REVIEW

nature chemical biology

Plant hormones are versatile chemical regulators of plant growth

Aaron Santner[1], Luz Irina A Calderon-Villalobos[2] & Mark Estelle[2]

The plant hormones are a structurally unrelated collection of small molecules derived from various essential metabolic pathways. These compounds are important regulators of plant growth and mediate responses to both biotic and abiotic stresses. During the last ten years there have been many exciting advances in our understanding of plant hormone biology, including new discoveries in the areas of hormone biosynthesis, transport, perception and response. Receptors for many of the major hormones have now been identified, providing new opportunities to study the chemical specificity of hormone signaling. These studies also reveal a surprisingly important role for the ubiquitin-proteasome pathway in hormone signaling. In addition, recent work confirms that hormone signaling interacts at multiple levels during plant growth and development. In the future, a major challenge will be to understand how the information conveyed by these simple compounds is integrated during plant growth.

Pioneering studies in the nineteenth century by Julius von Sachs and Charles Darwin demonstrated that various plant growth processes are regulated by "substances" that move from one part of the plant to another[1,2]. Over a century later we know that the substances in question are small molecules derived from various essential metabolic pathways. In general these compounds are present at very low concentrations and act either locally, at or near the site of synthesis, or in distant tissues. Over the years, the pantheon of plant hormones has been growing and now includes (but is not limited to) abscisic acid (ABA), indole-3-acetic acid (IAA or auxin), brassinosteroids (BRs), cytokinin, gibberellic acid (GA), ethylene, jasmonic acid (JA) and salicylic acid (**Fig. 1**). Collectively these compounds regulate every aspect of plant life, from pattern formation during development to responses to biotic and abiotic stress. Plants also use several peptide hormones to regulate various growth responses, but this class of hormones will not be discussed further in this review[3].

Although the physiological function of most of these compounds has been studied for decades, the last 10 to 15 years have seen dramatic progress in our understanding of the molecular mechanisms of hormone biosynthesis, transport and response. The biosynthetic pathways for most of the hormones are either well characterized (as in the case of ABA, BR and GA) or emerging (as for auxin and JA). What we are learning is that hormone levels are highly regulated and responsive to a changing environment. In the case of auxin, local and long-distance transport of the hormone have an essential role in many aspects of plant growth and development, whereas transport of volatile compounds such as ethylene and methyl jasmonate is important for plant defense.

There have also been dramatic advances in the area of hormone response, including the identification of receptors for most of the major hormones. Several themes have emerged from this work. First, plant hormone receptors are diverse and different from animal hormone receptors. Second, regulated protein degradation plays a central role in hormone signaling. Several receptors are themselves enzymes in the ubiquitin-protein conjugation pathway. In addition, the levels of key downstream signaling proteins are regulated by ubiquitin-dependent degradation. Third, hormone signaling generally leads to major changes in transcription. Although there are likely to be nongenomic hormonal responses, in general these are not as well characterized as the transcriptional responses. Fourth, the hormones interact at multiple levels to regulate various growth and defense processes. In this article we review some of the exciting recent advances in hormone biology.

Auxin

The major naturally occurring auxin is IAA. Synthetic auxins such as 2,4-dichlorophenoxyacetic acid are widely used as herbicides[4]. IAA has been implicated in virtually every aspect of plant growth and development, as well as defense responses. This diversity of function is reflected by the extraordinary complexity of IAA biosynthetic, transport and signaling pathways. IAA is synthesized from tryptophan via at least two pathways: the tryptamine (TAM) and indole-3-pyruvic acid (IPA) pathways[5]. At least in *Arabidopsis thaliana*, studies indicate that these pathways have overlapping functions because plants deficient in either pathway have similar growth defects. To further complicate matters, genes that function in the TAM and IPA pathways are present in gene families[5]. In addition, studies in *Arabidopsis* and maize indicate that IAA can also be synthesized from indole, bypassing tryptophan[6]. All of this suggests that auxin levels are highly regulated, which is consistent with its central role in plant growth.

Once synthesized, auxin is distributed throughout the plant via a sophisticated cell-to-cell transport system[7]. Cellular auxin influx and efflux carriers promote the formation of local auxin maxima and gradients that inform diverse growth and developmental processes. Just to name one example, the initiation of leaves on the flank of the shoot apical meristem requires local accumulation of auxin at the site of organ

[1]Molecular Kinetics, Inc., Indianapolis, Indiana, USA. [2]University of California San Diego, Section of Cell and Developmental Biology, La Jolla, California, USA. Correspondence should be addressed to M.E. (mestelle@ucsd.edu).

Published online 17 April 2009; doi:10.1038/nchembio.165

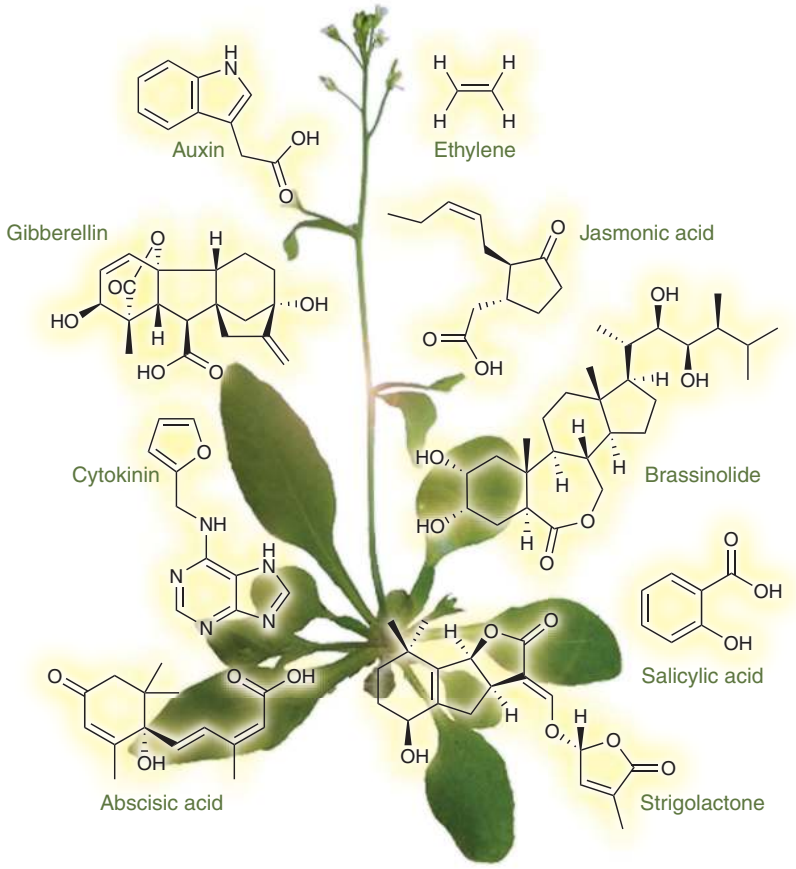

Figure 1 Phytohormones regulate all aspects of plant growth and development.

primordium formation[7]. The direction of auxin transport, and hence the position of local auxin maxima, is determined by asymmetric localization of transport proteins within cells. Cellular localization is highly dynamic and responsive to both developmental and environmental cues. Because of the importance of these processes to plant development, this is an extremely active and exciting area of investigation.

