The Past, Present, and Future of Chemical Biology in Auxin Research

Bert De Rybel[†,‡,||], **Dominique Audenaert**[†,‡,||], **Tom Beeckman**[†,‡,*], and **Stefan Kepinski**[§,*]

[†]Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Gent, Belgium, [‡]Department Plant Biotechnology and Genetics, Ghent University, Technologiepark 927, B-9052 Gent, Belgium, and [§]Centre for Plant Sciences, University of Leeds, Leeds LS2 9JT, U.K., [||]These authors contributed equally to this work.

The term "chemical biology" is inherent to the field of plant biology as plants use an extraordinarily vast range of small molecules (chemical) to adapt their developmental and physiological programs (biology) in response to ever-changing environmental conditions. The absence of cell movement in plants means that these low molecular weight mobile compounds have a vital role as signaling molecules. Plants are literally packed with endogenous small molecules such as hormones (auxin, cytokinins, ethylene, brassinosteroids, gibberellic acid, jasmonic acid, and others) and secondary metabolites. Because of their importance for plant growth and development, it is not surprising that research has turned to the immense chemical repertoire of small molecule libraries as an alternative way to study particular processes. Although the use of bioactive small molecules is still considered as an innovative approach in plant research, the two fields have a longer association than one might at first expect. Auxin research in particular has always been reliant on the use of many synthetic auxin analogues, antagonists, and transport inhibitors (Figure 1, Table 1). Screening for resistance to a range of auxin probes has built on this early work to provide most of the key players in auxin biosynthesis, signaling, and transport. Here, we give an overview of the small molecules used in auxin biology and indicate their site of action throughout the known auxin pathway.

Use of Auxin and Auxin-Like Molecules. Our current understanding of how the auxin signal is transduced is based largely on a collection of mutants resistant to 2,4-dichlorophenoxy acetic acid (2,4-D, Figure 1), a synthetic auxin invented more than 60 years ago (*1*). This auxinic herbicide is an aryloxyacetate molecule strikingly different from the indole ring structure of the most abundant auxin in plants (indole-3-acetic acid, IAA,

ABSTRACT Research into the plant hormone auxin has always been tightly linked with the use of small molecules. In fact, most of the known players in auxin signaling and transport in the model plant *Arabidopsis thaliana* were identified by screening for resistance to auxin analogues. The use of high-throughput screening technologies has since yielded many novel molecules, opening the way for the identification of new target proteins to further elucidate known pathways. Here, we give an overview of well-established and novel molecules used in auxin research and highlight the current status and future perspectives of chemical biology approaches to auxin biology.
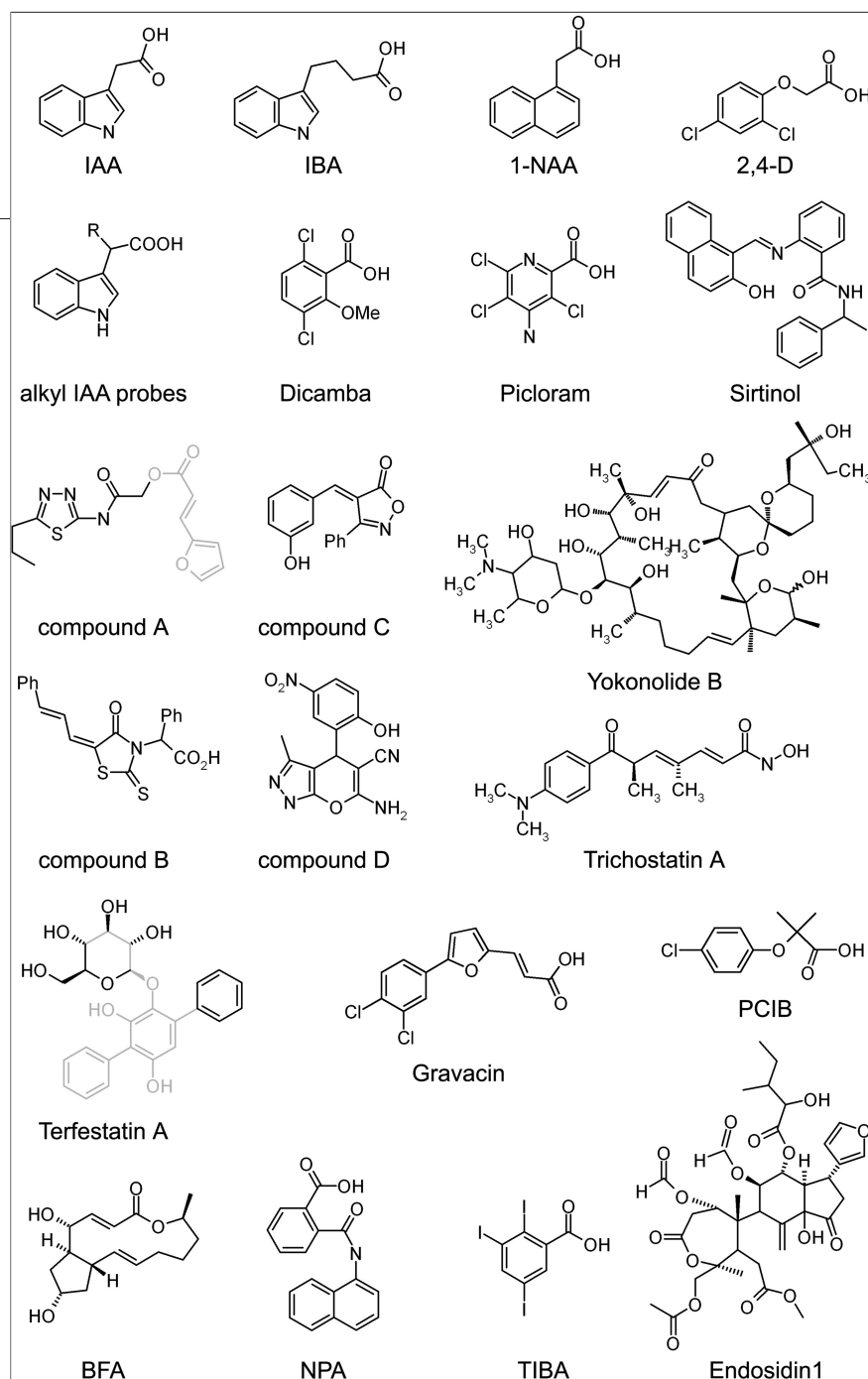
*Corresponding authors,
s.kepinski@leeds.ac.uk,
tom.beeckman@psb.vib-ugent.be.

Figure 1. Overview of small molecules used in auxin research (also see Table 1). Grey substructures indicate known active core structures of the originally identified molecules; compounds A–D from ref 34.

