# Auxin in action: signalling, transport and the control of plant growth and development

*William D. Teale, Ivan A. Paponov and Klaus Palme*

Abstract | Hormones have been at the centre of plant physiology research for more than a century. Research into plant hormones (phytohormones) has at times been considered as a rather vague subject, but the systematic application of genetic and molecular techniques has led to key insights that have revitalized the field. In this review, we will focus on the plant hormone auxin and its action. We will highlight recent mutagenesis and molecular studies, which have delineated the pathways of auxin transport, perception and signal transduction, and which together define the roles of auxin in controlling growth and patterning.

The word 'αυξάνω' in Greek, or 'to grow' in English, gives us 'auxin': the name of a small class of molecules with a powerful ability to induce growth responses in plants. In plants, growth is defined as an irreversible increase in size, and is achieved by the enlargement of individual cells driven by the uptake of water. Auxin refers to an important group of phytohormones that has been implicated in most of the quantitative growth changes that occur during a plant's life cycle. Exactly how these changes are brought about is now becoming clear after more than a century of research. We will give an overview of auxin research, and summarize the remarkable advances that have been made over the past few years. This recent progress will be the foundation on which our understanding of the molecular mechanisms of auxin signal transduction is built, and is beginning to explain how auxin can not only have a direct influence on cell growth, but also control numerous and diverse aspects of plant development.

## Auxin and its effects on plants

Since Julius von Sachs first discussed the concept of a phytohormone in 1887 (REF. 1), several chemical growth regulators have been identified in plants. Phytohormones are chemicals that have specific effects on plant growth, and are active at low concentrations. Plants use a wide variety of hormones, including steroids and peptides, as well as the five classical classes of phytohormones (auxins, abscisic acid, cytokinins, ethylene and gibberellins), which are all relatively small molecules. The extent and significance of phytohormone transport is not well understood for all of these classes, but is particularly significant to the action of auxin and to the story of its discovery. The effect of auxin was first documented when Charles and Francis Darwin published *The Power of Movement in Plants*. They noted that after the perception of light in one area of a grass coleoptile, an "influence is transported" that causes bending towards the light in another[2]. Forty-five years later, in 1926, this messenger was separated from plant tissues simply by being allowed to diffuse into agar blocks, which then retained a growth-promoting activity[3,4]. Three kinds of auxin were initially found in plants, of which one was also found in human urine. Subsequently, the first published reports began to appear on the crystallization and structural characteristics of auxin. In those early days, only one of the structures, that of indole-3-acetic acid (IAA), was correctly identified.

Auxin is now used as the generic name for a group of important molecules in plants, which can also be found in humans, animals and microorganisms. IAA is the predominant auxin in plants (TABLE 1), and is an indispensable phytohormone with a well-documented ability to regulate many aspects of plant development. Synthetic auxin derivatives are still important herbicides; for example, 2,4-dichlorophenoxyacetic acid is one of the world's most widely used weed-killers. The effect of auxin on a growing plant depends on the type of auxin applied and its concentration. Endogenous IAA has been implicated in embryonic and post-embryonic development, and tropisms such as movement in relation to light and gravity. So, auxin influences aspects of cell division, cell elongation and cell differentiation, although exactly how it is involved in each process (and to what extent they are intertwined) is not completely

*Institut für Biologie II/Botanik, Schänzlestrasse 1, 79104 Freiburg, Germany. Correspondence to K.P. e-mail: klaus.palme@biologie.uni-freiburg.de*
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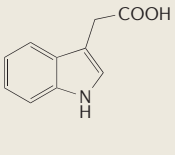
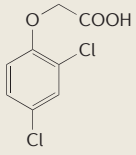
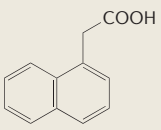
Table 1 | **Properties of three commonly used auxins**

| Properties | Natural | Synthetic | |
|---|---|---|---|
| Chemical structure |  |  |  |
| | IAA | 2,4-D | NAA |
| ***Affinity to receptors*** | | | |
| TIR1 binding ($K_d$) | High | Low | Middle |
| ABP1 binding ($K_d$) | Middle | Low | High |
| ***Transport capacity*** | | | |
| Influx carriers | Yes | Yes | No (by diffusion) |
| Efflux carriers | Yes | No | Yes |

Indole-3-acetic acid (IAA) is considered to be the most important natural auxin, 1-naphthaleneacetic acid (NAA) is a horticulturally important auxin, and 2,4-dichlorophenoxy-acetic acid (2,4-D) is a common selective herbicide.

understood. Its diverse effects in plants might also extend to animals, as photoactivated auxin seems to have potential as a cytotoxin in cancer therapy[5,6].

The reason why auxins have attracted so much attention for almost a century is not only that they have the capacity to influence growth, but that they have additional farther-reaching effects on the life cycle of plants. Recent evidence shows that, through a unique mechanism of perception and elicitation, the physiological responses that auxin governs are central to a plant's structure and functioning.

## Auxin-mediated regulation of gene expression

For a long time it was thought that, like steroid receptors in animals, dedicated plant-receptor proteins initiate the transduction of the auxin signal into numerous physiological responses. Finally, and after decades of research, two separate approaches yielded valuable information on the nature of how auxin functions. The first approach was based on observations made more than 20 years ago, which showed that auxin alters gene expression after only minutes in a selective and dramatic way[7–10]. The second approach was based on the analysis of a series of auxin-resistant mutations. Many of these different mutant plants lacked functional components of the ubiquitin-mediated proteolytic pathway, indicating that selective protein degradation is a crucial regulator of many aspects of the auxin response[11–13].

Whereas the levels of some mRNAs decrease many fold in response to auxin, those of other mRNAs increase many fold (for example, Aux/IAA, *GRETCHENHAGEN-3* (*GH3*) and members of the small auxin up RNA (SAUR) gene family)[10,14,15]. Furthermore, auxin activates regulons directly and rapidly. The genes that are activated or repressed during this process are ultimately responsible for the many physiological effects of auxin. The complex auxin responses are mediated by two groups of well-studied genes: the Aux/IAA genes, which consist of 29 members, and the auxin response factor (ARF) genes with 23 members, in *Arabidopsis thaliana*[16–18].

***Aux/IAA genes.*** The Aux/IAA family forms a group of early auxin-response genes. The variation in amino-acid identity among them is high and ranges from 10% to 83%. However, even poorly conserved family members seem to have compensatory functions, which means that despite their distinct induction kinetics, dose responses and expression profiles, obtaining conclusive functional information for the Aux/IAAs using loss-of-function mutants has been difficult[18]. Each individual Aux/IAA gene might have a set of non-essential functions, but these functions combine to form an important regulatory programme. Similar observations have been made in studies of the yeast oxysterol-binding protein family[19] (discussed in REF. 18), in which a functional analysis of various sets of different proteins, which individually perform non-essential functions, revealed that they combine to perform essential regulatory functions. In the future, extensive multidimensional expression maps and genetic studies of Aux/IAAs and ARFs might be necessary to tackle this difficult problem.

