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Small molecule approaches in plants

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Small molecules offer exciting opportunities for plant science. So far, bioactive small molecules have been identified as plant hormones, herbicides, growth regulators, or taken from animal research. Recently, plant scientists have started to explore further the chemical space for novel modulators of plant hormone signalling, and have followed up this work with exciting discoveries illustrating the potential of small molecules such as brassinazole and sirtinol. New chemical genetic screens have been designed to generate chemical tools for the investigation of membrane trafficking, gravitropism and plant immunity. Further novel 'chemetic' tools to identify targets and modes of action are currently generated through an intimate interdisciplinary collaboration between biologists and small molecule chemists.

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Introduction

Small molecules (<500 Da) are a common good in plants. In fact, plants thrive because of small molecules. Most important are the plant hormones auxin, salicylic acid, cytokinin, gibberellic acid, abscisic acid, brassinosteroid, jasmonate and ethylene, all of which are small molecules. Plants also produce a tremendously broad repertoire of other small molecules, called secondary metabolites [1]. Many of these molecules are produced under stress conditions, protecting plants against challenges such as cold, UV light or pathogens, yet the mode of action of most of these compounds is unknown.

Owing to the key role of plant hormones, the plant research community has a long history in studying small molecules on whole organisms. Also, the use of 'non-self' bioactive compounds has clearly penetrated plant research, and these compounds are now widely used to study the role of some enzymes or processes. Table 1 and Figure 1 provide examples of these bioactive compounds. Most of these bioactive compounds were first described in animal model systems, and later directly used in plant research. The effect of these compounds on plants is very often similar to that described in animal systems, indicating that the targeted proteins are well conserved. This observation, however, does not always hold true, because plants also have unique features. Mastoparan, for example, activates mitogen-activated protein (MAP) kinases by inhibiting G proteins in animals, but in plants it activates MAP kinases in the absence of G proteins [2•]. Furthermore, caspase inhibitors (e.g. VAD-cmk) in plants also inhibit vacuolar processing enzymes, subtilisin-like serine proteases and, most likely, other unidentified enzymes [3•,4•].

Screens for herbicides and growth regulators have provided the plant research community with yet another series of bioactive compounds. These screens were performed by agrochemical companies but the details of these screens have not entered the public domain. Examples are the herbicide isoxaben and the plant protectant benzothiadiazole (BTH, BION^{TM}). Isoxaben-insensitive mutants have provided essential information on the mechanism of cellulose production [5,6] and BTH is frequently employed in plant research to induce systemic acquired resistance [7].

Although it is evident that diverse plant processes can be manipulated with small molecules, systematic screening for novel modulators has been limited to only a few studies in recent years (Table 2). Some of these chemical genetic screens were followed up by characterization of chemical-insensitive mutants and identification of the corresponding genes. More chemical genetics screens have been initiated in the past year to explore other plant processes. Here, we summarize the most recent achievements, their impact on the field and the problems encountered.

Versatile cytochrome P450 inhibitors

The triazol-type cytochrome P450 inhibitors have offered tremendous opportunities for research on plant hormones. Originally, triazole-type inhibitors were selected as effective fungicides and herbicides because they block the steroid biosynthesis by inhibiting cytochrome P450 monooxygenases. It was later found that the fungicide uniconazole also inhibits the biosynthesis of the plant hormone gibberellic acid (Figure 2) [8], and subsequent research on these triazoles resulted in inhibitors of brassinosteroid biosynthesis and abscisic acid catabolism.

Table 1

Bioactive compounds used in plant research.