Figure 1) but has an extremely potent auxin-like action. Its high stability made it the molecule of choice for a series of *Arabidopsis* genetic screens for auxin resistance in the early 1980s (*1, 2*). This early example of a "chemical genetics" approach, in which the application of chemicals is combined with a search for suppressor mutants, revealed ubiquitin-mediated proteolysis to be a central mechanism in the regulation of gene expression by auxin. In fact, of six loci named *AXR1* (for <u>a</u>uxin <u>r</u>esistant1) to *AXR6*, only one (*AXR4*) was found not to be di-

rectly related to the control of the stability of a family of transcriptional repressor proteins called Aux/IAAs (*e.g.*, AXR2, -3, and -5) (*3, 4*). The ubiquitination and consequent proteolysis of Aux/IAA proteins in the 26S proteasome is catalyzed by an SCF-type E3 ubiquitin−ligase complex, of which SCF^{TIR1} is the prototype, There are predicted to be several hundred different SCF complexes in *Arabidopsis*, and the specificity of each complex for particular target proteins is determined by a subunit of the complex known as an F-box protein. In the case of the Aux/IAA repressors the F-box protein is TIR1 or one of a small family of closely related F-box proteins called AFBs (for <u>a</u>uxin-related <u>F-b</u>ox protein). Importantly the interaction between TIR1/AFBs and Aux/IAAs is promoted by the direct binding of auxin, placing SCF^{TIR1} and SCF^{AFBs} at the hub of a transcriptional derepression mechanism in which auxin prompts the rapid destruction of Aux/IAAs and thus the relief of repression of genes to which they are targeted (Figure 2). Structual analysis has shown why synthetic auxins such as naphthalene-1-acetic acid (1-NAA, Figure 1) and 2,4-D can act as auxins at the TIR1/AFB receptors: by binding to the base of an Aux/IAA pocket in TIR1, beneath the docked Aux/IAA, they increase the extent of hydrophobic interactions in the ternary complex, acting as a kind of "molecular glue" (Figure 3) (*5*) (also see refs 6 and 7 for detailed reviews).

These findings defined a new class of receptor in which an otherwise generic mechanism for protein ubiquitination is regulated by the direct binding of a small molecule. Recent chemical biological approaches have shed more light on the functioning of TIR1/AFB family of receptors. Capitalizing on the crystallographic data of TIR1 in complex with various auxins and an Aux/IAA degron, Hayashi and colleagues (*8*) designed and synthesized a range of small molecule agonists and antagonists of the TIR1-Aux/IAA interaction (Figures 1 and 2). Interestingly, their rational design approach resulted in the production of the first auxin probes that interacted specifically with TIR1 and most

TABLE 1. Overview and classification of all molecules used in auxin research[a] (also see Figure 1)

Type	Common name	IUPAC name	Target	Reference
Endogenous auxins				
	IAA	indole-3-acetic acid	TIR1/AFB1−3	4
	IBA	indole-3-butyric acid	TIR1/AFB1−3	4
Synthetic auxins and herbicides				
	1-NAA	naphthalene-1-acetic acid	TIR1/AFB1−3	4
	2,4-D	2,4-dichloro-phenoxyacetic acid	TIR1/AFB1−3	4
	2,4,5-T	2,4,5-trichloro-phenoxyacetic acid	TIR1/AFB1−3	4
	Picloram	4-amino-3,5,6-trichloropicolinic acid	AFB4−5	4
	Dicamba	2-methoxy-3,6-dichlorobenzoic acid	AFB4−5	4
	Sirtinol	2-[(2-hydroxy-naphthalen-1- ylmethylene)-amino]-N-(1-phenyl-ethyl)-benzamide	Unknown	23, 55
	DAS534	4-amino-3-chloro-6-(4-chlorophenyl)-5-fluoro-pyridine-2-carboxylic acid	AFB5	10
Inhibitors of auxin signaling				
	PCIB	p-chloro-phenoxy-isobutyric acid	Unknown	56
	Yokonolide B	complex natural product	Unknown	29
	Terfestatin A	active core: 3-butoxy-4-methylbiphenyl-2,6-diol	Unknown	30
	Compound A	[2-oxo-2-[(5-propyl-1,3,4-thiadiazol-2-yl)amino]ethyl]3-(furan-2-yl)prop-2-enoate; active core: 2-FAA, 2-furylacrylic acid	Unknown	34, 53
	Compound B	2-(5-cinnamylidene-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)-2-phenylacetic acid	Unknown	34
	Compound C	4-[(3-hydroxyphenyl)methylidene]-3-phenyl-1,2-oxazol-5-one	Unknown	34
	Compound D	6-amino-4-(6-nitro-3-hydroxyphenyl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile	Unknown	34
	Toyocamicin	complex natural product	Unknown	31
Inhibitors of auxin transport				
	NPA	naphthylphthalamic acid	PIN and PGP	57
	TIBA	2,3,5-triiodobenzoic acid	Unknown	57
	Gravacin	3-(5-[3,4-dichlorophenyl]-2-furyl)acrylic acid	PGP19	42, 43
Other functions				
	Juglone	Inhibitor of Aux/IAA-SCF[TIR1] interaction	PPIases	58, 59
	ES1	Inhibitor of endocytosis	Unknown	41
	BFA	Inhibitor of GNOM dependent exocytosis	GNOM	39, 40
	α-Alkyl IAA	Agonists or antagonists of Aux/IAA-SCF[TIR1] interaction	TIR1/AFB1-3	8
	Trichostatin A	Inhibitor of chromatin remodeling	Histone deacetylases	60

[a]The common name, IUPAC name, and target proteins, if identified, are listed.

likely other members of the AFB family of proteins. Importantly, these small molecules could not bind the only other putative auxin receptor, auxin binding protein 1 (ABP1) (9). These TIR1-specific probes opened the way for chemical biological analysis of auxin-regulated processes in a wide range of plant species as demonstrated by the existence of specific activation or inhibition of auxin responses in the moss *Physcomitrella patens*, suggesting that the TIR1-like-mediated auxin response might represent an ancient mechanism (8). This work also supported the "molecular glue" hypothesis of Tan *et al.* by demonstrating that in order to function as an
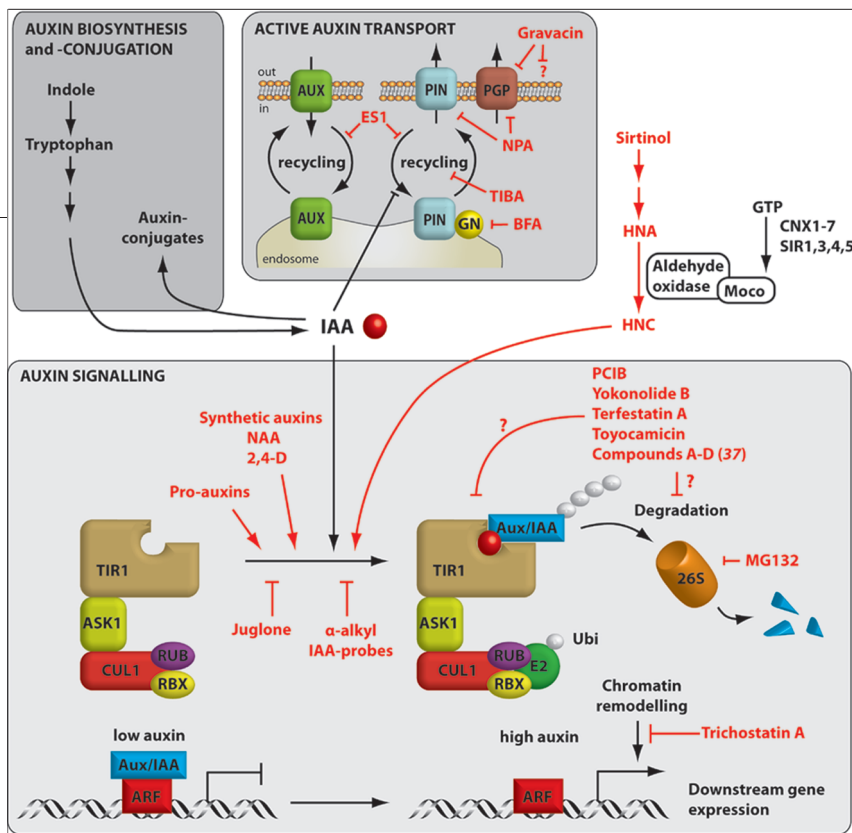
Figure 2. Overview of known small molecule interactions within active auxin transport, auxin signaling, and other auxin-related pathways. Red arrows and text indicate the discussed small molecules.

auxin, the active ring-structure core of the auxin probes needs to bind the receptor beneath the docked Aux/IAA degron (5).