Aux/IAA genes encode proteins that generally have nuclear localization signals and four conserved domains (I–IV). Domain III has a predicted ribbon–helix–helix DNA-binding domain that is found in bacterial transcriptional regulators (although it is not thought that Aux/IAAs bind DNA in plants)[20]. Aux/IAAs have indeed been found in the nucleus. Several Aux/IAA genes are transcribed within minutes of plants or cells being exposed to exogenous auxin or protein synthesis inhibitors. Most strikingly, they were shown to form homo- and heterodimers not only with one another, but also with members of the ARF family.

***ARF genes.*** ARFs are transcription factors that contain an amino-terminal B3-like DNA-binding domain, which binds to an auxin-responsive element (ARE; TGTCTC) in the promoter of auxin-response genes in an auxin-independent manner[21,22]. The carboxy-terminal domain is similar to the carboxy-terminal region of the Aux/IAA proteins and is likely to promote direct interaction between both groups of proteins while bound to the ARE[23]. This interaction blocks ARE-mediated transcription[20].

***Auxin-mediated gene regulation.*** Aux/IAA proteins have been shown to function as negative regulators of gene expression[20]. Semi-dominant alleles (that show intermediate phenotypes) with mutations in the GWPPV motif of the conserved domain II cause severe auxin-related phenotypes as they affect the stability of these repressors[24]. In some cases, this can result in contradictory regulatory phenomena in which the sensitivity of the mutant to exogenously applied auxin is reduced, whereas the auxin-related phenotype is simultaneously enhanced[25]. The core region of domain II was mapped to a 13-amino-acid sequence, known as the degron: a motif that is sufficient to confer instability, even when fused to other proteins such as β-glucuronidase or luciferase[26–28]. Proteins that contain this degron sequence are an efficient target of the $SCF^{TIR1}$ E3 ubiquitin ligase complex[29].

Regulon
A collection of separate genes, the expression of which is controlled as a unit by a specific signalling compound or factor.

Degron
A protein element, usually a sequence motif, that targets the protein for proteolytic degradation.

$SCF^{TIR1}$ E3 ubiquitin ligase
A multisubunit ubiquitin ligase that consists of SKP1, CUL1 and an F-box protein (TIR1 in this case) that confers substrate specificity, as well as a RING protein that is also known as HRT1, RBX1 or ROC1.

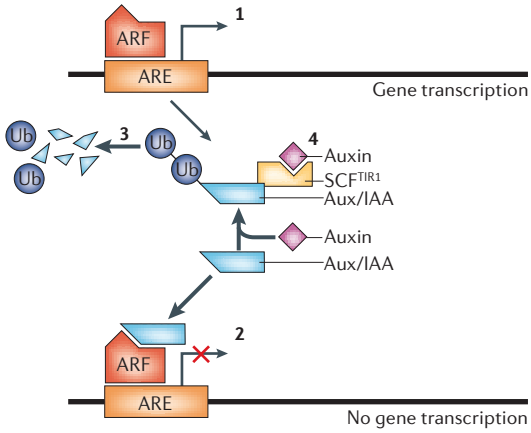



Figure 1 | **SCF$^{TIR1}$-mediated auxin signalling.** There are four distinct layers of regulation in auxin-mediated gene expression (steps 1–4). Members of the auxin response factor (ARF) family are transcription factors that bind to auxin-responsive elements (AREs) in the promoters of primary auxin-responsive genes, mediating their transcription (step 1). Aux/IAAs are early auxin-response proteins that bind ARFs, thereby inhibiting ARE-mediated gene transcription (step 2). Aux/IAAs are targets of 26S proteasome-mediated degradation, and this degradation is directed by the ubiquitylation (Ub) of Aux/IAAs; a reaction that is catalysed by an SCF E3 ubiquitin ligase (step 3). Ubiquitin-mediated proteolysis of Aux/IAA is stimulated by the binding of auxin to the F-box protein TIR1, the component of the SCF E3 ligase that specifically recognizes the proteins to be degraded (step 4). IAA, indole-3-acetic acid.

F-box protein
A component of the machinery for the ubiquitin-dependent degradation of proteins. F-box proteins recognize specific substrates and, with the help of other subunits of the E3 ubiquitin ligase, deliver them to the E2 ubiquitin-conjugating enzyme.

26S proteasome
A protein complex that is responsible for degrading intracellular proteins that have been tagged for destruction by the addition of ubiquitin.

COP9 signalosome
An eight-subunit protein complex that regulates protein ubiquitylation and turnover in a range of developmental and physiological contexts. Extensively characterized in plants but fundamental to all eukaryotes, this complex post-translationally modifies the cullin subunit of E3-ubiquitin ligases by cleaving off the covalently coupled peptide, Nedd8/RUB1.

T-DNA mutation
A mutation that is the result of the integration of DNA from *Agrobacterium tumefaciens* into plant genomes. The insertion is random and might therefore disrupt genes, causing a mutation at the insertion point.

The SCF complex, which is well characterized in many organisms including yeast and mammals, has an important role in a wide range of signal-transduction processes by ubiquitylating target proteins that are selected by F-box proteins[30,31]. These F-box-containing SCF complexes not only specifically select, but covalently modify their target proteins through the addition of several ubiquitin peptides, a process that marks the target proteins for degradation by the 26S proteasome. TIR1, an F-box protein that was identified in a screen for mutants that were resistant to a chemical inhibitor of active auxin transport, was shown to have a relatively mild auxin-insensitive phenotype when mutated[156], which indicated that other F-box proteins (subsequently termed AFB1, 2 and 3) also control the ubiquitylation of Aux/IAA proteins. Degradation of Aux/IAA proteins reduces the proportion of Aux/IAA-bound ARFs, thereby allowing ARE-mediated gene transcription to elicit an auxin response (FIG. 1).

The regulation of SCF activity is complex, and depends on a finely balanced network of post-translational modifications and protein–protein interactions. Empirical evidence for the influence of such components is occasionally counter-intuitive. For example, the COP9 signalosome (CSN) is a protein complex that removes RUB1 (a small ubiquitin-like protein that regulates SCF E3 ligase activity[32]) from the SCF complex. The inactivation of the CSN complex results in the stabilization of certain Aux/IAA proteins, which indicates that the auxin response can be regulated[33]. Furthermore, CAND1 (CULLIN-ASSOCIATED AND NEDDYLATION-DISSOCIATED), an SCF-binding protein, prevents F-box proteins from also binding to the SCF complex. Paradoxically, a *cand1* mutant causes the stabilization of Aux/IAA7 (REF. 34). It has been proposed that the rate of F-box recycling on the SCF is an important factor in controlling the proteasome. Several reviews illustrate the biological relevance of auxin-mediated protein degradation[29,35–42].

## F-box protein TIR1 is an auxin receptor

In one of the most important advances in plant biology of recent years, the F-box protein TIR1 has been identified as an auxin receptor[43,44]. Two crucial observations that led to this discovery were that auxin enhances the interaction between TIR1 and Aux/IAAs, as shown in a cell-free system[45], and that pretreatment of TIR1 with auxin enhances its binding to Aux/IAAs[27,46]. Two other recent studies demonstrated that auxin stabilizes the interaction between TIR1 and the Aux/IAAs, that auxin is continuously required for this effect, and that SCF$^{TIR1}$ binds auxin directly with a dissociation constant of between 20 and 80 nM (REFS 43,44).

