Simple hormones but complex signalling

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It has not been easy to make sense of the pleiotropic effects of plant hormones, especially of auxins; but now, it has become possible to study these effects within the framework of what we know about signal transduction in general. Changes in local auxin concentrations, perhaps even actively maintained auxin gradients, signal to networks of transcription factors, which in turn signal to downstream effectors. Transcription factors can also signal back to hormone biosynthetic pathways.

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Abbreviations

ARF	auxin-response factor
AtPIN2	*Arabidopsis thaliana* PIN-FORMED2
AUX	AUXIN
bdl	*bodenlos*
GA	gibberellic acid
IAA	indole-3-acetic acid
Kn	Knotted
MP	*MONOPTEROS*
NPA	*N*-1-naphthylphthalamic acid
Ntc12	*Nicotiana tabacum* c12
NTH15	*Nicotiana tabacum* homeobox15
SCF	Skp1/Cullin/F-box
SINAT5	seven-in-absentia of *Arabidopsis thaliana*5
STM	SHOOTMERISTEMLESS
TIR	Toll/interleukin1 receptor

Introduction

'Auxin does everything' [1]. A simple statement that sums up common knowledge not just about auxin but plant hormones in general. The classical plant hormones are small non-protein molecules that have specific effects on an astounding variety of developmental and physiological processes. How do they do it?

In a common view of signal transduction, an environmental or developmental signal activates a signalling cascade that recruits specific transcription factors. These transcription factors activate downstream executor genes, which in turn carry out the required response. In such a scheme, specificity is provided primarily by the transcription factors. There is excellent evidence to support such models [2] (for a non-plant view see [3,4]).

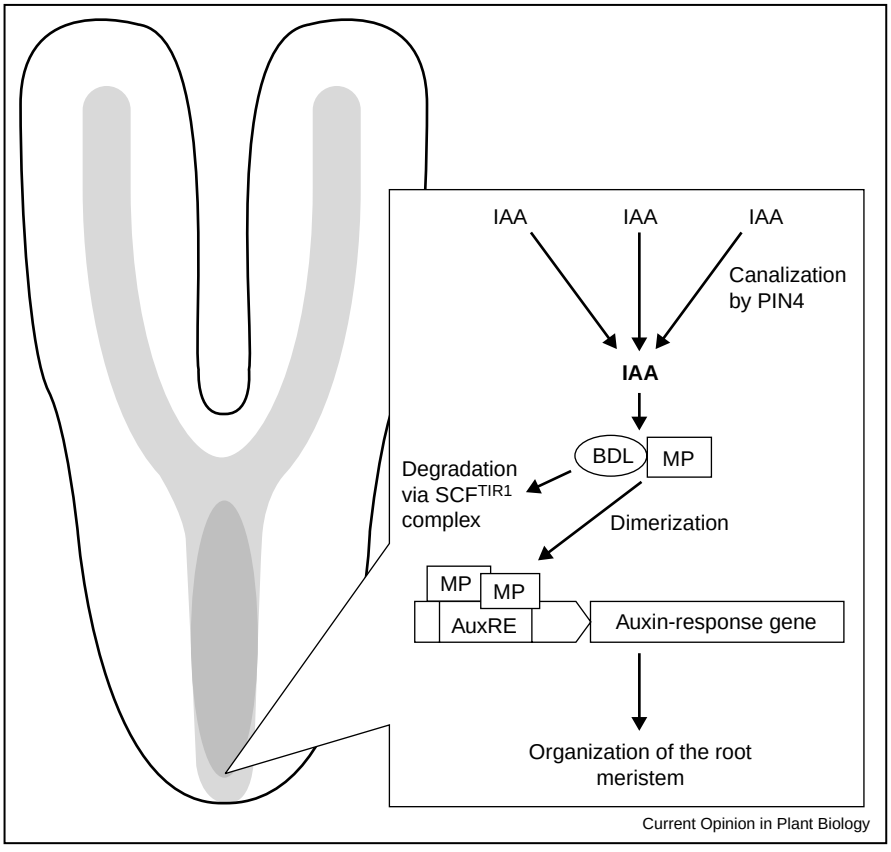
In this review, we discuss selected experiments that shed light on the specificity of signalling. Transcription factors are obviously important, but they are not the only elements that can provide specificity. There is clear evidence that the auxin distribution system contributes to the specificity of various auxin responses. Further, we review evidence for the roles of cytokinin and especially gibberellin downstream of transcription factors.