Chemical specificity within the TIR1/AFB family could be demonstrated by Walsh *et al.* (10), who used a proprietary picolinate molecule, related to the herbicide picloram, to show that loss of function of AFB5, a putative member of the auxin receptor family, was sufficient to confer resistance to picolinates but, crucially, not to 2,4-D or IAA. Intriguingly, the *afb5* mutant also showed slightly increased sensitivity to IAA, hinting at the possibility that AFB5 may possess a negative regulatory function in terms of responses to IAA (10). The absence of naturally occurring picolinate molecules involved in auxin response raises questions about the significance of this chemical specificity. Furthermore, it is not clear whether picloram can actually bind AFB5 and if so, if it can bind in a manner similar to IAA and 2,4-D in TIR1. It is also possible that AFB5 and the putative SCF^{AFB5} act in an unknown and picloram-regulated way to affect auxin perception and signaling *via* the canonical receptors TIR1/AFB1−3.

In addition to their role in identifying the molecular basis of transcriptional responses to auxin, auxin-like molecules have also been extremely important in unraveling the events of auxin movement and uptake by the cell. IAA can enter cells by at least two routes. First, be-

cause of the lower pH outside the cell, a pool of IAA molecules is protonated (IAAH) and able to diffuse passively through the plasma membrane (11). In contrast, the anion must be transported actively into the cell by a family of transmembrane amino acid permeases known as the AUX1/LAX family (12, 13). The characterization of mutants in this family of transporters has been helped greatly by the use of the synthetic auxin 1-NAA, which unlike IAA is able to pass freely across the membrane (14). Since the majority of genetic screens to date have been based on resistance to auxin, 1-NAA provides a ready way to distinguish mutations that affect auxin uptake. For example, *aux1* mutants can be partially rescued by growth on low levels of 1-NAA (12).

The synthetic auxinic herbicide 2,4-D provides a useful complementary tool for understanding the role of auxin movement in specific developmental events. In complete contrast to 1-NAA, 2,4-D can be imported into the cell *via* the AUX1/
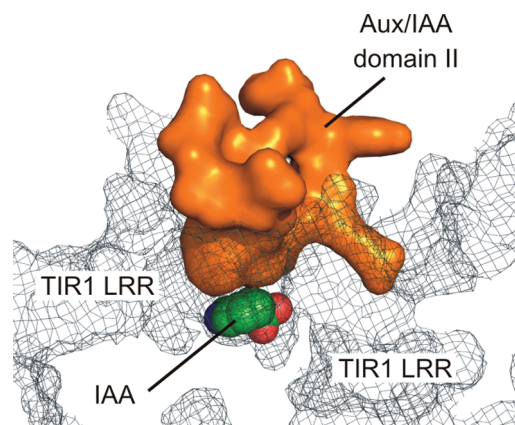
Figure 3. Auxin binding pocket of TIR1. The LRRs that form the auxin and Aux/IAA binding pocket are shown as a gray mesh. The IAA molecule (green) nestles in the base of the pocket beneath the bound Aux/IAA domain II degron (shown as a surface representation in orange). IAA or one of a range of auxinic molecules including 2,4-D and 1-NAA are auxins because they fit into the base of this pocket while still allowing the Aux/IAA to dock, increasing the extent of hydrophobic interactions in the ternary complex. This detains the Aux/IAA protein at the SCF^{TIR1} complex and thereby promoting its ubiquitination. Figure adapted from ref 5 and reprinted with permission.

LAX carriers, but crucially and also unlike 1-NAA, it is a very poor substrate for active auxin efflux *via* a system of transmembrane proteins known as the PIN/ABCB efflux facilitator system (*11, 15, 16*). The subcellular asymmetric localization of PIN efflux facilitators in particular is extremely important in development because it provides a mechanism to orientate the flow of auxin within tissues and thereby generate the gradients and accumulations of auxin that control so many developmental phenomena (*11, 17*). Therefore 2,4-D has provided a particularly effective way to disrupt these gradients of auxin; in addition to being difficult to move out of the cell, it is also metabolically stable (Box 1).

Chemical Tools for Auxin Research. The example of the auxin analogue picloram described above hints at the existence of at least some level of functional speci-

ficity among the TIR1/AFB receptors. This demonstrates that the application of auxin-like compounds that interfere with auxin binding at specific TIR1/AFB receptors can be used to study specific auxin-related processes. However, in general, auxin and auxin-like molecules exert a broad spectrum of activity and are involved in virtually every aspect of plant growth and development (Figure 4), mainly because many downstream processes diverge from the TIR1/AFB receptors. Therefore, interfering at the level of these receptors makes it extremely difficult to analyze one particular pathway without perturbing other closely connected processes. Targeting distinct auxin-related processes is more conveniently accomplished by interfering at the level of auxin signaling. Standard genetic techniques have been extremely informative in defining the basics of auxin signaling. In some

---

Box 1. What makes an auxin?

Although auxin is often mistakenly considered as one molecule, the word "auxin" in fact refers to a range of molecules with sometimes little in common structurally. Furthermore, application of common auxins such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), napthalene-1-acetic acid (NAA), and 2,4-dichlorophenoxy acetic acid (2,4-D) (Figure 1) result in strikingly divergent phenotypes on, for example, lateral root development (*4*). Also, the ways these auxins are transported through the plant are very different (*14*). Nevertheless, the most useful definition of an "auxin" is of a molecule that prompts plant responses qualitatively similar to those elicited by IAA, the predominant naturally occurring auxin. On this basis, several synthetic molecules (like 2,4-D, picloram, and dicamba; Table 1, Figure 1) have been developed and characterized as auxins, often because of their potential as herbicides. The question remains, why are these molecules auxins?