There is evidence for both genomic and nongenomic auxin responses, but the mechanisms of auxin-regulated transcription are much better characterized[8]. Auxin regulates transcription through the action of two large families of transcription factors called the auxin/indole-3-acetic acid (Aux/IAA) proteins and the AUXIN RESPONSE FACTORs (ARFs) (**Fig. 2**). ARFs directly bind DNA and either activate or inhibit transcription depending on the type of ARF[9]. The Aux/IAA proteins bind to the ARFs and repress auxin-regulated transcription by recruiting a co-repressor called TOPLESS (TPL)[10].

Importantly, the Aux/IAA proteins are short-lived and their degradation requires the ubiquitin-proteasome pathway[11]. This is a conserved proteolytic pathway in which proteins destined for degradation are tagged with the small protein modifier ubiquitin. Tagged proteins are typically recognized by the proteasome and degraded. Ubiquitin is covalently conjugated to protein substrates by the sequential activity of three enzymes: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and the ubiquitin protein ligase (E3)[12]. Most eukaryotes have one or two E1 isoforms, and a larger family of related E2 proteins. The E3 ligases are more diverse and can be divided into several classes based on mechanism of action and subunit composition. One large class of E3s is the cullin-based E3s. The best characterized cullin-based E3s are the SCFs, composed of CUL1, RBX1 (the RING protein), SKP1 (ASK in plants) and an F-box protein (FBP)[13]. E3s confer specificity to the pathway, and for SCF-type E3s, specificity is determined by the F-box protein. The *Arabidopsis* genome encodes more than 700 F-box proteins, which indicates that SCFs have a prominent role in plants[14]. Indeed, SCFs have been implicated in diverse processes including plant hormone signaling[15].

The ubiquitin-proteasome pathway, and an SCF in particular, was initially implicated in auxin response with the isolation and characterization of an auxin-resistant mutant in *Arabidopsis* called *transport inhibitor response 1* (*tir1*)[16]. The *TIR1* gene encodes an F-box protein that interacts directly with Aux/IAA proteins (**Fig. 2b**). Importantly, this binding is enhanced by auxin[17–19]. Ultimately, structural studies demonstrated that auxin forms a complex with TIR1 and the Aux/IAA proteins, thus stabilizing the interaction[20]. One important implication of the structure is that both TIR1 and the Aux/IAAs appear to contribute to high-affinity binding of auxin. In this sense, it may be more appropriate to call TIR1 and the Aux/IAA co-receptors. If true, this also implies that different combinations of F-box protein and substrate may have unique auxin-binding characteristics.

The discovery that TIR1 functions as an auxin receptor was a landmark event. This discovery indicates that F-box proteins, and perhaps other E3s, can function as receptors for small molecules. Indeed, further studies have demonstrated that this is the case (see JA signaling below). In addition, the realization that a small molecule can significantly enhance the E3-substrate binding offers a new approach for the development of drugs that target the ubiquitin-proteasome pathway[20]. Beyond the TIR1-Aux/IAA-ARF pathway, our knowledge of the auxin-regulated transcriptional network is limited. A DNA sequence that binds ARF proteins, called the *Auxin Response Element* (*AuxRE*), has been identified. However, the function of this element has only been established for a few genes. Further, the promoters of many auxin response genes don't have an *AuxRE*, which suggests that other DNA sequences are probably involved in ARF function[21]. Microarray experiments have shown that auxin rapidly regulates a very large number of genes with diverse cellular functions[22]. This list includes transcription factors that presumably function to regulate downstream gene cascades. In addition, auxin directly stimulates expression of the genes encoding the Aux/IAAs, which indicates that auxin response is subject to a negative feedback loop[11]. A variety of different cell wall–related genes are also induced by auxin. The proteins encoded by these genes probably facilitate cell wall changes associated with growth. However, it should be noted that the function of these genes in auxin response has not been determined.

Jasmonic acid

The oxylipin JA and its metabolites, collectively known as jasmonates, are important plant signaling molecules that mediate biotic and abiotic stress responses as well as aspects of growth and development[23]. In higher plants, JA is synthesized via the octadecanoid pathway. Once synthesized,

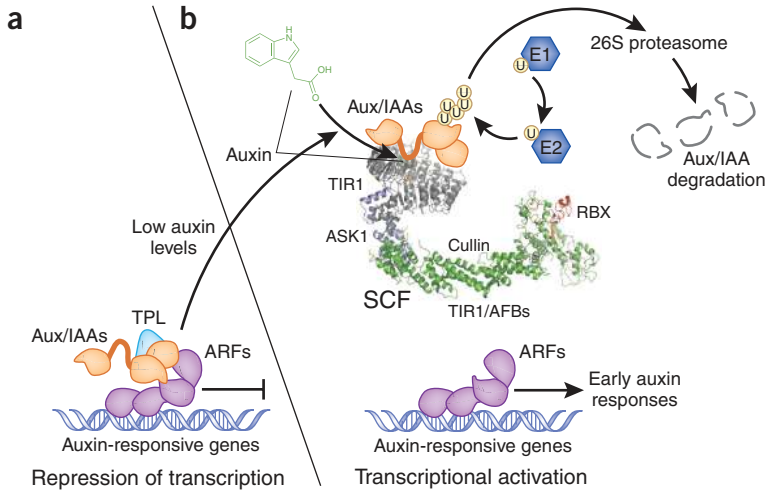

Figure 2 Auxin signaling in *Arabidopsis*. SCF^{TIR1} and related SCF complexes bind auxin and target Aux/IAA proteins for degradation. (**a**) At low cellular levels of auxin, transcription of auxin response genes is repressed by the Aux/IAAs. (**b**) When auxin cellular levels increase, auxin binds to TIR1, enhances its affinity for the Aux/IAAs, and promotes their ubiquitination and subsequent degradation, thus permitting the ARF proteins to promote transcription.