Although the mechanism by which the binding of auxin to TIR1 promotes its interaction with Aux/IAAs is not yet known, it is safe to conclude that SCF$^{TIR1}$ and the associated protein-degradation machinery, together with Aux/IAAs and ARFs, represent the full signal-transduction cascade from the auxin signal to gene expression, and that these F-box proteins represent a new class of receptors (FIG. 1). In *A. thaliana*, ~700 different F-box proteins are encoded by the genome, many more than have been found in any other non-plant eukaryotic organism. This creates an extremely large range of specificity for recognition and proteolytic targeting[47–49]. TIR1 itself is a member of a small clade of F-box proteins, other members of which also seem to mediate auxin-dependent developmental regulation[50]. Genetic studies indicate that AFB1, AFB2 and AFB3 (which are encoded by the three genes that are most closely related to TIR1 in the *A. thaliana* genome) function in a partially redundant manner in mediating the auxin response. They are expressed in overlapping patterns in seedlings, leaves and flowers[50]. All three contribute to the auxin response as shown by T-DNA mutations. Analysis of two of the T-DNA alleles (*afb2* and *afb3*) showed that the transgenic seedlings were more resistant to auxin. Higher-order mutants result in a progressive decrease in diverse auxin responses such as root elongation and lateral root formation[50]. A *tir1 afb2 afb3* triple mutant either arrested shortly after germination or developed a root that was partially resistant to auxin.

F-box proteins seem not only to be important receptors for the specific degradation of Aux/IAA proteins[51], as closely related family members also mediate the response to jasmonic acid (a hormone that is involved in wound healing and pathogen defence)[52] and gibberellin[53]. These results place the 26S proteasome at the hub of plant growth and development. Assigning functions to the relatively large number of F-box proteins that are encoded by the *A. thaliana* genome is a daunting task, but will be one that could potentially revolutionize the way we think about signalling and development in plants.

## ARFs, Aux/IAAs and the auxin response

With the potential involvement of many F-box proteins, proteasome-mediated auxin signalling is unexpectedly complicated. This auxin-receptor-binding signal is then relayed to the subsequent phase of auxin-responsive gene expression, which is anticipated to be an intricately intertwined set of processes. The effects of auxin are thought to depend on its concentration, with high and low doses eliciting different responses. A framework for understanding how auxin can have such different roles in plant development is now in place. At basal auxin levels, Aux/IAAs are relatively stable, homodimerize and heterodimerize with ARFs that can bind to AREs in the promoters of auxin-responsive genes[20,54]. The ARF-bound Aux/IAA proteins block transcription from auxin-responsive promoters by controlling the amount of free ARF transcription factors to the promoters[23].

An increase in auxin levels causes the proteasome-mediated degradation of Aux/IAAs, which in turn allows for a gradually increasing number of functionally active ARF proteins and the transcriptional activation of auxin regulons. ARFs can be grouped into three subsets and vary between 57 and 129 kDa in size. The amino-acid content in the variable middle region determines whether a particular ARF functions as a repressor or an activator[17,55]. Glutamine-rich ARFs such as ARF5 and ARF7 activate transcription. When mutated, they often give remarkable phenotypes; for example, those seen in *monopteros* (ARF5) (rootless) and those seen in *nonphototropic hypocotyl4* (ARF7) (unable to bend towards light)[56]. There is emerging evidence that ARFs that lack a glutamine-rich middle region function as transcriptional repressors[55].

The expression patterns of ARF and Aux/IAA genes vary and depend on the tissue and stage of development (FIGS 2,3). Moreover, some of the most related ARF and Aux/IAA proteins (ARF3 and ARF4, ARF6 and ARF8, ARF10 and ARF17, and ARF11 and ARF18; IAA6 and IAA19, IAA8 and IAA9, and IAA32 and IAA34) (REF. 16) also share similar expression patterns. ARFs are specific to particular plant responses; for example, ARF1 and ARF2 regulate floral senescence[57,58], and ARF7 and ARF19 regulate leaf expansion and lateral root development[17,59]. The picture is further complicated by the discovery that specific pairs of ARFs and Aux/IAAs can preferentially bind to each other and can also mediate specific processes[60]. Unravelling these further layers of regulation, analysing the possible combinations of ARF and Aux/IAA interaction, and assigning specific pairs of ARF and Aux/IAA proteins that have a functional significance *in planta* promises to take our understanding of auxin signalling to unprecedented levels of detail.

Why do plants need so many different ARF proteins, and what are their specific regulatory targets? As has been observed in the case of Aux/IAA loss-of-function mutants, systematic forward-genetics approaches with ARF genes has also failed to reveal additional growth phenotypes, which indicates that ARFs have redundant and probably compensatory functions. Direct evidence for this was provided by microarray studies, which showed that different ARF proteins as well as different ARF–ARF and ARF–Aux/IAA combinations function in particular developmental windows to form a regulatory code that programmes the expression of not only auxin-sensitive genes but also genes that are regulated by auxin in a more indirect manner[17,18]. Further global expression studies with multiple higher-order (for example, double or triple) mutants are likely to provide detailed expression maps of distinct tissues and cells that might point to the regulatory targets of these genes.

Auxin concentration also influences cell patterning, as the highest levels of auxin signalling proteins correspond to the sites of new lateral roots and leaves, which has been shown to be functionally significant[61,62]. In the *A. thaliana* root tip, the relationship between the formation and structuring of new organs has been studied in detail. Here, the Aux/IAA–ARF signalling pathway is necessary for the expression of transcription factors that determine the correct differentiation of the various cell types that are present in roots. PLETHORA proteins, for example, provide positional information that is necessary for proper root development and depend on auxin (through ARF5 and ARF7) for their expression[63].

## Alternative signalling pathways

Aux/IAA–ARE-mediated signalling is probably not the only pathway through which auxin functions. Compelling reasons come from many studies: those in which impermeable auxins show their effects without entering the cell[64], or in which auxin responses are simply too fast to be mediated by gene transcription, for example, in membrane depolarization[65]. Decades of auxin research have shown that auxin controls numerous cellular processes, although the features of some responses indicate that they might not be the subject of transcriptional control (see also BOX 1). The effects of auxin on the abundance and activity of the plasma-membrane-located $H^+$-ATPase are particularly well studied[66–69]. Other regulatory targets are the potassium and chloride channels and chloride-uptake transporters[70–73]. This auxin-mediated stimulation of ion uptake correlates with the idea that auxin either controls or sustains the intracellular turgor pressure that is necessary for plant cell growth[74].

In addition to the chain of events that is initiated by the binding of auxin to F-box proteins in the nucleus, alternative modes of auxin perception have been proposed. Many of these mechanisms are based on proteins that have been shown to bind auxin directly. Many auxin-binding studies have been carried out, and more than 30 years ago several binding sites were identified in the cellular membrane system (in line with the classical idea that a signal-transduction pathway begins with ligand binding to a membrane-bound receptor) — in the plasma membrane, the endoplasmic reticulum (ER) and the vacuole[75,76]. Although several proteins with clear binding specificities were identified, the functional characterization focused on one of them, AUXIN-BINDING PROTEIN-1 (ABP1), as it binds auxins with high specificity and affinity ($K_d$ in the $10^{-8}$ M range)[77].