A multitude of transcription factors

Two protein families play major roles in auxin-modulated gene expression. The auxin-response factors (ARFs), which are encoded by a gene family of 23 members in *Arabidopsis*, are DNA-binding proteins that can either activate or repress the transcription of target genes [5]. One group of ARFs contain a Q-rich middle region and are thought to be transcriptional activators, whereas ARFs with other middle regions are putative repressors [5,6]. The second group of auxin-modulated transcriptional regulators are the auxin/indole-3-acetic acid (AUX/IAA) proteins, which comprise 29 members in *Arabidopsis*. The Aux/IAA proteins do not bind to DNA directly [7] but contain conserved protein–protein binding domains, which are also present in ARFs. Aux/IAA proteins are thought to repress auxin-induced gene expression by heterodimerization with ARFs, thereby modulating the activity of these DNA-binding transcription factors [8••]. The rapid, auxin-dependent, turnover of AUX/IAA proteins that is mediated by the protein destabilization domain II and executed by a specialized branch of the ubiquitin-proteasome pathway makes Aux/IAA proteins highly responsive to changes in auxin signalling [9,10,11••,12].

Both the *ARF*- and the *Aux/IAA*-gene families are subdivided into different subfamilies, and the less-conserved regions of these proteins could have an influence on specific targets or functions [6,13]. Subtly different affinities of various ARFs for *cis*-acting elements in auxin-responsive target genes may endow individual ARFs with specialized functions. Although many ARFs are expressed in all major organs, some appear to be expressed in a more restricted fashion at the tissue level [14]. Most convincingly, single mutations in *ARF* genes give distinct phenotypes; for example, mutations in *ARF5/MONOPTEROS* (*MP*) affect early embryogenesis, lesions in *ARF3/ETTIN* (*ARF3/ETT*) specifically interfere with gynoecium development, whereas disruptions of *ARF7/NONPHOTOTROPIC HYPOCOTYL4* (*NPH4*)

Figure 1



Aux/IAA and ARF proteins act together to mediate auxin responses in the embryo. Auxin is canalized in the embryonic root by PIN4. When a certain auxin concentration is reached BDL–MP heterodimers dissociate. BDL is then degraded via the SCF^{TIR1} (Skp1/Cullin/F-box–Toll/interleukin1 receptor) complex; whereas MP builds homodimers that bind to auxin-responsive elements (AuxRE) on the promoters of unknown auxin-responsive genes whose expression thus is turned 'on'. Correct BDL–MP signalling is necessary for the organization of the root meristem. The expression patterns of MP (light gray) and BDL (dark gray) are shown in a torpedo-stage embryo.

cause defects in the differential growth of aerial tissues [15,16]. Thus, whether through differential expression, differential affinities for target promoters, or both, individual ARFs have distinct functions.

There is good evidence, for instance from the molecular analysis of the *bodenlos* (*bdl*/*iaa12*) mutation [17••], that the unique roles of individual Aux/IAAs are based on their differential expression. The *bdl* and *mp* mutants have similar embryonic phenotypes and are thought to affect the same developmental pathway. The mutant BDL/IAA2 protein has a characteristic amino-acid change in domain II (which is also found in IAA17/AUXIN-RESISTANT3-1 [AXR3-1] and IAA3/SHORT HYPOCOTYL2 [SHY2]), which leads to protein stabilization [18••,19]. Thus, stabilization of the IAA12/BDL protein has the same effect as a loss-of-function mutation in ARF5/MP, indicating that the function of IAA12/BDL is to inhibit the activator role of ARF5/MP (Figure 1). *IAA12/BDL* and *ARF5/MP* are co-expressed in early embryogenesis and their protein products interact in a two-hybrid assay. By contrast, *IAA3/SHY2* is not expressed in the embryo, and mutations in this gene have different phenotypic effects later in development [20,21].