JA can be conjugated to isoleucine to form JA-Ile or converted to the volatile methyl-JA. JA levels increase rapidly in response to a wide range of inductive signals, including herbivory, mechanical wounding and various other abiotic stresses. Increased hormone levels result in extensive changes in defense gene expression. Recent results indicate that JA signaling is strikingly similar to auxin signaling (**Fig. 3**). In the case of JA, the hormone promotes the degradation of transcriptional repressors called JAZ proteins, a process that requires an F-box protein called CORONATINE-INSENSITIVE1 (COI1)[24]. Coronatine is a phytotoxin that is structurally related to JA. The *coi1* mutant was recovered in a screen for coronatine resistance and is resistant to both coronatine and JA. These results suggested that jasmonate response requires SCF^{COI1}- dependent degradation of repressors, much like SCF^{TIR1} targets the Aux/IAAs. For JA, the relevant repressors are called jasmonate ZIM-domain (JAZ) proteins[25,26]. The JAZ proteins are degraded in a proteasome-dependent manner following jasmonate treatment but are stabilized in the *coi1*mutant. In addition, the JAZ proteins bind COI1 both *in vitro* and in a yeast two-hybrid test[25,26]. Finally, radiolabeled coronatine binds to COI1-JAZ complexes with high affinity, which strongly suggests that COI1 functions as a receptor[27]. Interestingly, these studies also demonstrated that JA-Ile is the active molecule in the plant rather than JA. These results were confirmed recently in experiments showing that COI1 is required for JA-Ile or coronatine binding to the COI1-JAZ complex[27,28].

Gibberellins

The GAs are a large family of tetracyclic, diterpenoid growth regulators. This hormone has a particularly interesting role in modern agriculture. It was originally isolated in 1938 as a metabolite from the rice fungal pathogen *Gibberella fujikuroi*[29]. Infection of rice by the fungus results in excessive stem elongation, ultimately causing the plant to fall over (lodging). In the 1960s and 1970s, the "green revolution" was associated with the adoption of new dwarf varieties. Recent molecular genetic studies show that these varieties are affected in now well-characterized components of GA signaling pathways[30]. Because these varieties have shorter stems, relatively more of the plants' resources are used to produce grain. In addition, the dwarf strains are more resistant to wind and other severe weather[30].

Like the other phytohormones, GAs play a major role in diverse growth processes including seed development, organ elongation and the control of flowering time[29]. GAs are synthesized from geranylgeranyl diphosphate in a multi-enzyme pathway that is subject to complex regulation. GA represses expression of several genes whose products are involved in its biosynthesis and promotes expression of genes involved in GA inactivation[29,31]. Further, GA levels are influenced by other hormones such as auxin and ethylene[29].

GA response is mediated by negative regulators of GA response called DELLA proteins. The DELLAs are named after the conserved N-terminal DELLA domain and also contain a C-terminal GRAS domain[32]. GAs promote degradation of DELLA proteins, thus inducing the GA transcriptional response[33].

The DELLAs accumulate in *Arabidopsis* and rice mutants mutated in the genes encoding F-box proteins: *sleepy1* (*sly1*) and *gibberellin-insensitive dwarf2* (*gid2*), respectively[34,35]. Thus, as for auxin and JA, GA appears to regulate the level of negative regulators through the action of SCF-type ligases (**Fig. 3**). However, unlike Aux/IAA and JAZ proteins, the DELLAs may not function directly as transcriptional repressors. Recent reports indicate that DELLAs inhibit cell elongation by binding to the DNA binding domain of a transcription factor called PHYTOCHROME INTERACTING FACTOR3 (PIF3), as well as PIF4 and PIF5. This binding prevents the PIF from interacting with promoter elements and stimulating transcription of growth-related genes[36,37].

Recently, the GA receptor was identified in rice[38]. The GIBBERELLIN INSENSTIVE DWARF1 (GID1) protein is localized in the nucleus and binds biologically active gibberellins[38]. Three orthologous genes (*GID1a*, *GID1b* and *GID1c*) are present in *Arabidopsis*[39,40]. The triple mutant lacking all three proteins is GA-insensitive, which indicates that GA responses require functional GID1 proteins in both rice and *Arabidopsis*[39]. The GID1s interact with DELLA proteins in a GA-dependent manner. Further, the DELLAs

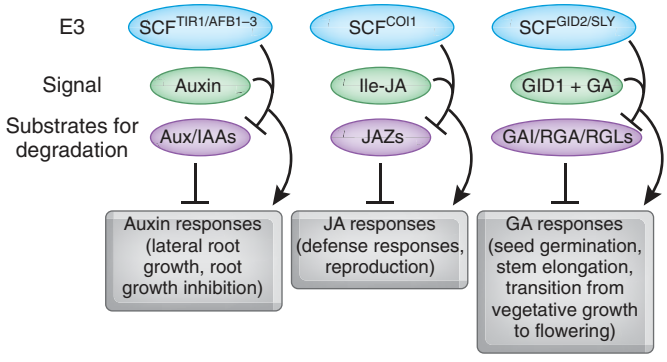

Figure 3 Similarities between auxin, JA and GA signaling. SCF-E3–type ubiquitin ligases promote the ubiquitination and degradation of repressors of auxin-, JA- and GA-regulated transcription. In the case of auxin and JA, the hormone directly binds to the SCF and promotes binding and ubiquitylation of the repressors. In the case of GA, an additional protein (the GA receptor GID1) facilitates substrate recognition by $SCF^{GID2/SLY}$.