ABP1 is a soluble, ER-located, dimeric glycoprotein, which forms a β-jellyroll barrel that carries auxin in a central hydrophobic pocket. It resembles the 7S seed-storage proteins of the ancient family of cupin proteins that is functionally highly diversified[78]. Cupins and triose isomerase barrel proteins form a superfamily

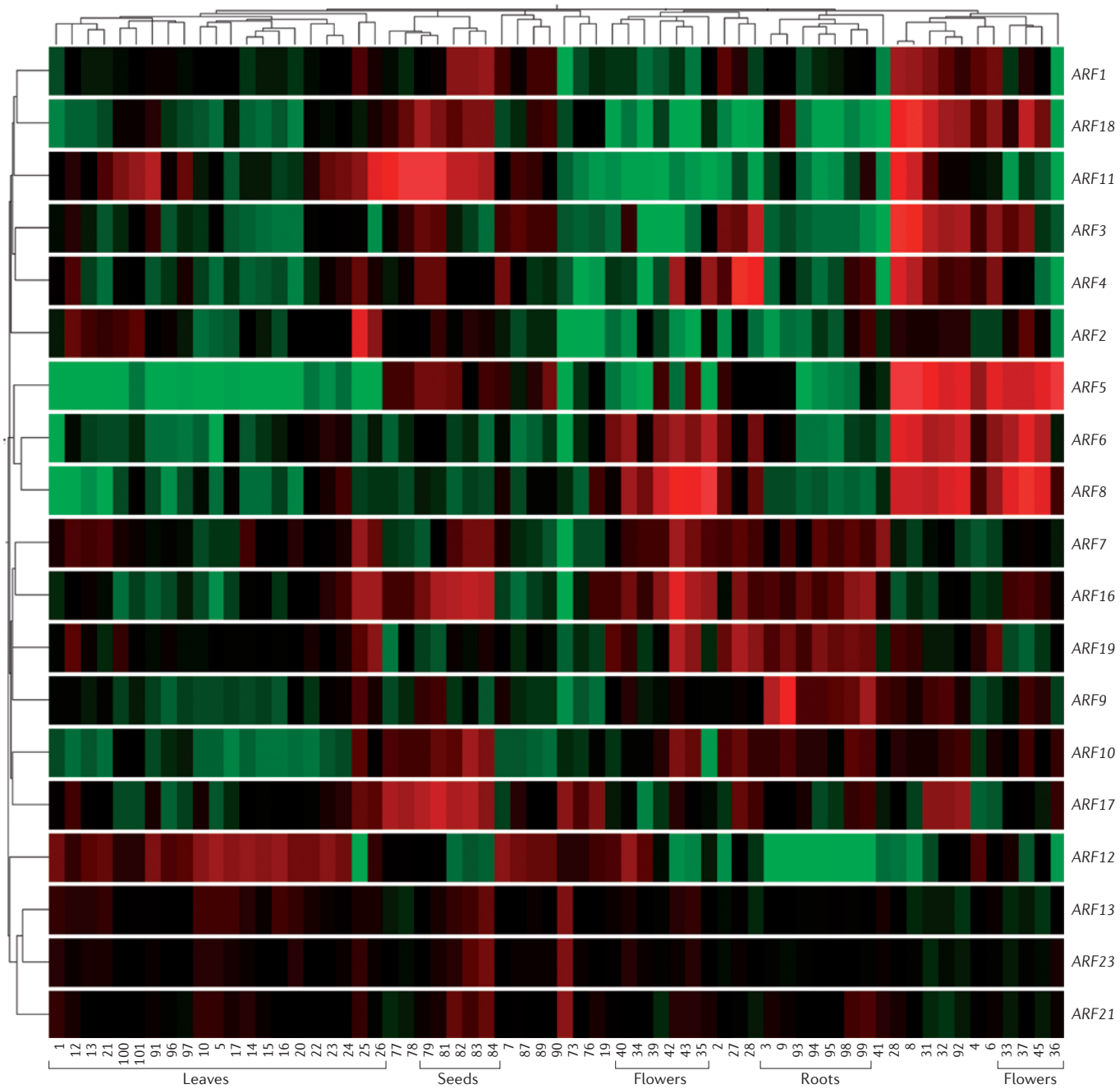



Figure 2 | **The 'auxin code' as determined by hierarchical cluster analysis of the expression patterns of ARF genes.** This figure illustrates the wide variety of expression patterns observed within the auxin response factor (ARF) gene family (FIG. 3 illustrates the wide variety of patterns observed within the Aux/IAA early auxin-response gene family). The *x* axis represents different tissues at different stages. Genes are listed on the *y* axis. In any specific tissue at any one time, a unique set of ARF genes are expressed. This heterogeneity has the potential to deliver an extremely specific signal and discriminate among all of the possible downstream effects that auxin can elicit. Bright green indicates low expression and bright red indicates high expression. Three replicates of 63 experiments were taken in different tissues and developmental stages of wild-type *Arabidopsis thaliana* (ecotype Columbia-0). The data file used for the expression analysis comes from the Developmental Affymetrix Gene Expression Atlas supplied by the Max Planck Institute, Tübingen (M. Schmid, J. Lohmann and D. Weigel laboratory ). The experimental design can be downloaded from TAIR (The *Arabidopsis* Information Resource). The data were clustered using a Pearson correlation for the condition tree and standard correlation for the gene tree (GeneSpring 7.2). When four or more of the same tissues cluster together they are labelled. IAA, indole-3-acetic acid.

in plants with possibly the widest range of biochemical functions known for any superfamily described to date[79]. It is thought that many of these proteins are important for cell survival through their involvement in cell-wall structure, modification or maintenance. In line with structural data, numerous observations show an involvement of ABP1 in cell expansion, stomatal closure, plasma-membrane hyperpolarization and cell division[68,80–84]. Although most of these responses are mild, some effects seem striking, with a complete loss-of-function mutation of the *A. thaliana ABP1* gene conferring embryo lethality, which indicates an essential