Much work has gone into trying to understand the structure–activity relationships of these molecules (*18*) but ultimately it is the cellular site and mode of action of the molecule that gives the greatest insight. The most complete picture is for perception and signaling *via* the TIR1/AFB family of auxin receptors. Any molecule that can fit into the base of the binding pocket in TIR1 and increase the extent of the hydrophobic interaction with the Aux/IAA will induce a physiological auxin response and can be considered an "auxin" (Figure 3). Molecules that can occupy the auxin-binding pocket with even a moderate affinity and either do not enhance or even preclude Aux/IAA interaction should act as anti-auxins. For events in the binding pocket of the TIR1/AFB receptors this is an excellent definition of what molecules must do to be an "auxin". However, and in keeping with the philosophy of chemical biology, there are an unknown number of ways to affect the other crucial events between receptor and phenotype, some of which may be modulated by the vast diversity of chemical probes available. Thus in the case of TIR1/AFB-mediated control of transcription, any molecule that negatively affects the function of Aux/IAA repressor proteins could mimic IAA when whole-plant responses are considered. It is important to note that TIR1/AFB-mediated auxin perception and signal transduction is not the only mechanism through which auxin can act. There are several auxin responses which cannot be adequately accounted for by this signaling network. For example, certain membrane responses to IAA seem likely to be too fast to be mediated by genomic responses, suggesting that other putative receptors are at work (a good candidate is ABP1, which binds IAA in a different orientation) and hence that other types of auxin-like molecules might be found.
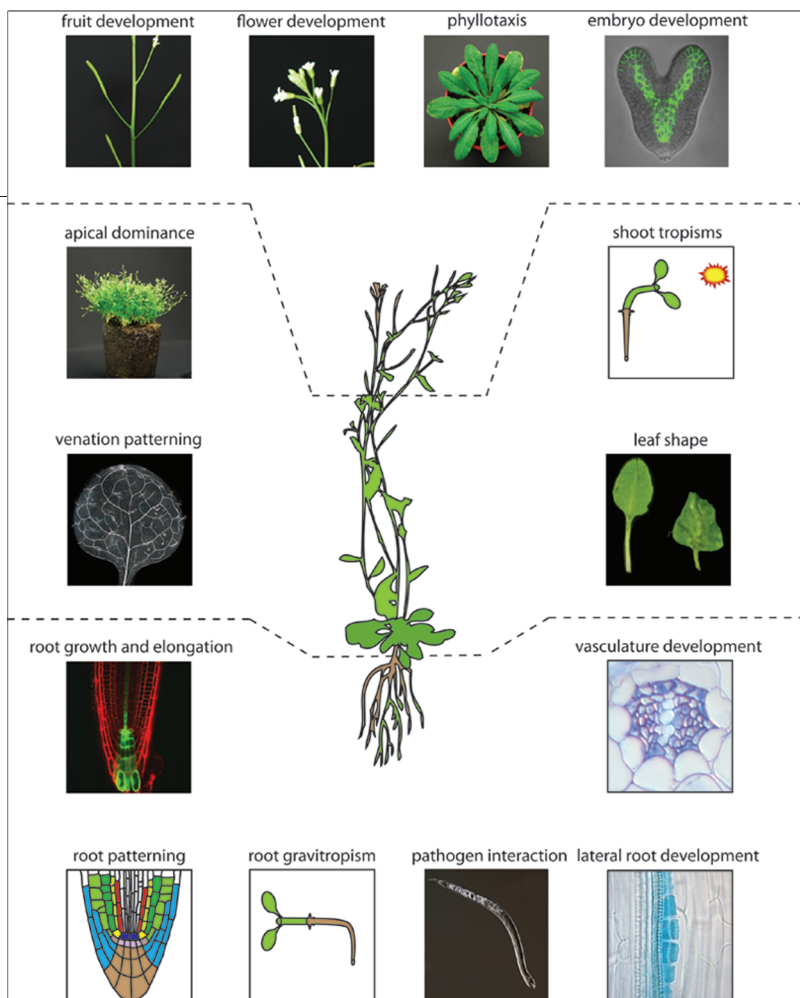
Figure 4. Roles of auxin on plant growth and development. In *Arabidopsis*, the plant hormone auxin has been implicated in virtually every aspect of growth and development throughout the lifespan of the plant. Chemical genetics offers a novel opportunity to dissect these distinct signaling pathways.

cases, however, these approaches are less readily applied to study the details of specific auxin responses because many signaling components belong to gene families (*19, 20*) with high levels of functional redundancy and partly overlapping tissue-specific expression patterns. The Aux/IAA and ARF families, for example, contain 29 and 23 members, respectively (*19, 20*), forming a highly complex network of regulators of auxin signal transduction. This complexity can be dissected by the use of small molecules that, unlike auxin and auxin analogues, are able to target distinct signaling cascades controlling specific aspects of plant growth and development (Figure 4). A chemical biology approach can also overcome the redundancy is-

sue if compounds are applied that interact with conserved regions in protein families, which is nicely illustrated by chemical biology studies in the abscisic acid (ABA) and brassinosteroid fields (*21, 22*). Genetic analysis of auxin signaling mutants is also hampered by the fact that the mutation is already present at the embryonic stage, which could lead to defects and severe pleiotropic phenotypes before the auxin-regulated developmental program in question has begun. The application of chemical tools circumvents these problems as they can be applied at a particular stage of development. Furthermore, unlike genetic tools, the application of chemicals is tunable, both in dosage and in treatment times. Taken together, the advantages of applying one or a combination of small molecules acting downstream of the auxin receptor at specific targets allows studying specific auxin-related processes at certain developmental stages without affecting other closely related pathways.

Screening CompoundCollections To Identify New Chemical Tools. The rational design of chemical probes can be very powerful if detailed structural information is available, as in the case of the TIR1-specific auxin probes discussed above. However, in the majority of cases the identification of new small molecules for auxin research is dependent on the ability to screen large compound collections. Ultimately, the goal of this type of screening is to have small molecules available that target specifically the activity of each protein of the proteome and to affect that protein's function *in vivo* in a conditional fashion. This implies the screening of not only a large but, even more importantly, a diverse collection of compounds to cover a wide range of chemical space with a minimal number of compounds. In addition, to be usable as a conditional research tool to study physiological processes in model organisms, compounds should be bioavailable, stable in *in vivo* conditions, and efficacious at low concentrations to reduce off-target effects. Since the mid-1990s, an increasing number of diverse commercial compound collections became available through several vendors (*e.g.*, Asinex, ChemBridge, Maybridge), making small molecule screening feasible in an academic setting. In addition, rapidly evolving technologies for high-throughput screening allowed the evaluation of larger compound collections.

Next to high-quality and diverse chemical libraries, a robust screening assay is essential to address specific biological questions. In agrochemical companies, much effort has been placed in pure phenotype-based "spray and pray" methodologies to identify new pesticides, insecticides, and fungicides. Although this strategy has proven its usefulness in plant biology and in the development of agrochemicals, it is a "brute force" approach that is not practical in an academic setting and does not always offer insight in the underlying molecular mechanisms. As an alternative strategy, biomarker-based assays can be applied in model organisms in which changes in expression of a marker gene report particular physiological or developmental events. This more sophisticated approach allows specific pathways to be targeted and more specific fundamental biological questions to be addressed.