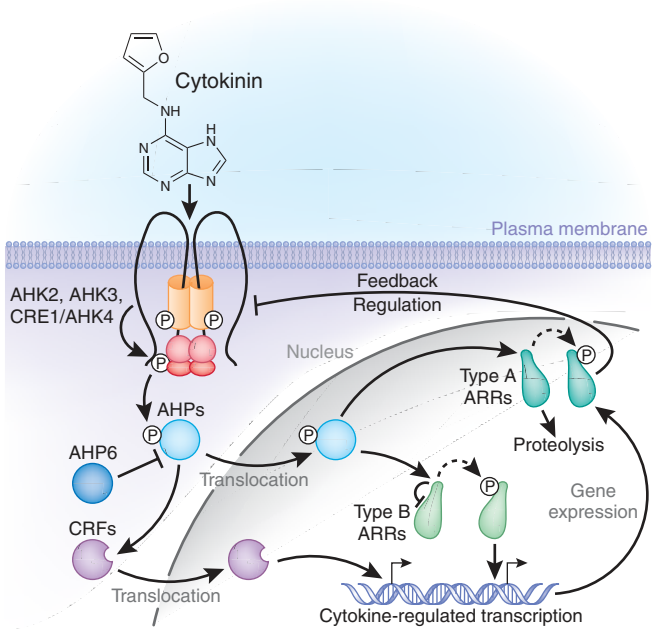

Figure 4 Model of cytokinin signal transduction. Cytokinin is perceived by the AHK plasma membrane receptors. Cytokinin signal is further amplified by phosphorelay events starting from AHKs, which lead to the activation and subsequent nuclear translocation of AHP proteins. AHP proteins transfer the phosphoryl group to type A or type B ARR proteins. The former act as repressors of cytokinin signaling, whereas the latter act as positive transcriptional regulators of cytokinin-induced genes, including those encoding type A ARRs. CRF proteins are also activated by cytokinin, and after translocation to the nucleus they act as activators of cytokinin-regulated genes.

are better able to interact with $SCF^{GID2/SLY}$ while in a complex with gibberellin-bound GID1 (refs. 38,39,41). This interaction ultimately leads to ubiquitination and degradation of the DELLA repressor, thus promoting GA-mediated transcription.

The structures of rice GID1 and *Arabidopsis* GID1a support this model[42,43]. GID1 is a member of the hormone-sensitive lipase (HSL) family[38]. The receptor contains a deep binding pocket whose access is regulated by a flexible lid[42,43]. GA binds to the pocket and probably causes the lid to fold back and interact with the DELLA domain[42,43]. It is not clear how complex formation enhances the DELLA-$SCF^{SLY/GID2}$ interaction, but it may involve a conformational change in the C-terminal GRAS domain of the DELLA proteins.

Cytokinins

The cytokinins are N6-substituted adenine-based molecules that were originally discovered in the 1950s by Carlos Miller on the basis of their ability to promote plant cell division[44]. They have since been implicated in a broad range of developmental processes including germination, root and shoot meristem function and leaf senescence[45]. In addition, cytokinin has an important role in the formation of nitrogen-fixing nodules and other plant-microbe interactions[46,47].

In general the first step in cytokinin biosynthesis is the production of N6-(Δ2-isopentenyl)adenine (iP) riboside 5′-tri-, 5′-di- or 5′-monophosphate by the enzyme adenosine phosphate-isopentenyltransferase (IPT)[48]. Active cytokinins are produced by a phosphoribohydrolase enzyme that converts the nucleotide to the free base[49]. The regulation of cytokinin levels is complex and involves changes in both synthesis and metabolism. Recent studies have focused on the complexity of *IPT* gene regulation and the role of other hormones, including auxin and ABA, in the regulation of cytokinin biosynthetic genes[48].

Cytokinin signaling is remarkably similar to the two-component signaling systems that are commonplace in bacterial species[45]. In two-component pathways, ligand binding initiates a phosphorelay that involves both histidine and aspartate kinases. Cytokinin is perceived by a membrane-associated hybrid kinase that transfers a phosphate to *ARABIDOPSIS* HIS PHOSPHOTRANSFER (AHP) proteins (**Fig. 4**). The AHPs are translocated into the nucleus where they phosphorylate *ARABIDOPSIS* RESPONSE REGULATOR (ARR) proteins. The ARRs can be either negative (type A ARRs) or positive (type B ARRs) effectors of cytokinin signaling. Microarray studies indicate that type B ARRs are direct regulators of the cytokinin transcriptional response. As for auxin, a large number of genes are regulated by cytokinin[22,50]. Among these are members of a family of transcription factor genes called *CYTOKININ RESPONSE FACTORS*. Mutants deficient in members of this family exhibit defects in cytokinin-regulated transcription, which indicates that these proteins mediate downstream effects of cytokinin on the transcriptional response[51].

Ethylene

The gaseous hormone ethylene is best known for its role in fruit ripening. In fact, the Greek philosopher Theophrastus recognized that sycamore figs don't ripen unless they are wounded by scraping with a metal tool[52]. It turns out that wounding causes the formation of ethylene, which in turn promotes ripening. We now know that ethylene has an important role in many other developmental processes[53]. It is synthesized from methionine via the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC)[54] in a pathway called the Yang cycle, for its discoverer, Shang Fa Yang[55]. Ethylene can be synthesized in essentially all plant organs, although the pathway is regulated by both environmental and developmental cues such as the onset of fruit ripening. One of the key enzyme families in the biosynthetic pathway, the ACC synthases, is a focus of this regulation. For example, auxin induces ethylene synthesis by directly promoting transcription of ACC synthase genes (*ACS*). In addition, various signals modulate ethylene levels by regulating the levels of the ACS proteins. This is accomplished through the action of another type of E3 enzyme called a BTB (broad-complex, Tramtrack and bric-a-brac) complex. In this case, the BTB protein is the specificity factor. Three BTB proteins, called ETHYLENE OVERPRODUCER1, ETO1-LIKE1 (EOL1) and EOL2, are responsible for promoting degradation of ACS proteins[56,57].

Like cytokinin, ethylene is perceived by a two-component protein kinase receptor[53]. In this case, however, the receptor is localized to the endoplasmic reticulum membrane. In the absence of ethylene, the receptor negatively regulates ethylene signaling via a Raf-like protein kinase called CONSTITUTIVE TRIPLE RESPONSE1 (CTR1). CTR1 in turn negatively regulates a second membrane protein called ETHYLENE INSENSITIVE2 (EIN2). Through an unknown mechanism, EIN2 activates members of a small family of transcription factors including EIN3. As for the other hormones, the ubiquitin pathway has an important role in ethylene signaling[53]. Both EIN2 and EIN3 levels are controlled by E3 ligases. In the absence of ethylene, $SCF^{ETP1/2}$ (with the F-box protein EIN2 TARGETING PROTEIN1 (ETP1) or ETP2) promotes degradation of EIN2, thus reducing the ethylene response[58]. Ethylene acts to decrease *ETP* gene expression, thus permitting the accumulation of EIN2. Similarly, another pair of F-box proteins called EIN3-BINDING F-BOX1 (EBF1) and EBF2 promote degradation of EIN3 at low levels of hormone. As hormone levels increase, EIN3 degradation decreases and ethylene-regulated transcription is stimulated. Finally, *EBF1* and *EBF2* RNA levels are regulated by an exoRNase called EIN5, or XRN4 (ref. 53).