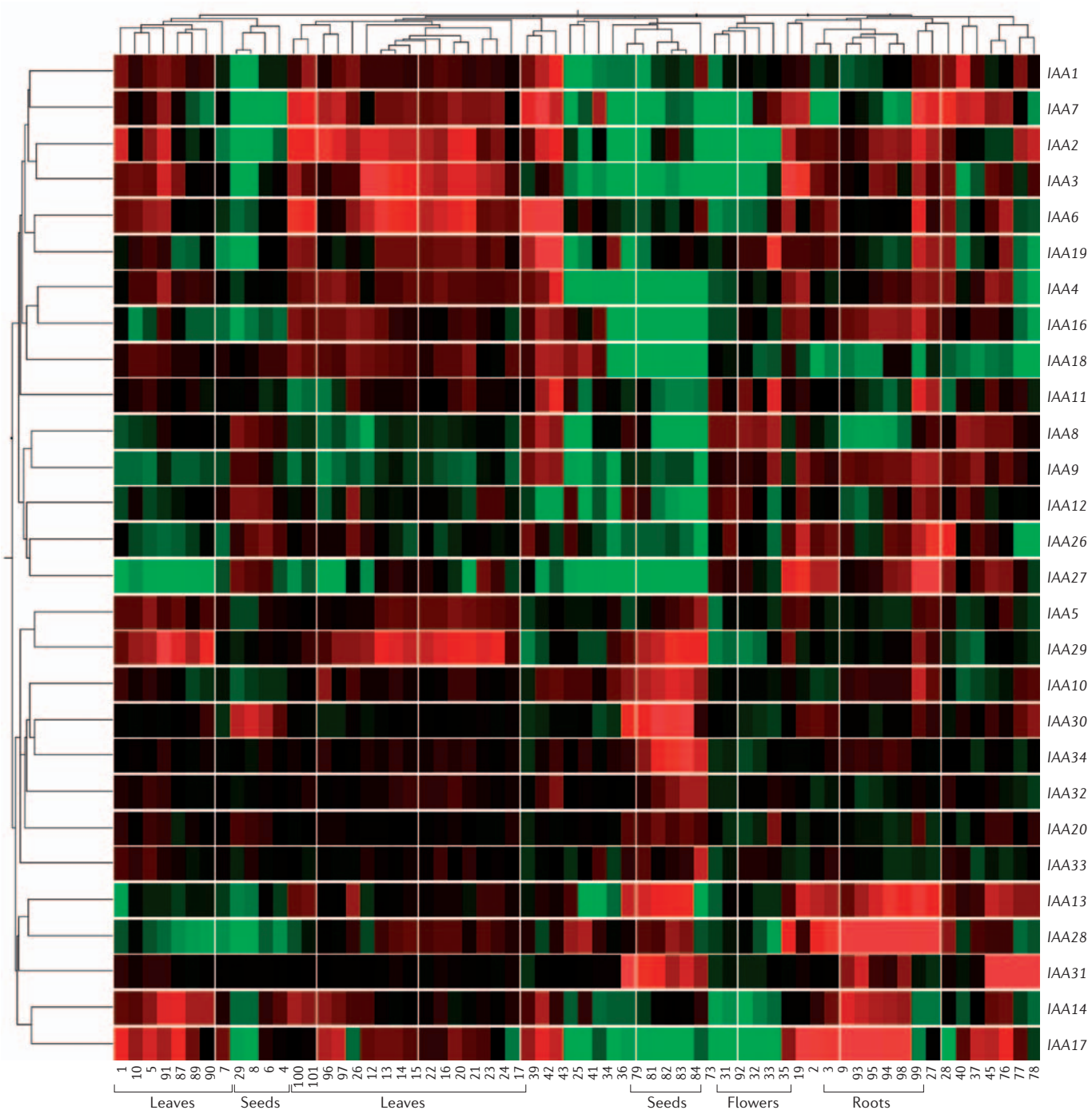



Figure 3 | **The 'auxin code' as determined by hierarchical cluster analysis of the expression patterns of Aux/IAA genes.** This figure illustrates the wide variety of expression patterns observed within the Aux/IAA early auxin-response gene family (FIG. 2 illustrates the wide variety of patterns observed within the auxin response factor (ARF) gene family). The *x* axis represents different tissues at different stages. Genes are listed on the *y* axis. In any specific tissue at any one time, a unique set of Aux/IAA genes are expressed. Bright green indicates low expression and bright red indicates high expression. IAA, indole-3-acetic acid. For further details, see FIG. 2.

Box 1 | **Post-transcriptional control of auxin signalling**

There are several points at which a plant's response to auxin is controlled. Transcriptional control is by far the best understood (see main text), but it is emerging that post-transcriptional control through microRNAs (miRNAs) and small interfering RNAs (siRNAs) also has a significant role by modulating the levels of auxin signalling proteins. The connection between RNA-mediated gene silencing and the auxin response was made after the mRNAs of five auxin response factors (ARFs) were predicted to be targets for miRNAs[147]. It has since emerged that, in the context of auxin signalling, ARFs seem to be particularly prevalent targets for small-RNA-mediated degradation. The potential of miRNAs to affect plant development through the stimulation of ARF RNA degradation was subsequently shown for a number of specific transcripts. For example, *ARF10* and *ARF16* are targeted by miR160 during root cap development[148], and *ARF3* is targeted by TAS3 (a *trans*-acting siRNA) in the juvenile-to-adult-phase transition[149]. Significantly, miR164 is induced by auxin and targets *NAC1*, an mRNA that is involved in downstream auxin signalling[150,151]. This regulation forms a homeostatic mechanism that controls the auxin response. However, as yet, the extent and exact developmental significance of specific mRNA degradation in auxin signalling is not fully understood.

miRNAs have also been shown to target mRNAs of the F-box auxin receptors TIR1, AFB2 and AFB3 in a study that gives our first functional insights into the relationship between auxin and miRNAs[152]. In this case, it was shown that exposure to a specific bacterial peptide results in downregulation of the auxin receptors. This is achieved by the induction of specific miRNAs by the peptide. Certain bacterial pathogens synthesize indole-3-acetic acid (IAA), which indicates that auxin aids the infection process. An attenuation of the auxin response by miRNA has therefore been described as part of a plant's natural immune response[152].

function for this protein in plant development[85]. There is no direct evidence for any downstream signalling events that are thought to occur after the binding of auxin to ABP1, or regarding the extent to which this signalling pathway mediates different auxin responses compared with the $SCF^{TIR1}$ pathway. However, given the almost instantaneous auxin responses that ABP1 can mediate, it is clear that gene expression need not be involved in certain aspects of auxin signalling.

## Downstream auxin signalling

The effects of auxin are many and diverse, and have been difficult to separate. They can, however, be divided into two broad categories: effects on cell expansion and effects on cell division. There is also evidence that auxin might have morphogenetic properties that are analogous to chemicals found in the animal kingdom, but the ability of auxin to change directly the developmental fate of cells has not yet been conclusively demonstrated.

***Auxin and cell expansion.*** Consequences of ABP1-mediated auxin signalling, such as cell expansion and membrane hyperpolarization, are evident at the cell periphery. Furthermore, genes that encode extracellular proteins — for example, those involved in cell-wall degradation, expansins and arabinogalactans (which are cell-wall proteins), extensins, Pro-rich proteins (which function as links between the cell wall and the plasma membrane), and class III peroxidases (which are involved in lignification, pathogen defence and wound healing) — are affected in a gain-of-function *iaa17* mutant[18]. This implicates at least two distinct auxin signalling pathways in plant structure and function through changes to the cell wall and the plasma membrane.

