplaze et al., 2007)
PIN1, PIN 3, PIN4 and PIN7	Auxin efflux transporter	Downregulated in shoot	(Laplaze et al., 2007)
PINOID	Regulator of subcellular PIN localization	Downregulated	(Brenner and Schmulling, 2012)

AHK, *Arabidopsis* histidine kinase; ARR, *Arabidopsis* response regulator; ASA1, anthranilate synthase, alpha subunit 1; AUX, auxin; AXR, auxin resistant; CYP79B2, cytochrome P450, family 792, subunit family B; IGPS, indole-3-glycerol phosphate synthase; IAA, indole-3-acetic acid inducible; ILL, IAA-leucine resistant (ILR)-like; PAT, phosphoribosylanthranilate transferase; PIN, pin-formed; TRP, tryptophan; WEI1, weak ethylene insensitive 2.

PIN4 and *PIN7* in the shoot. These findings led them to conclude that cytokinin disrupts PIN patterning during lateral root initiation.

Subsequent studies demonstrated that the size of the *Arabidopsis* primary root meristem is also carefully controlled by a balance between auxin, which controls the rate of cell division, and cytokinin, which controls the rate of cell differentiation (Fig. 3B). In a seminal paper, Dello Ioio et al. (Dello Ioio et al., 2008) investigated the mechanism behind this antagonism. Overexpression of *ARR1*, a type B ARR, led to a significant reduction in root meristem size. Earlier work (Taniguchi et al., 2007) had identified the Aux/IAA gene *IAA3*/*SHY2* as a target of *ARR1*. Dello Ioio et al. therefore examined *shy2* gain- and loss-of-function mutant plants; while the meristem was reduced in the gain-of-function mutant, the loss-of-function mutant had a larger root meristem along with faster growth, which led to longer roots that closely resembled *arr1* mutants. Expression of the *SHY2*::*GUS* reporter line was hardly detectable in the *arr1* mutant, in contrast with wild type where it was active in the vascular transition zone. Furthermore, induction of *ARR1* had no effect on the root meristem in loss-of-function *shy2* mutants, consistent with a role for *SHY2* downstream of *ARR1*. Finally, ChIP experiments confirmed a direct interaction between *ARR1* and *SHY2*. Noting that the expression of *PIN1*, *PIN3* and *PIN7* in the vascular tissues was downregulated by cytokinin, Dello Ioio et al. speculated that this might be mediated via *AHK3*, *ARR1* and *SHY2*. Indeed, expression of these PIN genes was cytokinin insensitive in the *arr1*, *ahk3* and *shy2* loss-of-function mutants; expression was also reduced in the *shy2* gain-of-function mutant. In addition to mediating cytokinin regulation of PIN genes, *SHY2* was also found to negatively regulate the cytokinin biosynthesis gene *IPT5*. Dello Ioio et al. therefore proposed a model in which the size of the root meristem is determined by an antagonism between auxin and cytokinin in the vascular transition zone; this antagonism is mediated by opposing regulation of *SHY2*, which in turn negatively regulates cytokinin biosynthesis while repressing auxin transport and signalling (Fig. 3B). This dual negative-feedback loop centred on *SHY2* establishes a robust mechanism for controlling meristem size. This model was later extended by Moubayidin et al. (Moubayidin et al., 2010) to include upregulation of *SHY2* by *ARR12* during meristem growth.

Further evidence that cytokinin modulates auxin transport by regulating PIN transporters came from a study by Růžička et al. (Růžička et al., 2009). As cytokinin and 2,4-D, which is poorly transported thanks to its low affinity with PIN transporters, have a similar effect on the root meristem, they speculated that the effect of cytokinin may be mediated via a reduction in auxin transport. Using tobacco cell cultures, they found that cytokinin treatment led to a decrease in auxin efflux. Following this, they showed that cytokinin decreases the expression of *PIN1* and *PIN3* in the *Arabidopsis* root meristem while increasing the expression of *PIN7*. The effect of cytokinin on *PIN1* was reduced in the *cre1* and *cre1 ahk2* cytokinin receptor mutants, confirming that this regulation is dependent on functional cytokinin signalling.

Following this work, a growing body of research describing the regulation of PIN transporters by cytokinin has accumulated. For example, Kalousek et al. (Kalousek et al., 2010), observed rapid upregulation of *PsPIN1* and *PsAUX1* expression in the axillary buds of pea plants following the application of cytokinin; cytokinin treatment also caused *PsPIN1* to become localised to the basal

membrane of competent cells. Similar changes were observed following decapitation, in keeping with earlier findings (Tanaka et al., 2006), showing that cytokinin-mediated regulation of polar auxin transport may play a role in regulating apical dominance.