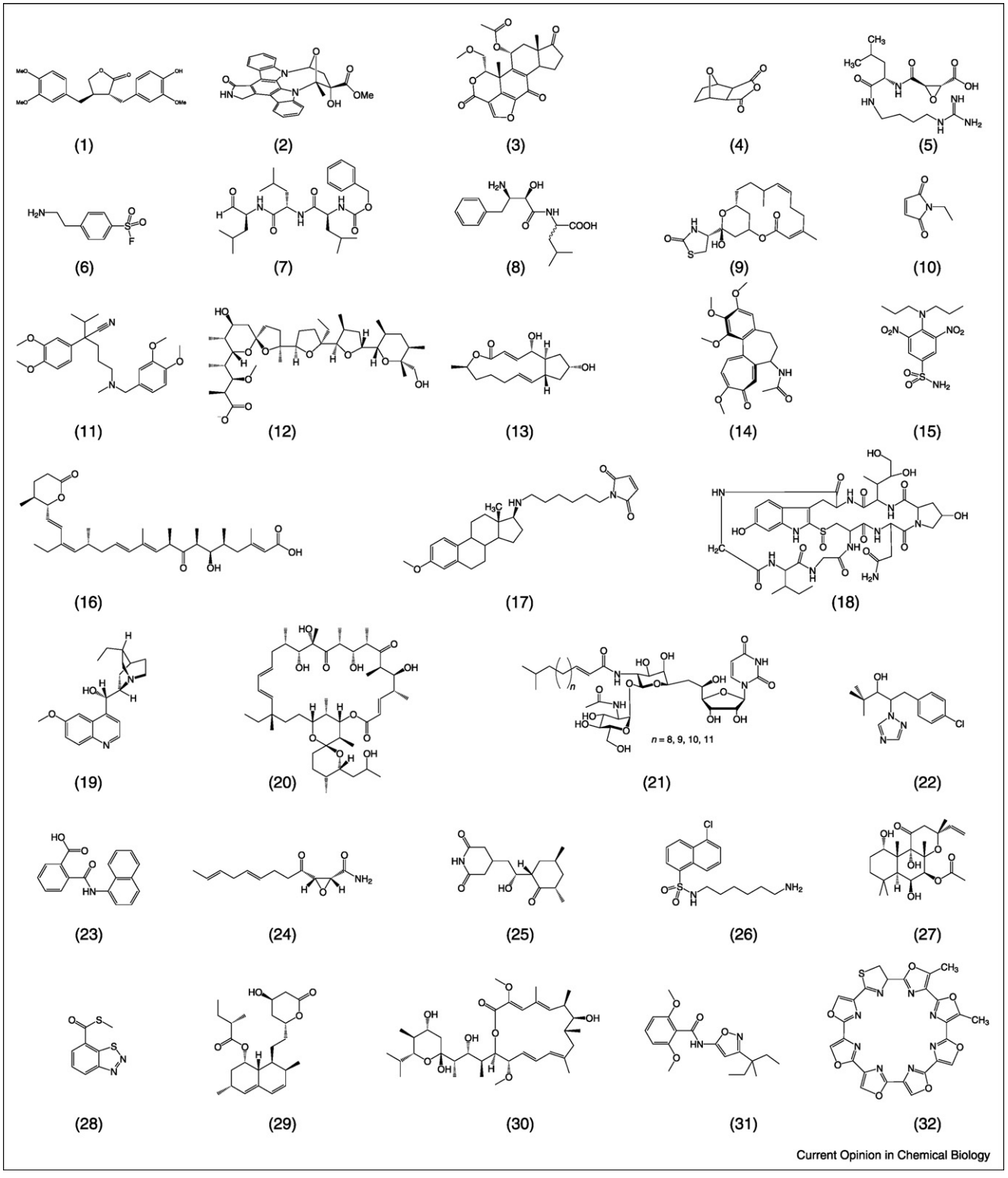
Number[a]	Compound	MW (Da)	Function/target	Refs
Modulators of phosphorylation				
–	Mastoparan	1478.8	MAP kinase activator	[2•]
1	PD98059	372.41	MAP kinase kinase inhibitor	[42]
2	K-252a	467.47	Kinase inhibitor	[43]
3	Wortmannin	428.43	Kinase inhibitor	[44]
4	Cantharidin	168.15	Phosphatase inhibitor	[45]
Modulators of proteolysis				
5	E-64	357.41	Inhibitor of cysteine proteases	[37•]
6	AEBSF	203.23	Inhibitor of serine proteases	[46]
–	DEVD-fmk	502.48	Inhibitor of caspase-like enzymes	[3•]
7	MG132	475.30	Proteasome inhibitor	[44]
8	Bestatin	308.17	Inhibitor of aminopeptidases	[41]
Modulators of membrane trafficking				
9	Latrunculin B	395.51	Inhibitor of actin polymerization	[44]
10	*N*-ethylmaleimide (NEM)	125.13	Inhibitor of exocytosis/actinomyosin complex	[47]
11	Verapamil	440.58	Inhibitor of exocytosis, Ca^{2+} channel blocker	[47]
12	Monensin	667.89	Inhibitor of intracellular protein transport	[47]
13	Brefeldin A	280.36	Inhibitor of vesicle trafficking	[48]
14	Colchicine	399.44	Inhibitor of microtubule assembly	[49]
15	Oryzalin	346.36	Inhibitor of tubulin polymerization	[44]
16	Leptomycin B	542.75	Inhibitor of nuclear export	[50]
Other modulators				
17	U73122	464.64	Inhibitor of phosphatidyl inositol phospholipase C	[51]
18	Amanitin	918.97	Inhibitor of RNA polymerase II	[52]
19	Quinidine	326.43	Inhibitor of root exudation	[53]
20	Oligomycin	805.09	Inhibitor of mitochondrial ATP synthase	[54]
21	Tunicamycin	830.92	Inhibitor of *N*-acetylglucosamine transferases	[55]
22	Paclobutrazol	293.79	Inhibitor of gibberellin biosynthesis	[20•]
23	Naphthylphthalamic acid	291.30	Inhibitor of auxin transport	[43]
24	Cerulenin	223.27	Inhibitor of fatty acid synthase	[56]
25	Cycloheximide	281.35	Inhibitor of protein synthesis	[57]
26	W7	340.87	Inhibitor of calmodulin-dependent proteins	[47]
27	Forskolin	410.50	Activator of adenyl cyclase	[47]
28	BTH	210.28	Induces systemic acquired resistance	[7]
29	Mevinolin, lovastatin	404.54	Inhibitor of the MVA pathway	[58]
30	Bafilomycin	622.83	Inhibitor of vacuolar proton-ATPase	[59]
31	Isoxaben	332.39	Inhibitor of cellulose biosynthesis	[60]
32	Telomestatin	582.50	Inhibitor of telomerase	[61]