Chemical Tools To Study Auxin Signaling. Some of the earliest chemical biology screens in auxin research were focused on finding modulators of auxin signaling with a biomarker-based approach by using auxin-inducible promoter-reporter constructs. Both yokonolide B (YkB) and terfestatin A (TrfA) (Figure 1 and Table 1) were isolated from yeast extracts and used in screens for inhibitors of BA3::GUS expression (*29, 30*). More recently, by screening culture extracts of *Actinomycetes*, toyocamycin was identified as an inhibitor of auxin responsive DR5::GUS and BA3::GUS expression (*31*). It was demonstrated that YkB, TrfA, and toyocamycin (Figure 1 and Table 1) interfered with Aux/IAA proteolysis but had no effect on 26S proteasome activity. In addition, these compounds inhibited the expression of auxin-induced genes and blocked physiological auxin responses *in planta*. Although it was shown that TrfA has no direct effect on auxin-mediated interaction between Aux/IAA proteins and TIR1 and YkB blocks Aux/IAA degradation upstream of TIR1, the exact mechanism of action and target proteins of these compounds have not yet been revealed. However, the molecules remain of further interest for the auxin research community as they provide new chemical tools to further dissect auxin signaling.

As exemplified with YkB, TrfA, and toyocamycin, screenings of natural product collections have yielded interesting tools to study auxin signaling. The chemical diversity of natural products is quite profound, in part due to the presence of three-dimensionality in their molecular structures (*32, 33*). Nevertheless, the bioactive component of a natural product extract is difficult to purify and synthesis of the molecule is hampered due to large structural complexity. This inspired Armstrong and co-workers (*34*) to combine a screen for the inhibition of the auxin-inducible BA3::GUS construct with a commercial synthetic library in a genuine high-throughput approach. Analysis of 10,000 molecules yielded 30 molecules that inhibited auxin induced BA3::GUS expression in the root, demonstrating the applicability of small synthetic molecules to identify novel and synthetically tractable signaling inhibitors. Four potent and structurally distinct molecules (compounds A−D) were studied in more detail (Figure 1 and Table 1). Compounds A, B, and C inhibited Aux/IAA proteolysis, as was also demonstrated with YkB, TrfA, and toyocamycin. Interestingly, compounds A and B induced similar developmental phenotypes and displayed similar global effects on gene expression, despite their structural dissimilarity. Identification of the protein target of these compounds will aid in understanding the underlying molecular mechanism of this finding. The availability of synthetic tractability of the compounds can be advantageous in this respect, because structure−activity analysis and the generation of optimized and more potent variants of these compounds can be more easily achieved.

Chemical Tools To Study Auxin Transport. As described above, auxin is unique among plant hormones in that it can be moved directionally within tissues by a system of membrane proteins consisting of the AUX1/LAX family of amino acid permeases for auxin influx (*12, 13*) and the PIN and ABCB (or PGP) proteins for auxin efflux (*35*). NPA and TIBA have long been used for their inhibitory effect on polar auxin transport (PAT) *via* these families of membrane proteins (*36, 37*). Although it has been shown that NPA can bind to PIN and ABCB proteins (*38*), the exact mode of action remains to be determined. Even though many of the earliest screenings were designed to target auxin signaling, auxin transport has also become a popular screening target.

One of the most used small molecules in auxin transport research is brefeldin A (BFA, Figure 1, Table 1), a well-studied inhibitor of secretion and subcellular trafficking. In plants, BFA has specifically been shown to inhibit PIN exocytosis to the plasma membrane by targeting the ARF-GEF GNOM (*39, 40*) (Figure 2). As a consequence, PIN proteins are retained in so-called BFA-bodies, resulting in auxin-dependent phenotypes, such as reduction in lateral root number and root agravitro-

pism (*39*). Recently, endosidin 1 (ES1, Figure 2, Table 1) was identified in a screen for inhibitors of pollen tube growth and was shown to selectively interfere with endocytosis of specific membrane proteins such as PIN2, AUX1 and BRI1, but not others, by inducing rapid agglomerations into a novel compartment (*41*). Although the target is unknown, the remarkable specificity of ES1 offers a selective tool to study endocytosis of these proteins involved in polar auxin transport.

Also the compound gravacin (Figure 1, Table 1) has an effect on auxin transport. Gravacin was identified as an inhibitor of vacuolar sorting and gravitropic response *in planta* (*42*) and was later shown to inhibit polar auxin transport mediated by the ABCB protein PGP19 and PGP19-PIN complexes (*43*). Although probably not the only target, PGP19 was convincingly shown to be a direct target of gravacin. Interestingly, gravicin was also shown to compete with NPA for binding sites on PGP19, suggesting a common target residue. As PIN proteins appeared not to be affected, gravacin was suggested to be more specific than NPA, providing a tool to study polar auxin transport at higher resolution.

Chemical Tools To Study Auxin Biosynthesis and Metabolism. Next to the regulation of auxin response at the level of auxin signaling and transport, auxin activity is also tightly controlled by diverse mechanisms including biosynthesis and metabolism. IAA is synthesized through tryptophan-dependent and tryptophan-independent pathways, and in each case the existence of multiple IAA biosynthetic cascades provides numerous opportunities to regulate IAA production (*4*). At the metabolic level, direct oxidation of the indole ring can occur providing a means to permanently inactivate IAA (*4, 44*). As an additional regulatory mechanism of IAA activity, the pool of IAA can be subjected to conjugation processes, which modify the carboxyl group of IAA, thereby leading to loss of biological activity. IAA can be conjugated to sugars through ester linkages (ester conjugates) or to peptides through amide linkages (amide conjugates). As these conjugates are inactive metabolites of auxin, the conjugation process reduces the size of bioactive auxin pool (*45*). However, a recent study has shown that, as well as merely reducing active auxin levels, tryptophan conjugates of IAA additionally affect auxin sensitivity, representing a previously unrecognized mechanism to regulate auxin action (*46*). Unfortunately, until now, there have been no compounds identified that specifically act on auxin biosynthesis or metabolism. The importance of such compounds is demonstrated by brassinazole, an inhibitor of the brassinosteroid biosynthesis protein DWF4 (*47*). Brassinazole is widely used as a tool in brassinosteroid research and played a key role in the identification of a new component in the signaling pathway, BZR1 (*48*). In addition, the physiological role of brassinosteroids in planta has been partially unraveled by using brassinazole (*49, 50*). Also in the field of ABA research, compounds that act on ABA metabolism have proven useful in understanding the role of ABA degradation in the regulation of ABA responses. Screening of known cytochrome P450 inhibitors in tobacco cell suspensions led to the identification of uniconazole as a potent inhibitor of ABA catabolism. Subsequent SAR analysis showed that diniconazole was a more effective ABA catabolic inhibitor (*51*). Treatment of *Arabidopsis* plants showed increased levels of ABA *in planta* and revealed that regulating ABA content through the degradation process can confer drought stress tolerance.

Target Identification: Which Way To Go? Identifying a compound bioactive for a specific phenotype or physiological response can be exciting, but the full potential of a chemical biology approach emerges only when the target of a compound is discovered. This is nicely illustrated by target identification of trapoxin, a natural compound that inhibits histone deacetylation, a process for which, at the time, the molecular entities responsible had not been identified. By using a trapoxin affinity matrix, Taunton and co-workers (*52*) isolated two trapoxin-binding proteins that co-purified with enzymatic histone deacetylase activity. Subsequent protein sequencing revealed the identity of both proteins of which one was molecularly and biochemically characterized as the molecular player responsible for histone deacetylation and was designated HD1 for histone deacetylase 1. HD1 was shown to be related to the yeast Rpd3p protein, a transcriptional regulator whose biochemical function was unknown, supporting a role of histone deacetylases as key regulators of eukaryotic gene transcription (*52*).