Not all of the transcription factors that are involved in auxin-dependent signalling are ARFs or AUX/IAA proteins. NAC1, which belongs to a different plant-specific family of transcription factors, induces the formation of lateral roots [22]. It is expressed in the same cells as SINAT5 and directly interacts with this E3 ubiquitin ligase. SINAT5 is involved in the proteolytic degradation of NAC1. Both *NAC1* and *SINAT5* are auxin-induced but the induction of *SINAT5* is slower than that of *NAC1*, thus a window for the transient activation of an auxin response is potentially created [23••].

Polar auxin transport is regulated by differentially expressed membrane proteins

Auxin is preferentially transported from the shoot tip downwards. This polar transport is energy-dependent and can be inhibited by specific inhibitors such as

N-1-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA). The chemiosmotic hypothesis, which was first formulated in 1974 [24], postulates the presence of an export carrier at the basal side of transporting cells. Over the past couple of years, such proteins have been identified and studied in great detail [25–27].

Several mutants have been isolated whose specific defects indicate that they have altered polar auxin transport. The *Arabidopsis thaliana* PIN-FORMED2 (AtPIN2) protein, which was identified in an agravitropic mutant, is a membrane protein that is expressed in a highly suggestive polar pattern. It localizes to the lower side of stele cells and to the upper side of epidermal cells, compatible with its having a role in auxin transport downward through the stele and back upward through the epidermis [28–30].

Most plant genes are present in multiple copies, and *AtPIN2* is no exception. What has been a major surprise, however, is that mutations in each individual member of the *AtPIN* family appear to cause distinct auxin-related phenotypes. A further member of the *AtPIN* gene family, *AtPIN3*, is involved in the gravitropic response. The response of *pin3* mutant plants to gravitropic stimuli is reduced. The AtPIN3 protein is expressed in the root tip and, within minutes of gravistimulation, accumulates at the lateral cell surface of the gravity-sensing tissues [31]. This is an impressive example of how the relocalisation of an auxin-transport protein (and by inference auxin redistribution) instructs a highly specific biological response.

In *Arabidopsis* roots, auxin accumulates to a distal maximum in columella initial cells. This maximum depends on polar transport as inhibitors that interrupt this transport disrupt the steep auxin gradient and lead to a broad distribution of auxin. A shift in the localization of the auxin peak interferes with patterns of cell fate that are distal to the re-localized auxin peak. In the most extreme case, the tissues proximal and distal to the auxin peak formed a mirror image of each other [32]. A further member of the *PIN* gene family is important in the establishment of this local auxin maximum in the root [33••]. *AtPIN4* is localized in developing and mature root meristems where it could create a sink for auxin below the quiescent centre. *Atpin4* mutants are able neither to create an endogenous auxin gradient nor to canalize exogenously supplied auxin. Again, defects in auxin distribution lead to patterning defects in the roots of the embryos and seedlings of *Atpin4* mutants.

AtPIN1 and AtPIN3 cycle rapidly between the basal membrane and intracellular compartments. This actin-dependent relocalisation of auxin efflux carriers gives a hint as to how plant organs are able to react quickly, via a redistribution of auxin, to changes in their growth direction (e.g. those induced by gravi- or phototropic stimuli) [31,34••].

Auxin import

Although not predicted by the chemiosmotic hypothesis, there is some indirect evidence of the existence of an active mechanism for auxin import [35].