Less clear, but probably just as significant, are the responses that might be mediated by other, less well-studied groups of enzymatic early auxin-response genes. Two of these groups are the glutathione-*S*-transferases, which are involved in the metabolism and detoxification of xenobiotic compounds, and the quinone reductases, which protect cells directly against oxidative stress by decreasing the formation of reactive oxygen species (ROS)[86]. ROS species mediate cell-wall loosening and extension growth[87] in a process that has been linked with auxin for a long time[88]. The effects of ROS could be caused either directly as a consequence of the oxidative effects of ROS on cell-wall proteins and structures[88], or indirectly through the activation of signalling pathways and intermediate kinases and phosphatases: these would in turn regulate gene expression[89]. It has been suggested that the translation of enzymes such as the quinone reductases protects the cell against damage from auxin-induced oxidative stress[86].

***Auxin and cell division.*** Auxin also promotes cell division. Although this relationship is well known, its exact molecular basis is not understood. It is not yet clear how closely auxin is linked to progression of the cell cycle, even though the expression of many cell-cycle genes is induced by auxin[90,91]. There is evidence that this induction is mediated through both proteasome-dependent[92] and ABP1-dependent[85] pathways, and that auxin has many potential targets. These targets include proteins that are involved in transitions throughout the cell cycle, for example, entry into S phase[92] and the G2–M transition[93].

## Biosynthesis and distribution of auxin

The fate of developing tissue can be determined by the sensitivity of the growing cells to auxin (as indicated by the relative expression of the various components of its signalling machinery), the concentration of active auxin and the relative concentrations of other phytohormones. This can also vary widely in different tissues at different developmental stages. Auxin is readily conjugated to a wide variety of larger molecules, rendering it inactive. Indeed, the majority of IAA in the plant is in the form of inactive conjugates. Auxin conjugation and catabolism can therefore decrease active auxin levels. *De novo* synthesis and hydrolysis of conjugates contribute to

Cupin proteins
A diverse family of plant proteins, all of which contain at least one double-stranded α-helix or jelly-roll structural motif. This motif is also present in all structurally characterized 2-oxoglutarate-dependent oxygenases, including the hypoxia-inducible factor (HIF) hydroxylases, and is characteristic of the Jumonji transcription factors.

Root cap
The layers of protective cells that cover the tip of the growing root.

Expansins
A class of proteins that are able to catalyse the loosening of the cell wall, enabling cell expansion.

Extensins
Proline-rich proteins that connect the cell wall and the plasma membrane.

the developmental regulation of auxin homeostasis by increasing active auxin levels[94–98]. There is a high capacity for auxin biosynthesis not only in young aerial tissues, but also in roots, particularly in the meristematic primary root tip[98]. Auxin is synthesized from indole through tryptophan-dependent and tryptophan-independent pathways, and has been recently reviewed[99]. The fact that no fully auxin-deficient mutant plants have been identified reflects the importance of auxin in plant development.

***Distribution of auxin.*** Auxin is unusual among phytohormones in that it has been shown to be specifically and actively transported. Indeed, the pattern of a plant's response to auxin is not so much determined by the relative rates of auxin synthesis and catabolism as by the cells' capacity for auxin influx and efflux. Although the rates of synthesis and conjugation are undoubtedly important for the overall auxin status of the plant, it is the fine concentration gradients across only a few cells that have powerful effects on plant development. These observations have made auxin transport one of the most studied topics in plant development.

Auxin redistribution involves many proteins[100–110]; among them, the most investigated belong to a family of polarly localized plasma-membrane proteins, the PIN proteins. PINs are found throughout the plant kingdom[111] and mediate auxin efflux[105–108,111–114]. Over short distances, PIN-dependent auxin transport mediates many developmental processes, including root development[106] and organogenesis[61,109].

***Sites of auxin transport.*** Ever since its discovery, auxin has been considered a highly mobile signalling molecule. Implicit in its role in tropic growth is a requirement for directional and responsive transport. Such a mechanism needs specific and active transporters, the existence of which was predicted long before their discovery. However, as the involvement of auxin in root specification illustrates, it is not limited to growth in response to external cues such as light and gravity, as was observed by Francis and Charles Darwin[2] — its capacity for redistribution is at the heart of plant development.

In trees, as well as moving passively in the bulk flow, auxin is transported actively through the vascular cambium — a cylinder of meristematic tissue that gives rise to (and is sandwiched between) the phloem and the xylem. In *A. thaliana*, a plant that normally contains no woody tissue, auxin is actively transported through the vascular parenchyma. This conclusion is supported by the cellular location of AUX1, an auxin cellular influx carrier that is seen in positions that are consistent with vascular loading (in the leaves) and unloading (in the roots)[115–117]. AUX1 transports auxin directly, and this capacity was demonstrated in *Xenopus laevis* oocytes at physiologically significant concentrations[118]. The asymmetrical subcellular localization of AUX1 (a member of the LAX family of transporters) is thought to have functional significance, and its localization is dependent on an ER protein, AXR4, which has been shown to be responsible specifically for AUX1 distribution[119]. AUX1 has a strongly agravitropic phenotype and has a role in auxin influx[110,117], but exactly how the phenotype is caused is unknown. The characterization of other members of the LAX gene family should improve our understanding of auxin influx.

PIN proteins are related to bacterial transporters and are commonly distributed polarly in the plasma membrane, which correlates with the expected direction of polar auxin flux[105–108,120]. Loss-of-function *pin1* mutants show a distinctive phenotype after the floral transition: they often grow a single pin-shaped stem, with none of the flowers or characteristic branching of the wild-type plant[112]. It has subsequently been shown that this phenotype is due to lower rates of auxin transport[107]. Indeed, if auxin is applied to this pin-shaped stem, lateral growth is stimulated[61].

## PIN-mediated auxin transport

There are eight PIN proteins in *A. thaliana*, and they are expressed at distinct times and locations[111]. Five have been well characterized and show a distinctive polar subcellular localization. Although data for PIN1 (the knockout of which gives rise to the most dramatic phenotype) have not been reported, when expressed in heterologous systems, two other PINs — PIN2 and PIN7 — transport IAA[121]. Therefore, it has been suggested that these proteins at least do not need any other plant-specific co-factors for auxin efflux. However, heterologous expression results in a reduction of their substrate specificity, which implies that plant-specific co-factors might still have a role. Furthermore, it has been shown that at least four PINs (PIN1, PIN4, PIN6 and PIN7) are rate-limiting for auxin efflux from plant cells[121].

The localization of PINs is extremely dynamic and can change rapidly; such changes and the associated repositioning of peaks of auxin concentration are associated with important developmental events, such as embryonic development[109] and the response to gravity[105]. The mechanism that underlies the polar localization of PIN proteins and their ability to relocalize rapidly is the recycling of PIN-containing endocytotic vesicles to and from the plasma membrane. There is evidence to indicate that auxin can directly influence endocytosis. After blocking the return of endosomes to the plasma membrane[122], visible changes in the distribution of PINs, as well as more general endosome markers, between the endosome and the plasma membrane could be observed at 5 μM auxin[123] (although this is a high concentration for root growth inhibition[99]). The discovery that the application of inhibitors of endosomal trafficking also inhibited polar auxin flux revealed a close relationship between the two processes[122]. It is still not clear to what extent these processes can be separated.