[a] Numbers refer to the structures indicated in Figure 1. An extended list of bioactive compounds used in plant research is available at www.plantchemetics.org.

Brassinosteroid hormones (BRs) are crucial for plant growth and development. To study the role of this hormone in the absence of genetic mutations and in other plant species, Yoshida and co-workers embarked on a mission to generate specific inhibitors of BR biosynthesis based on triazoles. A key enzyme in BR synthesis is the cytochrome P450 monooxygenase DWF4. Mutations in the *Dwf4* gene cause a dwarfing phenotype that can be rescued by BR treatment [9]. To identify DWF4 inhibitors, triazole derivatives were synthesized and tested in phenotypic screens. This resulted in brassinazole (Brz), which specifically binds to the DWF4 protein and blocks BR biosynthesis, causing a dwarf phenotype that can be rescued with BRs (Figure 2) [10,11]. The changes on the transcriptome induced by Brz are antagonistic to those induced by BR treatment [12], and cytological effects of Brz were identical to those in BR biosynthetic mutants [13]. The specificity of Brz is striking considering that there are numerous other cytochrome P450s in plants.

Brz has opened new avenues for research on the role of BRs because inhibition of BR biosynthesis can be done at any time point and with any doses of choice, and enables studies in other plant species. For example, Brz inhibits the development of secondary xylem in cress plants [14] and fibre development in cotton [15•]. Brz was also used in a screen for Brz-insensitive mutants, resulting in the identification of BRZ1 [16], which turned out to be highly interesting — it represents a new kind of plant-specific nuclear transcriptional repressor, thereby explaining the observed feedback regulation within the BR biosynthetic pathway [17•]. Quantitative trait locus (QTL) mapping revealed another Brz-insensitive locus, *Brz4*, which remains to be identified [18]. The recent discovery that a mutation in DWF4 in rice increases grain yield makes it

Figure 1



Examples of bioactive small molecules used in plant research. The numbers refer to those listed in Table 1. More examples are presented at http://www.plantchemetics.org.

Table 2

Chemical genetic screens discussed herein.