In plant biology and, more specifically, in auxin research, target identification and mechanism of action studies have been mainly accomplished by genetic approaches (*10, 23, 43*). In these cases, forward genetic screens resulted in the identification of compound-resistant mutants that are supposed to carry a mutation in the target protein or in a downstream component of the pathway in which the compound acts.

Box 2. Identification of sirtinol

Chemical genetics in plant biology and specifically in auxin research took a complicated turn with the identification of sirtinol (*23*), originally discovered as an inhibitor of the sirtuin family of NAD-dependent deacetylases in yeast (*24*) (Figure 1). Although sirtinol did not have the desired effects in mammalian systems, it did affect root and vascular development in *Arabidopsis* by inhibiting auxin signaling (*23*). The sirtinol resistant mutant, *sir1*, showed auxin-related developmental phenotypes and auxin-inducible genes were activated. The *SIR1* gene, annotated as a molybdopterin synthase sulfurylase, was shown to contain homology to an E1-like ubiquitin activating domain (*23*), making it a possible candidate as a mediator of the targeted degradation of Aux/IAA transcription factors at a time when TIR1 was not yet identified an auxin receptor. However, chemical analysis of sirtinol derivatives showed striking structural similarity between the synthetic auxin NAA and the active moiety of sirtinol (2-hydroxy-1-naphtoic acid or HCN) (*25*). Mapping other *sir* mutants revealed that the mutated genes also share a common role as enzymes in the biosynthesis of the molybdopterin cofactor, involved in the degradation of sirtinol into HCN, which acts as an auxin (*25*) (Figure 1). Nevertheless, many of the identified sirtinol- and auxin-resistant mutants were revealed to be part of the SCF^{TIR1} complex, which was later identified as an auxin receptor (*26, 27*). Another mutant carried mutations in CAND1, required for assembly and disassembly of the SCF complex (*28*).

Genetic mapping and subsequent mutational analysis eventually leads to the identification of the mutated component. Walsh and colleagues (*10*) screened for specific resistance to a picolinate-type of synthetic auxin, while sensitivity to NAA or 2,4-D was still intact. They identified two loci conferring resistance encoding AFB5, a TIR1 homologue, and SGT1b, encoding a protein associated with SCF-mediated ubiquitination. In the study by Rojas-Pierce and colleagues (*43*), positional cloning of EMS mutagenised plants that were insensitive to gravacin identified four different mutant alleles in the ABCB membrane protein PGP19, a multidrug resistance ABC transporter implicated in auxin efflux (*38*). While it was shown that gravacin interacts directly with PGP19, the effect on protein trafficking was independent of PGP19 activity, indicating that PGP19 is not the only target of gravacin (*43*). Although this study shows that screening for insensitive mutants can lead to the identification of a target that at least partially explains the compound-induced phenotype, this is not always the case, as illustrated by the sirtinol story (Box 2). Rather than identifying new elements in the auxin signaling cascade, forward genetics revealed the mechanism of action of sirtinol by identifying a pathway that is required to metabolize sirtinol to its active core moiety 2-hydroxy-1-naphtoic acid (HNC), a known auxin analogue (*23, 25*) (Figure 2). Conversion of sirtinol to its active core structure in turn activates the auxin signaling pathway. As HNC is an auxin analogue, this effect may

be attributed to direct binding to the auxin receptor, although this has yet to be demonstrated.

Although genetic approaches have been relatively successful for target identification purposes, the above-described example of trapoxin shows that affinity chromatography provides a powerful alternative. Indeed, affinity-based approaches offer a more direct method to identify proteins that interact physically with the compound, either directly or indirectly through the formation of protein complexes. This requires the immobilization of a compound to an affinity column through direct chemical linkage or by linking the compound chemically to biotin and using the biotin/streptavidin or a similar system. However, first a detailed structure−activity relationship (SAR) is essential to provide insight in the active moiety of a compound, allowing the design of tagged or column-linked compounds that are still active. In the study by Armstrong and colleagues (*34*), compound A was shown to inhibit auxin-responsive genes and to produce a phenotype indicative of an altered auxin response. SAR analysis showed that a substructure of compound A (2-furylacrylic acid, 2-FAA, Figure 1) is liberated by hydrolysis of an ester linkage and that 2-FAA is sufficient to induce the compound A phenotype (*53*). Identification of 2-FAA as the effector substructure now allows the synthesis of chimeric molecules that can be used for affinity-based target identification. Similarly, for Terfestatin A (TrfA), a specific inhibitor of auxin signaling (*30*), 3-butoxy-4-methylbiphenyl-

2,6-diol was identified as the active core structure by a detailed SAR study (*54*) (Figure 1). Similarly, this study provides an opportunity to design biotin-tagged TrfA or solid-support-linked TrfA for affinity chromatography of its target protein. At this point however, no studies have been published that used these modified compounds for target identification purposes.

Future Perspectives. The opportunities for chemical biology to impact significantly on plant biology and, more specifically, on auxin research are now more abundant than ever. Over the past decades, our knowledge of the auxin signaling pathway has increased tremendously. As could be predicted from the various distinct organ, tissue and cellular responses to auxin and the multitude of plant developmental processes under its control, auxin signaling appears to be extremely complicated. Because of the vast pleiotropic effect of auxin during plant growth and development (Figure 4), significant genetic redundancy, and embryo lethality, classical forward genetic approaches will not be sufficient to disentangle all aspects of its impact on plant growth and development. In contrast, there is still plenty of scope for new chemical screens, especially when coupled to the growing number of relevant reporter lines in *Arabidopsis*, which offer novel ways to further dissect the auxin signaling pathway.

Furthermore, the increasing availability of detailed structural information both for existing chemical probes and target proteins opens the door to the rational design of new molecules as exquisitely precise tools to discriminate the various signal transduction events. For example, in auxin biology a prominent question is of the extent to which known auxin responses can be attributed to auxin perception by the TIR1/AFB receptors. Indeed there are many such responses for which another mode of perception seems more likely, and it is here that probes that specifically can bind or specifically cannot bind TIR1/AFB receptors can be extremely informative. Also, within the TIR1/AFB family it is not clear to what extent these receptor proteins act redundantly or if specific roles in auxin signaling may be assigned to some of them. Compounds capable of inducing one specific or a limited subset of auxin responses can represent useful tools to unmask the contribution of the various known auxin perception and signaling components in single or multiple defined developmental processes and may even lead to the detection of alternative auxin response mechanisms.

REFERENCES

1. Maher, E. P., and Martindale, S. J. (1980) Mutants of *Arabidopsis thaliana* with altered responses to auxins and gravity, *Biochem. Genet. 18*, 1041−1053.

2. Estelle, M. A., and Somerville, C. R. (1987) Auxin resistant mutants of *Arabidopsis* with an altered morphology, *Mol. Gen. Genet. 206*, 200−206.