The relationship between PINs and other auxin transporters is still unclear. For example, in *A. thaliana* two multiple drug resistance/P-glycoprotein-like proteins (PGPs), PGP1 and MDR1, transport auxin[100]. It has been reported that PIN1 is mislocalized in a *pgp1 mdr1* double mutant, which indicates a certain extent of control over PIN function[124]. However, this finding has

Meristem
A zone (for example, the apex of the shoot) that contains undifferentiated cells that continue to divide, providing cells for further growth and differentiation.

Phloem
Vascular tissue that carries organic nutrients as well as information molecules such as hormones throughout the plant.

Xylem
Vascular tissue that delivers water and mineral nutrients, which are taken up by the root system, to aerial organs. It also provides mechanical support.

Agravitropic
When a plant is unable to either perceive or respond to gravity. Typically, the roots of agravitropic plants grow in all directions.

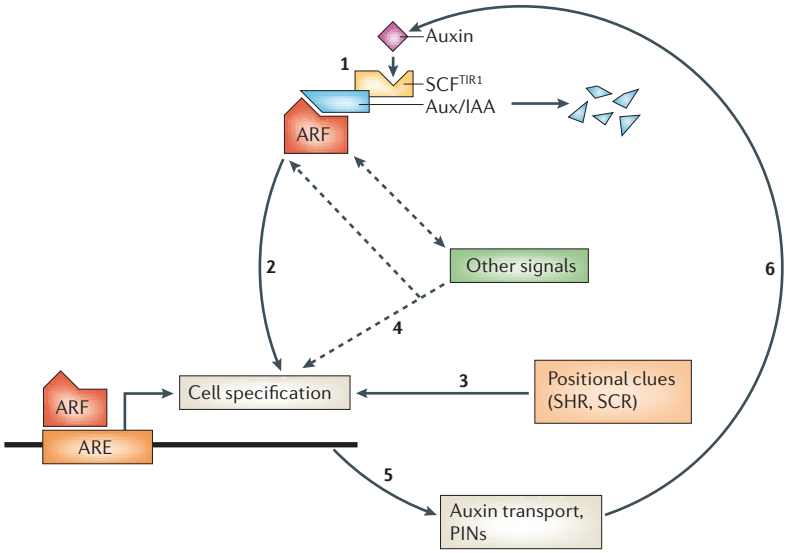



Figure 4 | **The developmental feedback loop of auxin signalling and auxin transport in roots.** The *Arabidopsis thaliana* root tip is a commonly used model system in plants. Here, we describe the relationships between auxin signalling, auxin transport and cell specification. In step **1**, auxin leads directly to the destabilization of Aux/IAA proteins, allowing auxin response factors (ARFs), which are transcription factors, to control the transcription of auxin-regulated genes (see also FIG. 1). In step **2**, the subsequent expression of certain auxin-inducible genes, such as *PLETHORA*, initiates a complex chain of cell-specification events. However, as in step **3**, cell specification is also dependent on self-generated positional cues, for example SHORTROOT (SHR) and SCARECROW (SCR), which mark the central cylinder and the endodermis/quiescent centre, respectively. In step **4**, the presence of auxin is not sufficient for cell specification. For example, even though it is required for the first events in root specification, auxin alone cannot induce root formation. Therefore, interactions between this and other signalling pathways define the final cell specification. In step **5**, the correct polarization of the auxin-transport machinery (the PIN proteins) is a result of as yet unknown polarized markers that are laid down by cell specification. In step **6**, changes in auxin concentration that are a result of auxin transport control the expression of the early auxin-responsive Aux/IAA genes and the regulation of auxin-inducible transcription by ARF transcription factors. IAA, indole-3-acetic acid.

been questioned by results showing that PIN1 function is not dependent on the presence of PGPs[121].

Although distinct roles for the PIN proteins have been described, there remains significant functional overlap among them. The most similar family members can complement one another in knockout mutants, and display some degree of adaptability in the cells in which they are expressed. For example, in the *A. thaliana pin1* mutant, PIN4 is seen to extend into the cells where PIN1 would have been present[114]. Auxin induces the expression of many PIN proteins in an Aux/IAA-dependent manner[125]. However, during the regeneration of a damaged root tip, the correct expression of PIN proteins has recently been shown to be dependent on pre-existing cell patterning rather than auxin concentration[126]. In addition, the presence of auxin alone is not sufficient for root specification in the absence of PIN-mediated auxin transport[127]. These two observations therefore make it unlikely that PINs alone directly determine either cell specification or polarity; instead, they indicate that PINs mediate distinct developmental signals as part of a wider developmental programme[126] (FIG. 4).

Guanine nucleotide exchange factor
A protein that facilitates the exchange of GDP (guanine diphosphate) for GTP (guanine triphosphate) in the nucleotide-binding pocket of a GTP-binding protein.

GTPase-activating protein
(GAP). A protein that stimulates the intrinsic ability of a GTPase to hydrolyse GTP to GDP. Therefore, GAPs negatively regulate GTPases by converting them from active (GTP-bound) to inactive (GDP-bound).

***Regulation of PIN expression.*** Of the many factors that have been shown to control PIN localization and function, there are two broad groups that have received much attention. The first regulates the vesicle-cycling machinery, and the second determines the phosphorylation status of the cell.

ADP ribosylation factors are monomeric GTPases that are involved in vesicular trafficking. Their activity is regulated by ADP ribosylation factor guanine nucleotide exchange factors (ARF GEFs) and ADP ribosylation factor GTPase-activating proteins (ARF GAPs). Loss-of-function alleles of these regulators, as well as of ARF1 (ADP RIBOSYLATION FACTOR-1) itself, can lead to defects in PIN function[128,129]. In particular, GNOM, an ARF GEF, controls many general aspects of polar auxin transport[130] and is involved in the mechanism that targets polar PIN1 to the appropriate end of the cell[129].

PIN expression is regulated by phosphorylation. The *ROOTS CURL IN NPA-1* (*RCN1*) gene encodes a regulatory subunit of protein phosphatase-2A, a heterotrimeric serine/threonine protein phosphatase[131]. Loss-of-function mutations in the *RCN1* gene exhibit an elevated level of root basipetal auxin transport[132]. Furthermore, treatment with inhibitors of protein phosphatases-1 and 2A mimics the phenotypes of *rcn1* mutants, including increased root basipetal auxin transport and an altered root gravity response[132,133]. However, at higher concentrations a significant reduction in root basipetal auxin transport was observed, which indicates a biphasic response of the root to dephosphorylation.

Loss-of-function mutations in *PINOID* (*PID*), a gene that encodes a protein kinase, cause flower stems similar to those of *pin1* mutants[134–136] and roots that are unable to respond to gravity[135,136]. Crucially, *pid* displays an apical–basal shift in the polarity of several PIN proteins[137], depleting the primary root meristem of auxin and causing a root meristem collapse. PID directly controls PIN polarity. It functions as a binary switch, with subthreshold PID levels leading to basal PIN localization and above-threshold PID levels leading to apical PIN localization. Although PID interactors have been identified, it is unclear whether they are targets for phosphorylation or whether they are upstream signalling components[138], although the central regulator PDK1 has recently been shown to bind and phosphorylate PID directly[139]. The elusive mechanism of PID in determining cell polarity makes it one of the most mysterious proteins in plants.