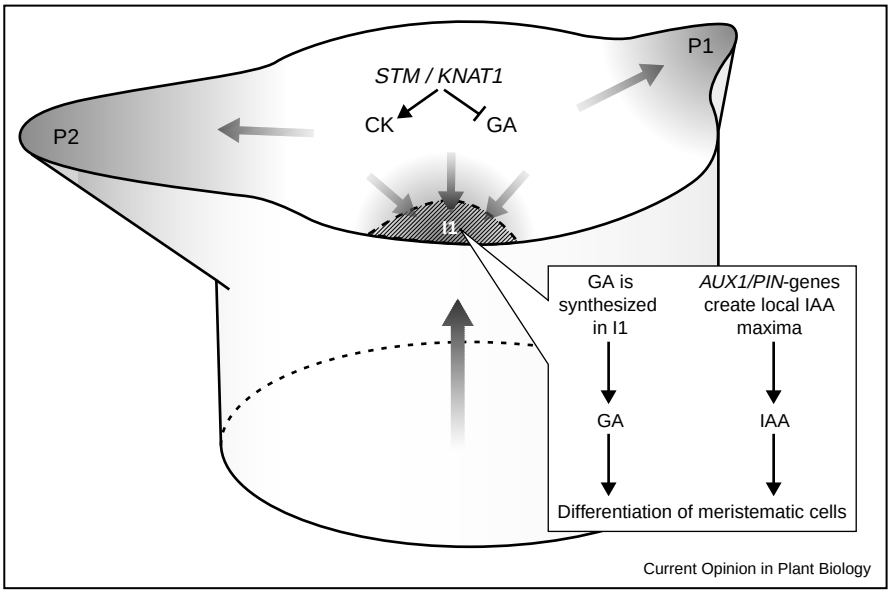
Considerable concentrations of free auxin in the phloem sap of *Ricinus communis* indicate that auxin may be transported over long distances through the phloem [36]. Similarly, NPA failed to inhibit the export of IAA from young leaves [37] and auxin accumulated in the apical tissue of NPA-treated roots [38], suggesting that cell-to-cell transport is not predominant in the long-distance transport of IAA. Recent work has provided evidence that the auxin influx carrier AUX1 is involved in phloem loading in leaf tissue [39], as well as in unloading in the roots [40••]. In the root itself, AUX1 is expressed in developing lateral root primordia [39]. The number of lateral roots that are induced in *aux1* mutants is only about half that in the wildtype [41], indicating that efficient phloem unloading and coordinated auxin import into lateral-root primordia is necessary for correct pattern formation.

Auxin gradients in the shoot apical meristem?

What about auxin responses that affect shoots? Little is known about auxin signalling in shoots as compared with roots, but it appears that the inhibition of auxin transport has quite different effects in roots and shoots. NPA application causes aberrant tissue development in the root tip; whereas in the shoot tip, it specifically interferes with organ initiation without affecting the anatomy of the meristem [32,42]. Treatment of tomato shoot apices with NPA had no effect on stem growth or on meristem maintenance, but completely stopped leaf formation. Microapplication of the natural auxin, IAA, to a defined position at the flank of one of the pin-shaped meristems that result from NPA treatment led to the formation of a new primordium at the site of application. A similar effect was seen in *Arabidopsis pin1* mutants, which have a defect in the auxin efflux carrier PIN1 [42]. In these mutants, the local application of IAA to the 'pins' caused the localized outgrowth of floral primordia. These experiments suggest that local auxin concentration determines the site of organ initiation in shoots in the circumferential dimension but not in the apical–basal dimension.

Interestingly, when pins that were formed as a result of NPA treatment were locally treated with the synthetic auxin naphthalene-1-acetic acid (NAA) rather than with IAA, the induction of primordia was not localized. Instead ring-shaped lateral structures that encompassed the meristem were formed. As NAA can enter cells by diffusion because of its lipophilic character, this may indicate that not only PIN proteins but also active auxin import is involved in organ positioning (PA Stieger *et al.*, unpublished data). Such data are compatible with models in

Figure 2



Auxin gradients in the shoot apical meristem regulate phyllotaxis. Auxin reaches the meristem via acropetal transport (upward pointing arrow). The existing leaf primordia (P1 and P2) act as auxin sinks that create a local auxin maximum (arrows) at the position of the next incipient leaf (I1). Homeobox genes (*STM*, *KNAT1*) that are expressed in the meristem inhibit the biosynthesis of GA and promote cytokinin (CK) production. They are not expressed at I1, leading to GA-production in this position (dashed line). Cells start to differentiate at I1 in response to GA and auxin, and a new leaf is formed.

which organ positioning in the shoot apical meristem is controlled by auxin gradients (Figure 2). At present, the evidence for such gradients is indirect. Visualization of auxin concentrations in the shoot apical meristem with the aid of auxin markers has not yet been possible; neither has the localization of auxin import and export proteins been determined. Each new leaf initiates at a position approximately 137° displaced relative to the previous primordium, so an auxin gradient that could control organ positioning in the shoot apical meristem would need to be highly dynamic in time and space.