Effect on	Organism	Library	Hit	Refs
Biosynthesis of BRs	Rice	10 triazoles	Brz (Figure 2)	[10]
Catabolism of ABA	*Arabidopsis*	9 triazoles	Diniconazole (Figure 2)	[20•,21•]
Seedling growth defect	*Arabidopsis*	57 biaryls	(P)-4k (Figure 3)	[22]
Auxin reporter gene	*Arabidopsis*		YkB (Figure 3)	[24,25]
Auxin reporter gene	*Arabidopsis*	10 000 diverse	Compounds (a)–(d) in Figure 3	[26••]
Inhibition of sirtuin	Yeast	1600 diverse	Sirtinol (Figure 4)	[27]
Secretion of vacuolar marker CPY	Yeast	4800 diverse	Sortin-1 (Figure 5a)	[34••]
Gravitropism	*Arabidopsis*	10 000 diverse	Compounds in Figure 5b	[35••]
Immune response	*Arabidopsis*	120 bioactive	Compounds in Figure 5c	[36•]

conceivable that BR biosynthesis inhibitors might even have commercial prospects [19].

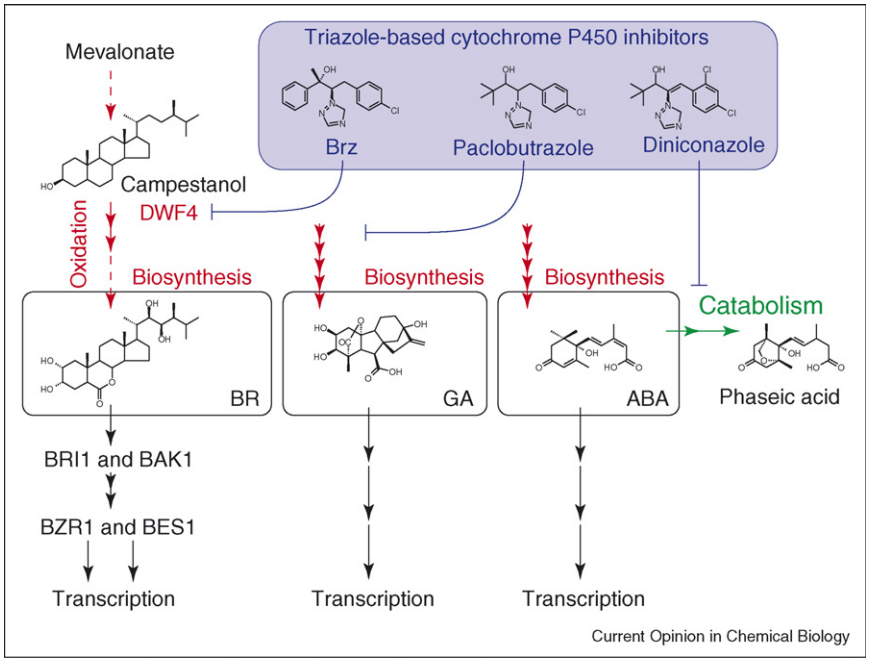
But inhibitors of cytochrome P450s had even more to offer. Cyp707A3 is a cytochrome P450 that hydrolyzes abscisic acid (ABA), a plant hormone regulating many developmental processes including the stimulation of stomatal closure, which protects plants from drought. A screen of the same triazole library used to identify BR biosynthesis inhibitors identified uniconazole and diniconazole, two potent inhibitors of ABA catabolism (Figure 2) [20•,21•]. *Arabidopsis* plants treated with these compounds showed increased levels of ABA and an increased drought tolerance [20•,21•]. Further studies with these ABA catabolism inhibitors are a promise for the near future.

In conclusion, the specificity and versatility of the triazole compounds is remarkable because these compounds are very similar but yet specifically modify the levels of three different plant hormones (GA, BR and ABA) by inhibiting different cytochrome P450s.

The quest for auxin-signalling inhibitors

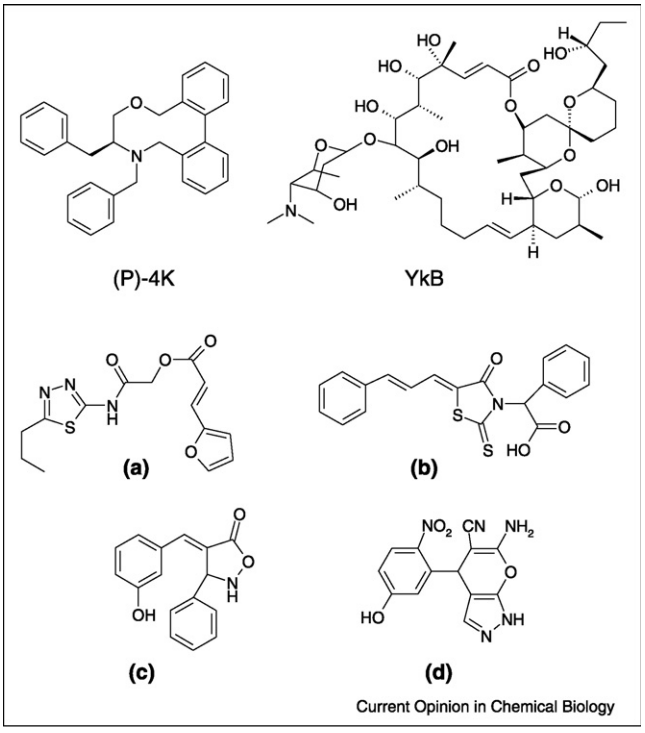
The plant hormone auxin has such an important role throughout the life of a plant that many genes required for