More recently, Bishopp et al. (Bishopp et al., 2011a) described a mutually inhibitory feedback loop between auxin and cytokinin that is involved in patterning the *Arabidopsis* stele. The vascular cylinder of the *Arabidopsis* root is precisely and consistently patterned; a central xylem axis is flanked by intervening procambial cells with the phloem poles located perpendicular to the xylem axis. Bishopp et al. observed an auxin signalling maximum throughout the xylem axis and a complementary cytokinin signalling domain in the procambium. Further investigation revealed that treatment with the polar auxin transport inhibitor NPA excluded the auxin response from the stele and altered the expression pattern of the pseudo-phosphotransferase *AHP6*, which was shown to be regulated by auxin. Because NPA inhibits auxin efflux, Bishopp et al. next investigated the role of PIN transporters in this patterning process. A weak vascular phenotype was found in *pin1* but not in the other single *pin* mutants; however, the *pin3 pin7* double mutant showed unstable formation of the auxin signalling maximum. Furthermore, the expression patterns of *PIN1*, *PIN3* and *PIN7* were shown to depend on cytokinin signalling. Reduction of cytokinin signalling in the cytokinin receptor mutants or by induction of *CKI* expression in the vascular domain led to a reduction in the *PIN7* expression domain and altered *PIN3* expression; lateral localization of *PIN1* in vascular cells may also depend on cytokinin signalling, as vascular cells of *wol* mutants only have basally localized *PIN1*. Based on these observations, Bishopp et al. proposed a model wherein high cytokinin signalling in the procambial cells generates a bisymmetric pattern of PIN localisation that directs the radial transport of auxin into the central axis, where high auxin signalling promotes the expression of *AHP6*, which reciprocally restricts the domain of high cytokinin signalling. Thus, a feedback loop (Fig. 3B) involving hormone transport dynamics and mutually inhibitory hormone interactions provides a mechanism to sharpen the boundary between the hormone domains and generate positional information for patterning.

Researchers have recently begun to elucidate some of the ways that cytokinin may regulate the PIN transporters. Zhang et al. (Zhang et al., 2011) compared the protein and mRNA levels of *PIN1*, *PIN3*, *PIN4* and *PIN7* in wild-type plants and in the multiple type A *arr3, arr4, arr5, arr6, arr7, arr8, arr15* mutant both with and without cytokinin. The mRNA levels did not differ significantly between the wild-type and mutant plants; *PIN1* and *PIN3* mRNA levels did not change upon cytokinin treatment, whereas *PIN4* mRNA levels increased. By contrast, expression of all three GFP-fusion proteins decreased with cytokinin treatment. These discrepancies led Zhang et al. to conclude that cytokinin-mediated regulation of these PIN transporters occurs primarily at a post-transcriptional level. In line with this, Marhavý et al. (Marhavý et al., 2011) found that cytokinin downregulated *PIN1* at the plasma membrane even when it was driven by the constitutive *35S* promoter, indicating that this regulation occurs at the protein level. Downregulation of *PIN1* by cytokinin occurred very quickly, with a 40% reduction in less than 2 hours. They observed transport of PIN1-GFP to the vacuole and were able to prevent this trafficking by pharmacological depolymerisation of actin or inhibition of vacuolar targeting. The BFA-visualized endocytosis trafficking defective mutants *ben1* and *ben2*, which are both defective in PIN1 endocytosis, did not show a decrease in PIN1-GFP following cytokinin treatment. Furthermore, cytokinin degradation of PIN1 was robust in the *ahk2* and *ahk3* single and double mutants but disrupted in the *cre1/ahk4* mutant; cytokinin also failed to degrade PIN1 in *arr2* and *arr12* mutants. Together, these data indicate that cytokinin signalling mediated via the *AHK4* receptor, ARR2 and ARR12 leads to endocytic trafficking of PIN1 from the plasma membrane to lytic vacuoles for degradation.

Cytokinin-gibberellin crosstalk

Gibberellins are phytohormones involved in a wide range of processes in plant development, from stem elongation and seed germination to regulating meristem size. Exogenous gibberellin application, which increases the size of the root meristem, was found to strongly downregulate *ARR1* expression in 5-day-old plants but not in 3-day-old plants. Root meristems were uniformly insensitive to gibberellin 3 days after germination; 2 days later, the meristems of *arr1-3* plants remained insensitive while wild-type meristems showed a response. The DELLA protein Repressor of GA 1-3 is expressed in the root transition zone and shows an increase in expression between 3 and 5 days after germination; the loss-of-function *rga-24* mutant was found to have decreased levels of *ARR1* and consequently of *SHY2* 5 days after germination. Taken together, these data indicate that a reduction of gibberellin levels 5 days after germination releases *ARR1* from repression, which upregulates *SHY2* (Fig. 4A) and leads to an increase in cell differentiation, which balances with cell division to control the size of the root meristem (Moubayidin et al., 2010).