Transcription factors can regulate local hormone levels

In this section, we draw attention to cases in which hormones seem to act downstream of specific transcription factors. Knotted (Kn)-type homeodomain transcription factors, such as SHOOTMERISTEMLESS (STM) and KNAT1, are required for meristem maintenance [43–45]. The genes that encode these transcription factors are highly expressed in the meristem, but their transcripts are absent from incipient leaf primordia. Ori and co-workers [46] expressed the maize homeodomain transcription factor Kn1 under the control of a senescence-specific promoter in tobacco. The transgenic plants showed a remarkable delay in senescence compared to the control. Moreover, their phenotype was similar to that of plants overexpressing a cytokinin biosynthetic gene with the same promoter. Indeed, cytokinin concentrations were up to 15 times greater in leaves expressing Kn1 than in comparable leaves of control plants. These results strongly suggest that Kn1 induces the biosynthesis of cytokinin, although the increase in cytokinin concentrations might be a secondary effect of Kn1 expression [46]. Overexpression of the isopentenyl transferase gene (*ipt*), on the other hand, led to the overproduction of cytokinin and to an increase in *KNAT1* and *STM* mRNA levels [47].

More direct evidence that hormone biosynthesis can be controlled by transcription factors comes from studies on gibberellin. In tobacco, the induction of *Nicotiana tabacum* homeobox 15 (NTH15; a tobacco Kn1 homologue) leads to the suppression of a gene that encodes a gibberellic acid (GA) 20-oxidase (i.e. *Nicotiana tabacum* c12 [Ntc12], a GA biosynthetic enzyme), which is followed by a decrease in bioactive GA_1 levels. NTH15 binds directly to a *cis*-element in the first intron of Ntc12 that is required for the NTH15-dependent suppression of Ntc12 [48••]. Conversely, the lobed-leaf phenotype that is caused by overexpression of Kn-type genes in *Arabidopsis* is antagonized by GA. In the tomato mutant *Mouse ears*, overexpression of a Kn-homologue causes the spectacular bisection of the leaf blade, a phenotype that can be partially rescued by GA application or by introducing the constitutive GA-signalling mutation *procera* [49••]. Taken together, these data clearly show that Kn-like transcription factors promote meristematic activity by controlling the homeostasis of growth factors.

Conclusions

Plant hormones are important intercellular signals, but until recently we could only speculate about the molecular mechanisms that transduce these signals into plant responses. In this review, we have selected a few highlights of modern hormone research in the belief that they serve as examples of the impressive progress made in the wide field of hormone signal transduction. One message is the importance of transcription factors as critical links in the signal transduction chain. The other message is that the sequence from signal to transcription factor to downstream effector may not be at all linear; transcription factors can feed back on hormone synthesis and distribution, thereby creating complex yet highly specific networks. Much remains to be learned about hormone signal transduction. For instance, we have only a single candidate for an auxin receptor. This essential protein, AUXIN-BINDING PROTEIN1 (ABP1), has an unusual structure for a receptor and does not yet link to known signal transduction pathways [50,51]. The *pin* mutants are associated with defects in auxin transport, but our understanding of the biochemistry of the PIN membrane proteins is virtually non-existent. We have gained considerable insight into tissue differentiation in the root, but we are only beginning to understand the role of auxin in organ development at the shoot meristem. Evidently, the key to understanding each auxin-dependent biological response will be to identify and study in detail the molecular components involved.

Acknowledgements

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- •• of outstanding interest

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