## The auxin signalling–transport relationship

Active auxin transport mediates cellular auxin concentration and is therefore a crucial component in the coordination of plant development (FIG. 5). However, the specific relationship between auxin signalling and auxin transport is poorly understood. Various kinases are regulated by auxins[140–142]; recently, elements of the oxidative stress MAP kinase cascade including MAP KINASE KINASE-7 (MKK7) have been identified that control plant architecture through the negative regulation of polar auxin transport[143]. It has been suggested that the auxin transporter also serves as an auxin receptor, but the suggestive correlative data currently

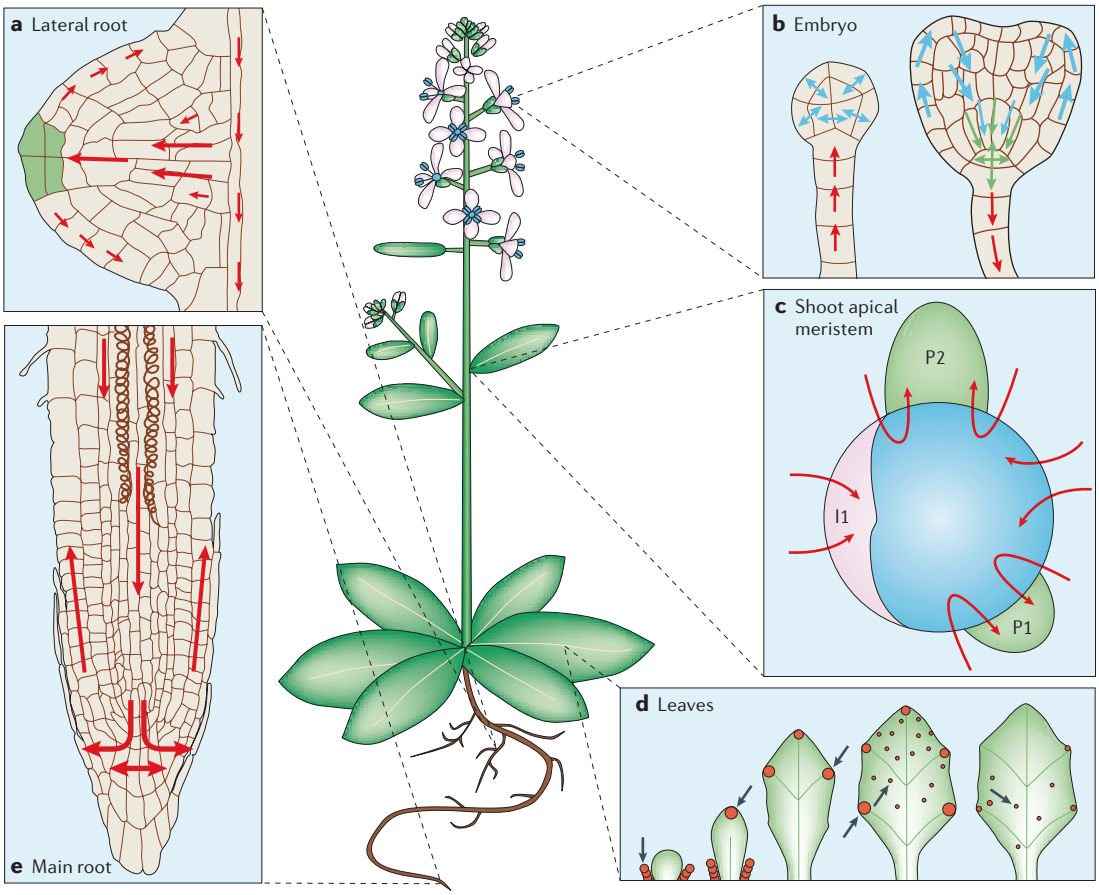



Figure 5 | **The developmental processes that are controlled by auxin flux. a** | Lateral root. PINs conduct auxin from the centre of the root (stele) to the new root tip (auxin is indicated in green and auxin transport is indicated by red arrows), and then away again through the epidermis. This forms the basis of the 'fountain' model of lateral root formation[62]. **b** | Embryo. Auxin is taken to the very young embryo by PIN7 (left). At a later stage (right), the auxin flux is reversed as PIN1, PIN4 and PIN7 conduct auxin out of the embryo. Transport by PIN1, PIN4 and PIN7 is indicated by blue, green and red arrows, in corresponding order. **c** | Shoot apical meristem. Auxin is redirected towards the site of new leaf formation (primordial P1 and P2 and the incipient primordium I1) in the epidermal layer. The shoot apex is indicated in blue. **d** | Leaves. Auxin mediates vascular tissue development (indicated as uninterrupted green lines) and patterning in the developing leaf through non-polar PIN1. The arrows indicate sites of auxin production and the red circles indicate auxin accumulation. **e** | Main root. PINs determine the flux of auxin towards the root tip in the centre of the root, and back again in the epidermis. This movement forms the basis of the root's ability to respond quickly to gravity. Parts **a**–**e** adapted, with permission, from REFS 62,125,153–155 © (2003) Cell Press, (2005) Company of Biologists Ltd, (2005) Current Biology Ltd, (2004) Kluwer Academic Publishers, and (2005) Scandinavian Society for Plant Physiology, in corresponding order.

only underline the gaps in our understanding of these processes[144]. Another surprising link indicates a role for Rac-like (ROP) GTPases in auxin action. Auxin activates ROP3 (REF. 145); and both auxin and active ROP3 (or RAC1) cause Aux/IAA proteins to aggregate into discrete nuclear bodies. These structures are proteolytically active, resulting in the 26S proteasome-mediated degradation of Aux/IAA proteins. This potentially provides a link between auxin perception at the plasma membrane and the regulation of genes in the nucleus[145,146]. Proteasome-dependent auxin signalling does not seem to be in exclusive control of auxin transport. As previously discussed, synthetic auxins have been shown to slow the rate of PIN endocytosis, thereby increasing the amount of PIN at the plasma membrane. This mechanism outlines a potential negative-feedback loop for the maintenance of cellular auxin concentration but, significantly, has not been shown to be affected by $SCF^{TIR1}$ (REF. 123). The regulation of auxin transport, and specifically the role of auxin itself in its own transport, is beginning to be uncovered. This area of research promises to teach us much about how plant growth is controlled.

## Concluding remarks

Almost 40 years ago it was proposed that the auxin efflux carriers and auxin receptors were closely linked, if not identical[157]. And although this proposition has not found widespread support, the fact that it cannot be fully dismissed illustrates just how closely auxin signalling and auxin transport are intertwined. Indeed, many data now point to a crucial and central role of

auxin carriers such as the PIN proteins in mediating the bewildering array of developmental processes triggered by auxin. Moreover, the recent findings on the role of endo- and exocytotic processes that are involved in the polar localization of auxin efflux carriers opens new and surprising perspectives for understanding auxin action and cell polarity control. Along with multiple layers of transcriptional control, these ideas will need to be incorporated into any tentative new models that attempt to explain auxin action.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### Competing interests statement
The authors declare no competing financial interests.

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