Ethylene-cytokinin crosstalk

It has been known for some time that cytokinin also regulates the biosynthesis of ethylene, which serves as a hormone in plants. As the application of cytokinin to dark-grown plants resulted in the ‘triple response’ that is characteristic of ethylene, Cary et al. (Cary et al., 1995) tested the role of ethylene in the cytokinin response of seedlings. They treated the ethylene-resistant mutants *ein1* and *ein2* with cytokinin and observed that these mutants were resistant to cytokinin-induced reduction of root and hypocotyl growth. Furthermore, the effects of cytokinin treatment in wild-type plants were reduced by the application of inhibitors of ethylene signalling or biosynthesis. These results demonstrate that the effect of cytokinin on root and hypocotyl growth is mediated by ethylene. Consistent with this, Penmetsa et al. (Penmetsa et al., 2008) observed that ethylene-resistant mutants in *Medicago* were resistant to cytokinin-mediated effects on root growth.

Following this, Vogel et al. (Vogel et al., 1998) suggested that cytokinin post-transcriptionally increases the activity of the ethylene biosynthesis gene *1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE 5* (*ACS5*). Subsequently, Woeste et al. (Woeste et al., 1999) demonstrated that treatment of etiolated seedlings with either cytokinin or auxin led to increased ethylene levels, while treatment with both hormones had a synergistic effect. Because they found that auxin, but not cytokinin, led to increased levels of *ACS4* mRNA, Woeste et al. suggested that cytokinin acts post-transcriptionally to increase *ACS4* function.

Continuing work by Joseph Kieber’s group has further elucidated the mechanics of this interaction (Fig. 4B). The *Arabidopsis* mutants *eto1*, *eto2* and *eto3* were identified as ethylene over-producing mutants owing to a constitutive triple-response phenotype (Guzmán and Ecker, 1990; Kieber et al., 1993). Positional cloning identified *eto2* and *eto3* as mutations in genes from the ACS family (Vogel et al., 1998; Chae et al., 2003), which encode enzymes that catalyse the first committed, and generally rate-limiting, step in ethylene biosynthesis. The *eto2* and *eto3*