3. Dharmasiri, S., Swarup, R., Mockaitis, K., Dharmasiri, N., Singh, S. K., Kowalchyk, M., Marchant, A., Mills, S., Sandberg, G., Bennett, M. J., and Estelle, M. (2006) AXR4 is required for localization of the auxin influx facilitator AUX1, *Science 312*, 1218−1220.

4. Woodward, A. W., and Bartel, B. (2005) Auxin: regulation, action, and interaction, *Ann. Bot. (Oxford, U.K.) 95*, 707−735.

5. Tan, X., Calderon-Villalobos, L. I., Sharon, M., Zheng, C., Robinson, C. V., Estelle, M., and Zheng, N. (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase, *Nature 446*, 640−645.

6. Santner, A., Calderon-Villalobos, L. I., and Estelle, M. (2009) Plant hormones are versatile chemical regulators of plant growth, *Nat. Chem. Biol. 5*, 301−307.

7. Santner, A., and Estelle, M. (2009) Recent advances and emerging trends in plant hormone signalling, *Nature 459*, 1071−1078.

8. Hayashi, K., Tan, X., Zheng, N., Hatate, T., Kimura, Y., Kepinski, S., and Nozaki, H. (2008) Small-molecule agonists and antagonists of F-box protein-substrate interactions in auxin perception and signaling, *Proc. Natl. Acad. Sci. U.S.A. 105*, 5632−5637.

9. Woo, E. J., Marshall, J., Bauly, J., Chen, J. G., Venis, M., Napier, R. M., and Pickersgill, R. W. (2002) Crystal structure of auxin-binding protein 1 in complex with auxin, *EMBO J. 21*, 2877−2885.

10. Walsh, T. A., Neal, R., Merlo, A. O., Honma, M., Hicks, G. R., Wolff, K., Matsumura, W., and Davies, J. P. (2006) Mutations in an auxin receptor homolog AFB5 and in SGT1b confer resistance to synthetic picolinate auxins and not to 2,4-dichlorophenoxyacetic acid or indole-3-acetic acid in *Arabidopsis*, *Plant Physiol. 142*, 542−552.

11. Vanneste, S., and Friml, J. (2009) Auxin: a trigger for change in plant development, *Cell 136*, 1005−1016.

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

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

14. Marchant, A., Kargul, J., May, S. T., Muller, P., Delbarre, A., Perrot-Rechenmann, C., and Bennett, M. J. (1999) AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root apical tissues, *The EMBO Journal 18* 2066–2073.

15. Delbarre, A., Muller, P., Imhoff, V., and Guern, J. (1996) Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells, *Planta 198*, 532–541.

16. Petrasek, J., Mravec, J., Bouchard, R., Blakeslee, J. J., Abas, M., Seifertova, D., Wisniewska, J., Tadele, Z., Kubes, M., Covanova, M., Dhonukshe, P., Skupa, P., Benkova, E., Perry, L., Krecek, P., Lee, O. R., Fink, G. R., Geisler, M., Murphy, A. S., Luschnig, C., Zazimalova, E., and Friml, J. (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux, *Science 312*, 914–918.

17. Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G., and Friml, J. (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation, *Cell 115*, 591–602.

18. Ferro, N., Gallegos, A., Bultinck, P., Jacobsen, H. J., Carbo-Dorca, R., and Reinard, T. (2006) Coulomb and overlap self-similarities: a comparative selectivity analysis of structure-function relationships for auxin-like molecules, *J Chem Inf Model 46*, 1751–1762.

19. Okushima, Y., Overvoorde, P. J., Arima, K., Alonso, J. M., Chan, A., Chang, C., Ecker, J. R., Hughes, B., Lui, A., Nguyen, D., Onodera, C., Quach, H., Smith, A., Yu, G., and Theologis, A. (2005) Functional Genomic Analysis of the AUXIN RESPONSE FACTOR Gene Family Members in *Arabidopsis thaliana*: Unique and Overlapping Functions of ARF7 and ARF19, *Plant Cell 17*, 444–463.

20. Overvoorde, P. J., Okushima, Y., Alonso, J. M., Chan, A., Chang, C., Ecker, J. R., Hughes, B., Liu, A., Onodera, C., Quach, H., Smith, A., Yu, G., and Theologis, A. (2005) Functional genomic analysis of the AUXIN/INDOLE-3-ACETIC ACID gene family members in *Arabidopsis thaliana*, *Plant Cell 17*, 3282–3300.

21. Park, S. Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T. F., Alfred, S. E., Bonetta, D., Finkelstein, R., Provart, N. J., Desveaux, D., Rodriguez, P. L., McCourt, P., Zhu, J. K., Schroeder, J. I., Volkman, B. F., and Cutler, S. R. (2009) Abscisic acid inhibits type 2C protein phosphatases *via* the PYR/PYL family of START proteins, *Science (New York, N.Y.) 324*, 1068–1071.

22. De Rybel, B., Audenaert, D., Vert, G., Rozhon, W., Mayerhofer, J., Peelman, F., Coutuer, S., Denayer, T., Jansen, L., Nguyen, L., Vanhoutte, I., Beemster, G. T. S., Vleminckx, K., Jonak, C., Chory, J., Inzé, D., Russinova, E., and Beeckman, T. (2009) Chemical inhibition of a subset of *Arabidopsis thaliana* GSK3-like kinases activates brassinosteroid signaling, *Chem Biol 16*, 594–604.

23. Zhao, Y., Dai, X., Blackwell, H. E., Schreiber, S. L., and Chory, J. (2003) SIR1, an upstream component in auxin signaling identified by chemical genetics, *Science 301*, 1107–1110.

24. Grozinger, C. M., Chao, E. D., Blackwell, H. E., Moazed, D., and Schreiber, S. L. (2001) Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening, *J. Biol. Chem. 276*, 38837–38843.

25. Dai, X., Hayashi, K., Nozaki, H., Cheng, Y., and Zhao, Y. (2005) Genetic and chemical analyses of the action mechanisms of sirtinol in *Arabidopsis*, *Proc. Natl. Acad. Sci. U.S.A. 102*, 3129–3134.

26. Dharmasiri, N., Dharmasiri, S., and Estelle, M. (2005) The F-box protein TIR1 is an auxin receptor, *Nature 435*, 441–445.

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

28. Cheng, Y., Dai, X., and Zhao, Y. (2004) AtCAND1, a HEAT-repeat protein that participates in auxin signaling in *Arabidopsis*, *Plant Physiol. 135*, 1020–1026.

29. Hayashi, K., Jones, A. M., Ogino, K., Yamazoe, A., Oono, Y., Inoguchi, M., Kondo, H., and Nozaki, H. (2003) Yokonolide B, a novel inhibitor of auxin action, blocks degradation of AUX/IAA factors, *J. Biol. Chem. 278*, 23797–23806.

30. Yamazoe, A., Hayashi, K., Kepinski, S., Leyser, O., and Nozaki, H. (2005) Characterization of terfestatin A, a new specific inhibitor for auxin signaling, *Plant Physiol. 139*, 779–789.

31. Hayashi, K., Kamio, S., Oono, Y., Townsend, L. B., and Nozaki, H. (2009) Toyocamycin specifically inhibits auxin signaling mediated by SCFTIR1 pathway, *Phytochemistry 70*, 190–197.