Abscisic acid

ABA is an isoprenoid compound associated with seed dormancy, drought responses and other growth processes[59,60]. The ABA biosynthetic pathway was defined through genetic studies of mutants with seed dormancy defects, primarily in maize and *Arabidopsis*. It is thought that nearly all of the genes in the pathway have now been identified[59]. ABA levels are regulated by a variety of environmental conditions. Particular attention has been paid to changes in ABA levels during seed maturation and in response to drought conditions. In both processes, changes in biosynthesis and catabolism appear to be important for ABA regulation.

Although several candidate ABA receptors have been proposed in recent years, these reports are controversial. Magnesium protoporphyrin-IX chelatase H subunit (CHLH; also called GENOMES UNCOUPLED5 or GUN5 in *Arabidopsis*) is an unconventional site of hormone perception[61]. This protein is localized to chloroplasts and was reported to have specific ABA-binding activity. Plants with decreased CHLH levels have ABA-insensitive germination and stomatal aperture phenotypes, whereas plants overexpressing CHLH are ABA-hypersenstive in these assays. However, at this point it is unclear how CHLH mediates these responses. Further, a recent study reported that CHLH from barley does not bind ABA, and that mutants with reduced CHLH levels do not display an ABA-related phenotype, thus calling into question the function of this protein as an ABA receptor[62].

A second report of a candidate ABA receptor involves a putative G protein–coupled receptor from *Arabidopsis* called G-PROTEIN COUPLED RECEPTOR2 (GCR2)[63]. This finding is also controversial because it is not clear that GCR2 has a transmembrane domain[64]. In addition, genetic analysis of the *GCR2* family failed to detect an ABA-related phenotype[65,66]. At this point, GCR2 appears unlikely to function as an ABA receptor.

The most recent report of ABA receptor function concerns another pair of G protein–coupled receptors called GTG1 (GPCR-type G protein 1) and GTG2 (ref. 67). Plants lacking both GTG1 and GTG2 are deficient in ABA responses during germination, flowering, root elongation and stomatal closure[67]. In addition, both proteins bind biologically active ABA *in vitro*. Whether or not GTG1 and GTG2 are the only ABA receptors is a question for the future. ABA responses are diverse, and it is possible that this diversity requires multiple sites of perception[68].

ABA signal transduction pathways are complex and involve a variety of small molecules and proteins. Two protein phosphatase 2C proteins called ABI1 and ABI2 have a central role in ABA response, as mutations in either gene affect all ABA responses. In addition, a variety of kinases, RNA-modifying enzymes and transcription factors have been proposed to function in ABA signaling[69,70]. In the guard cell, Ca^{2+}, ROS and NO have all been implicated in ABA promotion of stomatal closing[69]. Finally, the ubiquitin-proteasome pathway is known to be important. Two RING E3 ligases, ABI3-interacting protein (AIP2) and Keep on Going (KEG), promote normal ABA signaling by regulating the abundance of ABA-responsive transcription factors, namely ABA-insensitive 3 (ABI3) and ABI5 (refs. 71,72).

Salicylic acid

Salicylic acid (SA) is best known for its central role in plant defense response[73,74]. SA is synthesized from chorismate via isochorismate. Infection of the plants by a broad range of pathogens results in an increase in SA levels both at the site of infection and in distant tissues. Many of the details of SA signaling have not been worked out, including the mechanism of SA perception. However, it is clear that SA response depends on a protein called NONEXPRESSER OF PR genes (NPR1). When SA levels increase, NPR1 is translocated into the nucleus where it promotes the transcription of a large family of *PATHOGENESIS RELATED* (*PR*) genes. Some PR proteins have antimicrobial activity, but in general the function of these proteins has not been clearly defined. The precise mechanism of NPR1 function is also not clear. Interestingly, NPR1 is a member of the BTB domain family of proteins, and like ETO1 it is probably a subunit in an E3 ligase[74,75], which implies that SA action also involves regulated protein degradation.

One of the most interesting aspects of SA biology is its role in systemic acquired resistance (SAR)[74]. SAR is a defense pathway that provides systemic protection to a broad range of pathogens. As mentioned above, pathogen attack results in an increase in SA levels at the site of infection and at distant sites. The response appears to require the synthesis of the volatile compound methyl salicylate (MeSA) at the infection site. MeSA moves to other parts of the plant, where it is converted to SA by the protein SA BINDING PROTEIN2 (SABP2).

Interactions between hormones

Abundant physiological data suggest that all plant hormones interact with one or more additional hormones. Depending on context, hormone interaction can involve changes in hormone level or response. For example, several genes required for auxin biosynthesis are under transcriptional control of ethylene[76–78], while auxin can also influence ethylene biosynthesis by inducing expression of genes encoding ACC synthase[79].

Hormone interactions also occur at the level of hormone distribution. A good illustration of this type of crosstalk is the opposing action of auxin and cytokinin during lateral root initiation. It is widely known that polar auxin transport and the establishment of an auxin gradient is a very important determinant of plant growth and morphological patterning[80]. During root development, auxin promotes lateral root initiation while cytokinin opposes this response. One way that cytokinin does this is to influence the expression of the *PINFORMED* (*PIN*) auxin efflux carrier genes[81]. At least five *PIN* genes work collectively to establish auxin gradients in roots by controlling the direction of polar-auxin transport[82]. By reducing *PIN* expression, cytokinin disrupts local auxin gradient formation in lateral root founder cells, thereby inhibiting lateral root initiation[81].

Hormonal signaling pathways are also known to interact at the level of gene expression. For example, studies show that there is significant overlap between auxin- and brassinosteroid-responsive gene sets[21,83]. Generally, common target genes repressed by auxin are also repressed by brassinosteroids, and genes induced by auxin are induced by brassinosteroids, which suggests coordination between the signaling pathways. Furthermore, transcriptional profiling revealed that very few auxin response genes responded normally to auxin in the BR-deficient mutant *brevis radix* (*brx*)[84]. Conversely, many brassinosteroid-responsive genes are misregulated in the *yucca* mutant that overproduces auxin[21]. Taken together, the data suggest that auxin and brassinosteroid signaling pathways partially converge on a set of common target genes.