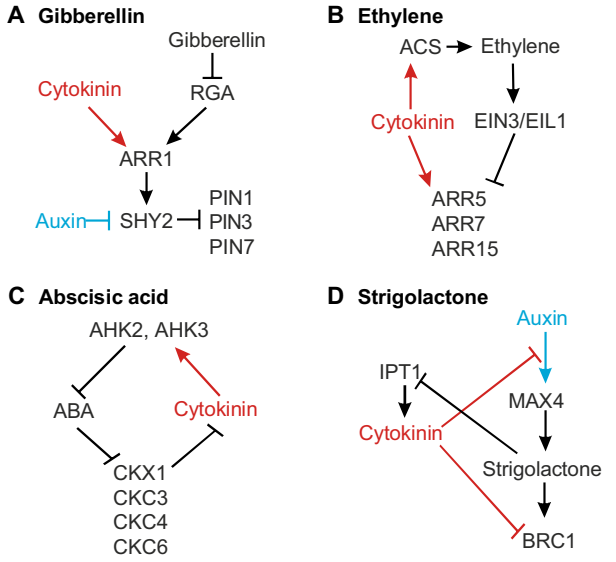

Fig. 4. Interactions between cytokinin and other hormones. (**A**) Cytokinin and gibberellin signalling come together in the regulation of *ARR1*, the expression of which is repressed by gibberellin (via degradation of the DELLA protein RGA, which promotes *ARR1* expression) and promoted by cytokinin. The regulation of *SHY2* by *ARR1* also represents a point of crosstalk with auxin, thus connecting three hormones in one network. (**B**) Ethylene and cytokinin signalling converge in the control of the type A ARRs *ARR5*, *ARR7* and *ARR15*, which play a role in freezing tolerance. Cytokinin also increases ethylene biosynthesis by increasing the stability of ACS proteins, which catalyse the rate-limiting step in ethylene biosynthesis. (**C**) Components of the cytokinin two-component signalling system interact with abscisic acid (ABA), regulating salinity and drought response. ABA represses several CKX genes, whereas *AHK2* and *AHK3* downregulate many ABA-responsive genes. The loop shown in the figure will buffer against changes in the concentration of either hormone, though the interaction is likely to be more complex in reality. (**D**) Cytokinin interacts with strigolactones to regulate the outgrowth of axillary buds. In addition to inhibiting the transcription factor *BRC1*, which acts downstream of strigolactones, cytokinins prevent auxin-mediated regulation of the strigolactone biosynthesis gene *MAX4*. Strigolactones, in turn, negatively regulate cytokinin biosynthesis through *IPT1*. Auxin and auxin activity are depicted in blue; cytokinin and cytokinin activity are depicted in red. ACS, aminocyclopropane-1-carboxylic acid synthase; AHK, *Arabidopsis* histidine kinase 4; ARR, *Arabidopsis* response regulator; BRC1, branched 1; EIL1, ethylene-insensitive 3-like 1; EIN3, ethylene-insensitive 3; IPT1, isopentenyltransferase 1; MAX4, more axillary branching; PIN, pin-formed; SHY, short hypocotyl.

mutations are found within the C-terminal domains of *ACS5* and *ACS9*, respectively, and seem to influence post-transcriptional regulation of the proteins. Chae et al. (Chae et al., 2003) showed that *eto2* does not affect the activity of *ACS5*; using a myc-tagged, dexamethasone-inducible *ACS5*, they were able to show that the *eto2* mutation increases the stability of the ACS5 protein. In wild-type plants, cytokinin was found to increase the synthesis and half-life of the myc-tagged construct; exogenous cytokinin also increased the steady-state ACS5 levels in the myc-tagged *eto2* mutant, leading Chae et al. to suggest that the effect of cytokinin and the *eto2* mutation are at least partially independent.

Shi et al. (Shi et al., 2012) reported that ethylene, in turn, modulates cytokinin signalling. They observed increased transcription of *ARR5*, *ARR7* and *ARR15* in the *ein3 eil1* double mutant compared with wild type; cold induction of these ARRs was also consistently higher in the mutant than in wild-type plants. These results indicate that the ethylene-responsive transcription factor *EIN3* represses these ARRs, a finding that was confirmed by direct binding assays. Furthermore, plants overexpressing these ARRs showed enhanced tolerance to freezing treatments; exogenous cytokinin treatment also enhanced the survival of freezing treatments. Treatment with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) led to a dramatic reduction in the expression of these ARRs and blocked their induction by cold temperatures, thereby reducing the freezing tolerance of the plants. Shi et al. therefore proposed that *ARR5*, *ARR7* and *ARR15* represent a key node that integrates ethylene and cytokinin signalling in regulating the freezing response of plants.

Crosstalk with abscisic acid

Abscisic acid (ABA) is a plant hormone involved in seed dormancy, growth inhibition and stress response. Though not much is known about the interaction between cytokinin and ABA, several experiments have indicated that crosstalk occurs (Fig. 4C). Microarray and RT-PCR experiments demonstrated that *CKX1*, *CKX3*, *CKX4* and *CKX6* are downregulated by ABA (Werner et al., 2006). In addition, cytokinin receptor mutants show increased drought and salinity tolerance, a process known to involve ABA; a genome-wide expression analysis identified many upregulated ABA-responsive genes in the *ahk2 ahk3* double mutant (Tran et al., 2007). Furthermore, Jeon et al. (Jeon et al., 2010) showed that *AHK2* and *AHK3* are involved in mediating the cold stress response by inhibiting ABA signalling via type A ARRs, although this may occur independently of endogenous cytokinin.

Crosstalk with strigolactone

Originally identified from branching mutants of *Arabidopsis thaliana*, *Pisum sativa* and other plants, strigolactones are a class of phytohormones that inhibit the outgrowth of axillary buds. Cytokinins are also known to play a role in axillary branching in the shoot. Increased levels of strigolactones in pea and *Arabidopsis* lead to reduced cytokinin levels in the xylem sap, although levels in the shoot remain unchanged (Foo et al., 2007). Dun et al. (Dun et al., 2009) found that the buds of strigolactone-insensitive plants also have reduced sensitivity to cytokinin supplied from the vasculature. Cytokinin may also prevent auxin upregulation of *MAX4*, a strigolactone biosynthesis gene (Bainbridge et al., 2005). In a recent study, Dun et al. (Dun et al., 2012) found that the *rms1* and *rms4* pea mutants, which are deficient in and insensitive to strigolactones, respectively, had increased levels of *PsIPT1* expression in the nodes and internodes of the shoot. The *rms1* mutant was also more sensitive to low levels of cytokinin supplied to the vasculature or directly to the bud than wild type; buds grew out at lower levels of cytokinin in *rms1* plants than in wild type. Taken together, these data suggest that cytokinin and strigolactone may act antagonistically in regulating bud outgrowth. Consistent with this, exogenous application of the synthetic strigolactone GR24 combined with cytokinin attenuated the effect of cytokinin on bud growth in *rms1* but not in *rms4* mutants, indicating that the effect of strigolactone on cytokinin is mediated by *RAMOSUS4* (*RMS4*). The antagonism between strigolactone and cytokinin results from their common target, *PsBRC1*, which encodes a strigolactone-responsive TCP transcription factor thought to act downstream of strigolactones in non-growing buds. *PsBRC1* expression, which is negatively correlated with bud growth (Aguilar-Martínez et al., 2007), was found to be upregulated by GR24 and downregulated by cytokinin even in the presence of the protein-synthesis inhibitor cycloheximide,