32. Fischbach, M. A., and Walsh, C. T. (2006) Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: logic, machinery, and mechanisms, *Chem. Rev. 106*, 3468–3496.

33. Smukste, I., and Stockwell, B. R. (2005) Advances in chemical genetics, *Annu. Rev. Genomics Hum. Genet. 6*, 261–286.

34. Armstrong, J. I., Yuan, S., Dale, J. M., Tanner, V. N., and Theologis, A. (2004) Identification of inhibitors of auxin transcriptional activation by means of chemical genetics in *Arabidopsis*, *Proc. Natl. Acad. Sci. U.S.A. 101*, 14978–14983.

35. Robert, H. S., and Friml, J. (2009) Auxin and other signals on the move in plants, *Nat. Chem. Biol. 5*, 325–332.

36. Bouchard, R., Bailly, A., Blakeslee, J. J., Oehring, S. C., Vincenzetti, V., Lee, O. R., Paponov, I., Palme, K., Mancuso, S., Murphy, A. S., Schulz, B., and Geisler, M. (2006) Immunophilin-like TWISTED DWARF1 modulates auxin efflux activities of *Arabidopsis* P-glycoproteins, *J. Biol. Chem. 281*, 30603–30612.

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

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

39. Geldner, N., Friml, J., Stierhof, Y. D., Jürgens, G., and Palme, K. (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking, *Nature 413*, 425–428.

40. Steinmann, T., Geldner, N., Grebe, M., Mangold, S., Jackson, C. L., Paris, S., Galweiler, L., Palme, K., and Jürgens, G. (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF, *Science 286*, 316–318.

41. Robert, S., Chary, S. N., Drakakaki, G., Li, S., Yang, Z., Raikhel, N. V., and Hicks, G. R. (2008) Endosidin1 defines a compartment involved in endocytosis of the brassinosteroid receptor BRI1 and the auxin transporters PIN2 and AUX1, *Proc. Natl. Acad. Sci. U.S.A. 105*, 8464–8469.

42. Surpin, M., Rojas-Pierce, M., Carter, C., Hicks, G. R., Vasquez, J., and Raikhel, N. V. (2005) The power of chemical genomics to study the link between endomembrane system components and the gravitropic response, *Proc. Natl. Acad. Sci. U.S.A. 102*, 4902–4907.

43. Rojas-Pierce, M., Titapiwatanakun, B., Sohn, E. J., Fang, F., Larive, C. K., Blakeslee, J., Cheng, Y., Cutler, S. R., Peer, W. A., Murphy, A. S., and Raikhel, N. V. (2007) *Arabidopsis* P-glycoprotein19 participates in the inhibition of gravitropism by gravacin, *Chem. Biol. 14*, 1366–1376.

44. Ostin, A., Kowalyczk, M., Bhalerao, R. P., and Sandberg, G. (1998) Metabolism of indole-3-acetic acid in *Arabidopsis*, *Plant Physiol. 118*, 285–296.

45. Ljung, K., Hull, A. K., Kowalczyk, M., Marchant, A., Celenza, J., Cohen, J. D., and Sandberg, G. (2002) Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*, *Plant Mol. Biol. 49*, 249–272.

46. Staswick, P. E. (2009) The tryptophan conjugates of jasmonic and indole-3-acetic acids are endogenous auxin inhibitors, *Plant Physiol. 150*, 1310–1321.

47. Asami, T., Mizutani, M., Fujioka, S., Goda, H., Min, Y. K., Shimada, Y., Nakano, T., Takatsuto, S., Matsuyama, T., Nagata, N., Sakata, K., and Yoshida, S. (2001) Selective interaction of triazole derivatives with DWF4, a cytochrome P450 monooxygenase of the brassinosteroid biosynthetic pathway, correlates with brassinosteroid deficiency *in planta*, *J. Biol. Chem. 276*, 25687–25691.

48. Wang, Z. Y., Nakano, T., Gendron, J., He, J., Chen, M., Vafeados, D., Yang, Y., Fujioka, S., Yoshida, S., Asami, T., and Chory, J. (2002) Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis, *Dev. Cell 2*, 505–513.

49. Nagata, N., Asami, T., and Yoshida, S. (2001) Brassinazole, an inhibitor of brassinosteroid biosynthesis, inhibits development of secondary xylem in cress plants (*Lepidium sativum*), *Plant Cell Physio.l 42*, 1006–1011.

50. Nagata, N., Min, Y. K., Nakano, T., Asami, T., and Yoshida, S. (2000) Treatment of dark-grown *Arabidopsis thaliana* with a brassinosteroid-biosynthesis inhibitor, brassinazole, induces some characteristics of light-grown plants, *Planta 211*, 781–790.

51. Kitahata, N., Saito, S., Miyazawa, Y., Umezawa, T., Shimada, Y., Min, Y. K., Mizutani, M., Hirai, N., Shinozaki, K., Yoshida, S., and Asami, T. (2005) Chemical regulation of abscisic acid catabolism in plants by cytochrome P450 inhibitors, *Bioorg. Med. Chem. 13*, 4491–4498.

52. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p, *Science 272*, 408–411.

53. Sungur, C., Miller, S., Bergholz, J., Hoye, R. C., Brisbois, R. G., and Overvoorde, P. (2007) The small molecule 2-furylacrylic acid inhibits auxin-mediated responses in *Arabidopsis thaliana*, *Plant Cell Physiol. 48*, 1693–1701.

54. Hayashi, K., Yamazoe, A., Ishibashi, Y., Kusaka, N., Oono, Y., and Nozaki, H. (2008) Active core structure of terfestatin A, a new specific inhibitor of auxin signaling, *Bioorg. Med. Chem. 16*, 5331–5344.

55. Dai, X., Hayashi, K., Nozaki, H., Cheng, Y., and Zhao, Y. (2005) Genetic and chemical analyses of the action mechanisms of sirtinol in *Arabidopsis*, *Proc. Natl. Acad. Sci. U.S.A. 102*, 3129–3134.

56. Oono, Y., Ooura, C., Rahman, A., Aspuria, E. T., Hayashi, K., Tanaka, A., and Uchimiya, H. (2003) *p*-Chlorophenoxyisobutyric acid impairs auxin response in *Arabidopsis* root, *Plant Physiol. 133*, 1135–1147.

57. Keitt, G. W., and Baker, R. A. (1966) Auxin activity of substituted benzoic acids and their effect on polar auxin transport, *Plant Physiol. 41*, 1561–1569.

58. Kepinski, S., and Leyser, O. (2004) Auxin-induced SCF[TIR1]-Aux/IAA interaction involves stable modification of the SCF[TIR1] complex, *Proc. Natl. Acad. Sci. U.S.A. 101*, 12381–12386.

59. Tian, Q., Nagpal, P., and Reed, J. W. (2003) Regulation of *Arabidopsis* SHY2/IAA3 protein turnover, *Plant J. 36*, 643–651.

60. Fukaki, H., Taniguchi, N., and Tasaka, M. (2006) PICKLE is required for SOLITARY-ROOT/IAA14-mediated repression of ARF7 and ARF19 activity during *Arabidopsis* lateral root initiation, *Plant J. 48*, 380–389.