Control over key components of signaling pathways by other hormone signals is another common example of crosstalk strategy. As described above, DELLA proteins are central regulators of the GA-mediated signaling pathway and appear to be a common crosstalk node for several interacting hormones including auxin, ethylene and ABA[85]. GA signaling during root elongation is known to require auxin, as disruption of polar auxin transport or signaling diminishes the effects of GA on root elongation[86]. The attenuated growth response corresponds with reduced RGA degradation in root cells. These observations indicate that auxin promotes the GA-induced destabilization of some of the DELLA proteins to affect GA responses[86]. Similarly, ethylene and ABA may also target DELLA proteins to exert antagonistic actions with GA during root growth[85,87].

Emerging chemical signals

It seems likely that the signaling compounds described here are not the end of the story. Given the metabolic complexity of plants, there are probably more, perhaps many more, small molecules with signaling function. Perhaps the most striking example of this is the recent discovery that the carotenoid-derived molecule strigolactone is a shoot branching hormone[88–90]. It has been known for many years that auxin synthesized in the apex of the plant inhibits the growth of lateral branches. This phenomenon is called apical dominance. More recently, genetic studies in *Arabidopsis*, pea, petunia and rice have shown that a signal originating in the root also inhibits shoot branching[88]. This signal turns out to be strigolactone. The mechanism of strigolactone action is still unclear, but some details are starting to emerge. Experiments in *Arabidopsis* indicate that strigolactone inhibits auxin transport, which suggests a complex interaction between these two hormones[91]. Further, one of the genes required for strigolactone-dependent inhibition of branching, *MORE AXILLARY BRANCHES* (*MAX2*), encodes an F-box protein, which indicates that once again, protein degradation is an important component of this regulatory system[88,92]. In addition to strigolactone, there is accumulating evidence that nitric oxide has a role in plant defense and perhaps in aspects of growth[73,93].

Conclusions and future directions

During the last ten years, our understanding of the molecular mechanisms of hormone biosynthesis, perception and response has improved dramatically. Knowledge of hormone metabolic and transport pathways will lead to new opportunities to manipulate hormone levels and thus regulate plant growth. Receptors for many of the hormone classes have been identified, thus leading to exciting new models of hormone perception. Detailed knowledge of receptor function may stimulate the development of new plant growth regulators[94]. Similarly, many downstream signaling components have been identified. In the future a major challenge will be to understand how these signaling pathways are integrated during plant growth. For example, we know that auxin, GA and BR all promote cell elongation in the plant stem. However, the relative contribution of each signaling pathway to growth regulation in response to changes in the environment is uncertain. Similarly, it is not known whether cell elongation involves the same set of genes for each hormone signal. Finally, as we learn more about the mechanisms of hormone action, our ability to generate predictive models of plant growth will improve. Already robust models for auxin-dependent regulation of shoot and root growth have been developed[95–99]. Ultimately we can expect these models to incorporate the diversity of hormone and environmental signals and thus enable an integrated view of plant growth and development.

ACKNOWLEDGMENTS

The authors' work is supported by grants to M.E. from the US National Institutes of Health (GM 43644), the US Department of Energy (De-FG02-02ER15312) and the US National Science Foundation (MCB-0519970, IOS-0849069).