Table 3. Interactions between cytokinin and other plant hormones

Component	Function	Interaction	Reference
ARR1	Cytokinin signalling (type B ARR)	Repressed by GA	(Moubayidin et al., 2010)
ARR5, ARR7 and ARR15	Cytokinin signalling (type A ARR)	Repressed by ethylene	(Shi et al., 2012)
CKX1, CKX3, CKX4 and CKX6	Cytokinin degradation	Downregulated by ABA	(Werner et al., 2006)
PsIPT1	Cytokinin biosynthesis	Upregulated by strigolactone	(Dun et al., 2012)
ACS4	Ethylene biosynthesis	Cytokinin increases protein activity	(Woeste et al., 1994)
ACS5 and ACS9	Ethylene biosynthesis	Cytokinin increases protein stability	(Chae et al., 2003)
Forty genes	ABA responsive genes	Repressed by cytokinin	(Tran et al., 2007)
MAX4	Strigolactone biosynthesis	Auxin upregulation is inhibited by cytokinin	(Bainbridge et al., 2005)
PsBRC1	Strigolactone responsive transcription factor	Downregulated by cytokinin	(Dun et al., 2012)

ACS, 1-aminocyclopropane-1-arboxylic acid synthase; ARR, *Arabidopsis* response regulator; BRC1, branched 1; CKX, cytokinin oxidase; IPT, isopentyl transferase; MAX4, more axillary branching 4.

indicating that this regulation does not require protein synthesis. Furthermore, *PsBRC1* expression was found to increase upon treatment with cycloheximide, suggesting that it may be repressed by a rapidly turned over protein. Dun et al. (Dun et al., 2012) suggest that strigolactones and cytokinin may interact quite closely with BRC1 or with the stable proteins that regulate it (Fig. 3B; Fig. 4D; Table 3).

Conclusions

The past 5 years have seen significant advances in our understanding of the extensive crosstalk between cytokinin and various other phytohormones. Antagonisms and interactions that have been known from early physiological and anatomical experiments are now being understood at a molecular and genetic level. The picture that is emerging is one of dizzyingly interconnected networks at all levels, from synthesis and degradation to perception and transport. As the number of interconnections and the complexity of the feedbacks grow, it becomes increasingly challenging to understand intuitively the dynamics of these networks and form insightful hypotheses about their behaviour. The recent growth of computational and systems biology approaches is a welcome development that can help to alleviate these difficulties. Modelling hormonal interaction networks and simulating them *in silico* will be invaluable in helping us to understand their dynamics, allowing us to formulate better hypotheses and even to predict possible novel interactions and partners (Ibañes et al., 2009; Chickarmane et al., 2012; Naseem et al., 2012).

As we learn more about how tightly intertwined the hormone synthesis, metabolism, signalling and transport networks are, the intriguing possibility of considering them as one entity emerges. Auxin has a dominant presence in plant morphogenesis; it is an inescapable player in many developmental processes and a central component of the crosstalk networks, as well as being the first class of phytohormone described. Could it be that the main role of cytokinin and the other hormones is, in fact, to modulate auxin transport, signalling and homeostasis? Given the pervasive crosstalk between auxin and cytokinin, it is possible that most or all of the developmental effects of cytokinin somehow involve auxin, raising the issue of whether there exist any truly auxin-independent cytokinin-driven processes. If so, should auxin be considered the primary driver in plant development? Or should it instead be considered a read-out, integrating information about the total hormonal status of the plant? As we continue to discover how intertwined and self-referential the hormone networks are, we may come to consider both sides of the coin at once and find that it acts as simultaneously as a driver and as a readout.

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Competing interests statement

The authors declare no competing financial interests.

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