Published online at http://www.nature.com/naturechemicalbiology/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Sachs, *J. Stoff und Form der Pflanzenorgane. Arb. Bot. Inst. Würzburg* **2**, 452–488 (1880).
2. Darwin, C. *The Power of Movement in Plants* (John Murray, London, 1880).
3. Jun, J.H., Fiume, E. & Fletcher, J.C. The CLE family of plant polypeptide signaling molecules. *Cell. Mol. Life Sci.* **65**, 743–755 (2008).
4. Davies, P.J. in *Plant Hormones Physiology, Biochemistry and Molecular Biology* (ed. Davies, P.J.) 1–12 (Kluwer Academic Publishers, Dordrecht, The Netherlands, 1995).
5. Strader, L.C. & Bartel, B. A new path to auxin. *Nat. Chem. Biol.* **4**, 337–339 (2008).
6. Woodward, A.W. & Bartel, B. Auxin: regulation, action, and interaction. *Ann. Bot. (Lond.)* **95**, 707–735 (2005).
7. Vieten, A., Sauer, M., Brewer, P.B. & Friml, J. Molecular and cellular aspects of auxin-transport-mediated development. *Trends Plant Sci.* **12**, 160–168 (2007).
8. Badescu, G.O. & Napier, R.M. Receptors for auxin: will it all end in TIRs? *Trends Plant Sci.* **11**, 217–223 (2006).
9. Guilfoyle, T.J. & Hagen, G. Auxin response factors. *Curr. Opin. Plant Biol.* **10**, 453–460 (2007).
10. Szemenyei, H., Hannon, M. & Long, J.A. TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. *Science* **319**, 1384–1386 (2008).
11. Mockaitis, K. & Estelle, M. Auxin receptors and plant development: a new signaling paradigm. *Annu. Rev. Cell Dev. Biol.* **24**, 55–80 (2008).
12. Pickart, C.M. Mechanisms underlying ubiquitination. *Annu. Rev. Biochem.* **70**, 503–533 (2001).
13. Deshaies, R.J. SCF and Cullin/Ring H2-based ubiquitin ligases. *Annu. Rev. Cell Dev. Biol.* **15**, 435–467 (1999).
14. Gagne, J.M., Downes, B.P., Shiu, S.H. Durshi, A.M. & Vierstra, R.D. The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **99**, 11519–11524 (2002).
15. Lechner, E., Achard, P., Vansiri, A., Potuschak, T. & Genschik, P. F-box proteins everywhere. *Curr. Opin. Plant Biol.* **9**, 631–638 (2006).
16. Ruegger, M. *et al.* The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes Dev.* **12**, 198–207 (1998).
17. Gray, W.M., Kepinski, S., Rouse, D., Leyser, O. & Estelle, M. Auxin regulates SCFTIR1-dependent degradation of AUX/IAA proteins. *Nature* **414**, 271–276 (2001).
18. Dharmasiri, N., Dharmasiri, S., Jones, A.M. & Estelle, M. Auxin action in a cell-free system. *Curr. Biol.* **13**, 1418–1422 (2003).
19. Kepinski, S. & Leyser, O. Auxin-induced SCFTIR1-Aux/IAA interaction involves stable modification of the SCFTIR1 complex. *Proc. Natl. Acad. Sci. USA* **101**, 12381–12386 (2004).
20. Tan, X. *et al.* Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* **446**, 640–645 (2007).
21. Nemhauser, J.L., Mockler, T.C. & Chory, J. Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. *PLoS Biol.* **2**, E258 (2004).
22. Peng, Z.Y. *et al.* *Arabidopsis* Hormone Database: a comprehensive genetic and phenotypic information database for plant hormone research in *Arabidopsis*. *Nucleic Acids Res.* **37**, D975–D982 (2009).
23. Wasternack, C. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann. Bot. (Lond.)* **100**, 681–697 (2007).
24. Xie, D.X., Feys, B.F., James, S., Nieto-Rostro, M. & Turner, J.G. COI1: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science* **280**, 1091–1094 (1998).
25. Thines, B. *et al.* JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. *Nature* **448**, 661–665 (2007).
26. Chini, A. *et al.* The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* **448**, 666–671 (2007).
27. Melotto, M. *et al.* A critical role of two positively charged amino acids in the Jas motif of *Arabidopsis* JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. *Plant J.* **55**, 979–988 (2008).
28. Katsir, L., Schilmiller, A.L., Staswick, P.E., He, S.Y. & Howe, G.A. COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proc. Natl. Acad. Sci. USA* **105**, 7100–7105 (2008).
29. Yamaguchi, S. Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* **59**, 225–251 (2008).
30. Peng, J. *et al.* 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400**, 256–261 (1999).
31. Fleet, C.M. & Sun, T.P.A. DELLAcate balance: the role of gibberellin in plant morphogenesis. *Curr. Opin. Plant Biol.* **8**, 77–85 (2005).
32. Schwechheimer, C. Understanding gibberellic acid signaling–are we there yet? *Curr. Opin. Plant Biol.* **11**, 9–15 (2008).
33. Itoh, H., Matsuoka, M. & Steber, C.M. A role for the ubiquitin-26S-proteasome pathway in gibberellin signaling. *Trends Plant Sci.* **8**, 492–497 (2003).
34. McGinnis, K.M. *et al.* The *Arabidopsis* SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. *Plant Cell* **15**, 1120–1130 (2003).
35. Sasaki, A. *et al.* Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. *Science* **299**, 1896–1898 (2003).
36. Feng, S. *et al.* Coordinated regulation of *Arabidopsis* thaliana development by light and gibberellins. *Nature* **451**, 475–479 (2008).
37. de Lucas, M. *et al.* A molecular framework for light and gibberellin control of cell elongation. *Nature* **451**, 480–484 (2008).
38. Ueguchi-Tanaka, M. *et al.* GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. *Nature* **437**, 693–698 (2005).
39. Griffiths, J. *et al.* Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. *Plant Cell* **18**, 3399–3414 (2006).
40. Nakajima, M. *et al.* Identification and characterization of *Arabidopsis* gibberellin receptors. *Plant J.* **46**, 880–889 (2006).
41. Willige, B.C. *et al.* The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of *Arabidopsis*. *Plant Cell* **19**, 1209–1220 (2007).
42. Shimada, A. *et al.* Structural basis for gibberellin recognition by its receptor GID1. *Nature* **456**, 520–523 (2008).

43. Murase, K., Hirano, Y., Sun, T.P. & Hakoshima, T. Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* **456**, 459–463 (2008).
44. Miller, C., Skoog, F., Saltza, M.V. & Strong, M. Kinetic, a cell division factor from deoxyribonucleic acid. *J. Am. Chem. Soc.* **77**, 1392 (1955).
45. To, J.P. & Kieber, J.J. Cytokinin signaling: two-components and more. *Trends Plant Sci.* **13**, 85–92 (2008).
46. Murray, J.D. *et al.* A cytokinin perception mutant colonized by Rhizobium in the absence of nodule organogenesis. *Science* **315**, 101–104 (2007).
47. Frugier, F., Kosuta, S., Murray, J.D., Crespi, M. & Szczyglowski, K. Cytokinin: secret agent of symbiosis. *Trends Plant Sci.* **13**, 115–120 (2008).
48. Sakakibara, H. Cytokinins: activity, biosynthesis, and translocation. *Annu. Rev. Plant Biol.* **57**, 431–449 (2006).
49. Kurakawa, T. *et al.* Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* **445**, 652–655 (2007).
50. Rashotte, A.M., Carson, S.D., To, J.P. & Kieber, J.J. Expression profiling of cytokinin action in *Arabidopsis*. *Plant Physiol.* **132**, 1998–2011 (2003).
51. Rashotte, A.M. *et al.* 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 (2006).
52. Theologis, A. One rotten apple spoils the whole bushel: the role of ethylene in fruit ripening. *Cell* **70**, 181–184 (1992).
53. Kendrick, M.D. & Chang, C. Ethylene signaling: new levels of complexity and regulation. *Curr. Opin. Plant Biol.* **11**, 479–485 (2008).
54. De Paepe, A. & Van der Straeten, D. Ethylene biosynthesis and signaling: an overview. *Vitam. Horm.* **72**, 399–430 (2005).
55. Adams, D.O. & Yang, S.F. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proc. Natl. Acad. Sci. USA* **76**, 170–174 (1979).
56. Wang, K.L., Yoshida, H., Lurin, C. & Ecker, J.R. Regulation of ethylene gas biosynthesis by the *Arabidopsis* ETO1 protein. *Nature* **428**, 945–950 (2004).
57. Christians, M.J. *et al.* The BTB ubiquitin ligases ETO1, EOL1 and EOL2 act collectively to regulate ethylene biosynthesis in *Arabidopsis* by controlling type-2 ACC synthase levels. *Plant J.* **57**, 332–345 (2009).
58. Qiao, H., Chang, K.N., Yazaki, J. & Ecker, J.R. Interplay between ethylene, ETP1/ETP2 F-box proteins, and degradation of EIN2 triggers ethylene responses in *Arabidopsis*. *Genes Dev.* **23**, 512–521 (2009).
59. Nambara, E. & Marion-Poll, A. Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* **56**, 165–185 (2005).
60. Israelsson, M. *et al.* Guard cell ABA and CO2 signaling network updates and Ca2+ sensor priming hypothesis. *Curr. Opin. Plant Biol.* **9**, 654–663 (2006).
61. Shen, Y.Y. *et al.* The Mg-chelatase H subunit is an abscisic acid receptor. *Nature* **443**, 823–826 (2006).
62. Muller, A.H. & Hansson, M. The barley magnesium chelatase 150-kDa subunit is not an abscisic acid receptor. *Plant Physiol.* published online, doi:10.1104/pp.109.135277 (28 January 2009).
63. Liu, X. *et al.* A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. *Science* **315**, 1712–1716 (2007).
64. Johnston, C.A. *et al.* Comment on "A G protein coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid". *Science* **318**, 914, author reply 914 (2007).
65. Guo, J., Zeng, Q., Emami, M., Ellis, B.E. & Chen, J.G. The GCR2 gene family is not required for ABA control of seed germination and early seedling development in *Arabidopsis*. *PLoS One* **3**, e2982 (2008).
66. Gao, Y. *et al.* Genetic characterization reveals no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in *Arabidopsis*. *Plant J.* **52**, 1001–1013 (2007).
67. Pandey, S., Nelson, D.C. & Assmann, S.M. Two novel GPCR-type G proteins are abscisic acid receptors in *Arabidopsis*. *Cell* **136**, 136–148 (2009).
68. McCourt, P. & Creelman, R. The ABA receptors–we report you decide. *Curr. Opin. Plant Biol.* **11**, 474–478 (2008).
69. Hirayama, T. & Shinozaki, K. Perception and transduction of abscisic acid signals: keys to the function of the versatile plant hormone ABA. *Trends Plant Sci.* **12**, 343–351 (2007).
70. Finkelstein, R., Reeves, W., Ariizumi, T. & Steber, C. Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* **59**, 387–415 (2008).
71. Stone, S.L., Williams, L.A., Farmer, L.M., Vierstra, R.D. & Callis, J. KEEP ON GOING, a RING E3 ligase essential for *Arabidopsis* growth and development, is involved in abscisic acid signaling. *Plant Cell* **18**, 3415–3428 (2006).
72. Zhang, X., Garreton, V. & Chua, N.H. The AIP2 E3 ligase acts as a novel negative regulator of ABA signaling by promoting ABI3 degradation. *Genes Dev.* **19**, 1532–1543 (2005).
73. Vlot, A.C., Klessig, D.F. & Park, S.W. Systemic acquired resistance: the elusive signal(s). *Curr. Opin. Plant Biol.* **11**, 436–442 (2008).
74. Durrant, W.E. & Dong, X. Systemic acquired resistance. *Annu. Rev. Phytopathol.* **42**, 185–209 (2004).
75. Gingerich, D.J. *et al.* Cullins 3a and 3b assemble with members of the broad complex/tramtrack/bric-a-brac (BTB) protein family to form essential ubiquitin-protein ligases (E3s) in *Arabidopsis*. *J. Biol. Chem.* **280**, 18810–18821 (2005).
76. Stepanova, A.N., Hoyt, J.M., Hamilton, A.A. & Alonso, J.M. A Link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in *Arabidopsis*. *Plant Cell* **17**, 2230–2242 (2005).
77. Stepanova, A.N. *et al.* TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* **133**, 177–191 (2008).
78. Tao, Y. *et al.* Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* **133**, 164–176 (2008).
79. Tsuchisaka, A. & Theologis, A. Unique and overlapping expression patterns among the *Arabidopsis* 1-amino-cyclopropane-1-carboxylate synthase gene family members. *Plant Physiol.* **136**, 2982–3000 (2004).
80. Feraru, E. & Friml, J. PIN polar targeting. *Plant Physiol.* **147**, 1553–1559 (2008).
81. Laplaze, L. *et al.* Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* **19**, 3889–3900 (2007).
82. Blilou, I. *et al.* The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature* **433**, 39–44 (2005).
83. Goda, H. *et al.* Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in *Arabidopsis*. *Plant Physiol.* **134**, 1555–1573 (2004).
84. Mouchel, C.F., Osmont, K.S. & Hardtke, C.S. BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. *Nature* **443**, 458–461 (2006).
85. Weiss, D. & Ori, N. Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiol.* **144**, 1240–1246 (2007).
86. Fu, X. & Harberd, N.P. Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. *Nature* **421**, 740–743 (2003).
87. Achard, P., Vriezen, W.H., Van Der Straeten, D. & Harberd, N.P. Ethylene regulates *Arabidopsis* development via the modulation of DELLA protein growth repressor function. *Plant Cell* **15**, 2816–2825 (2003).
88. Leyser, O. The control of shoot branching: an example of plant information processing. *Plant Cell Environ.* published online, doi:10.1111/j.1365–3040.2009.01930.x (1 January 2009).
89. Gomez-Roldan, V. *et al.* Strigolactone inhibition of shoot branching. *Nature* **455**, 189–194 (2008).
90. Umehara, M. *et al.* Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**, 195–200 (2008).
91. Bennett, T. *et al.* The *Arabidopsis* MAX pathway controls shoot branching by regulating auxin transport. *Curr. Biol.* **16**, 553–563 (2006).
92. Stirnberg, P., van De Sande, K. & Leyser, H.M. MAX1 and MAX2 control shoot lateral branching in *Arabidopsis*. *Development* **129**, 1131–1141 (2002).
93. Flores, T. *et al.* Arginase-negative mutants of *Arabidopsis* exhibit increased nitric oxide signaling in root development. *Plant Physiol.* **147**, 1936–1946 (2008).
94. Hayashi, K. *et al.* Small-molecule agonists and antagonists of F-box protein-substrate interactions in auxin perception and signaling. *Proc. Natl. Acad. Sci. USA* **105**, 5632–5637 (2008).
95. Jönsson, H., Heisler, M.G., Shapiro, B.E., Meyerowitz, E.M. & Mjolsness, E. An auxin-driven polarized transport model for phyllotaxis. *Proc. Natl. Acad. Sci. USA* **103**, 1633–1638 (2006).
96. Hamant, O. *et al.* Developmental patterning by mechanical signals in *Arabidopsis*. *Science* **322**, 1650–1655 (2008).
97. Grieneisen, V.A., Xu, J., Maree, A.F., Hogeweg, P. & Scheres, B. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* **449**, 1008–1013 (2007).
98. Kramer, E.M., Draye, X. & Bennett, M.J. Modelling root growth and development. *SEB Exp. Biol. Ser.* **61**, 195–211 (2008).
99. Smith, R.S. *et al.* A plausible model of phyllotaxis. *Proc. Natl. Acad. Sci. USA* **103**, 1301–1306 (2006).