Figure 2



Specific interference of different triazoles with plant hormones. Brassinazole (Brz) inhibits DWF4, a cytochrome P450 that confers initial oxidation of campestanol, leading to the hormone brassinolide (BR). BR acts through its receptors BRI1 and BAK1, leading to activation of transcription factors BZR1 and BES1, resulting in transcription of BR-responsive genes. Paclobutrazole inhibits cytochrome P450s in the biosynthetic pathway leading to gibberellic acid (GA), a plant hormone that regulates transcription of GA-responsive genes. Diniconazole inhibits Cyp707A3, a cytochrome P450 that hydrolysis abscisic acid (ABA), a hormone regulating transcription of ABA-responsive genes.

Figure 3



Structures of auxin-signalling inhibitors. (P)-4K has an overall effect on germination of *Arabidopsis*, yokolonide B (YkB) and compounds **(a)** to **(d)** inhibit induction of an auxin-responsive reporter gene in *Arabidopsis* seedlings.

auxin signalling are difficult to detect by mutagenesis because their loss causes severe pleiotropic phenotypes. To bypass this pitfall, researchers harnessed the power of chemical genetics to identify potent inhibitors of auxin signalling. In an early study, a compound library of 57 biaryl compounds was screened for those causing phenotypic effects on *Arabidopsis* seed germination [22]. One compound, (P)-4k (Figure 3), caused stunted development of *Arabidopsis* seedlings and loss of pigmentation. Although it was speculated that these phenotypes could result from inhibition of auxin signalling [22,23], more specific screens had to be designed.

Hayashi *et al.* [24] performed such a specific screen with transgenic *Arabidopsis* plants harbouring an auxin-responsive GUS reporter gene. They screened a natural library of fermentation products from the soil bacterium *Streptomyces diastatochromogenes* and isolated two spiroketal macrolides, yokolonide A and B, as potent inhibitors of auxin response [24,25]. Subsequent studies on yokolonide B (YkB [Figure 3]) revealed that it prevents auxin-induced degradation of the AUX/IAA transcription factors without inhibiting proteasome activity, indicating that YkB targets probably act upstream of the degradation of AUX/IAA proteins [25]. The widespread application of YkB, however, is hampered by the fact that it is a complex natural product that is not readily available.

The need for more simple bioactive compounds prompted Armstrong *et al.* [26••] to perform a forward chemical screen with a commercially available library of 10 000 chemically diverse compounds. They identified 30 compounds that inhibited the expression of an auxin-responsive GUS reporter gene in the root elongation zone. Four of these compounds (Figure 3a–d) were further characterized because they were structurally diverse and showed a strong inhibitory capacity in the micromolar range. Compounds (a), (b) and (c) impaired auxin-mediated proteolytic degradation of the AUX/IAA transcription factors, a hallmark for auxin signalling. Compounds (a) and (b) showed similar phenotypes and had similar global effects on the transcriptome, but RT–PCR experiments indicated that these small molecules also had distinct tissue specific effects. Testing these compounds on auxin-signalling mutants will be an obvious next step but this work by Armstrong *et al.* [26••] illustrates the potential of chemical screens.

The deceiving action of sirtinol

Sirtinol is certainly the most deceiving small molecule that has been identified by chemical genetic screens. The actual screen was performed in yeast, aimed at finding inhibitors of the sirtuin class of histone deacetylases that could be used to study the role of sirtuins in other organisms. Disappointingly, sirtinol (sirtuin-inhibiting

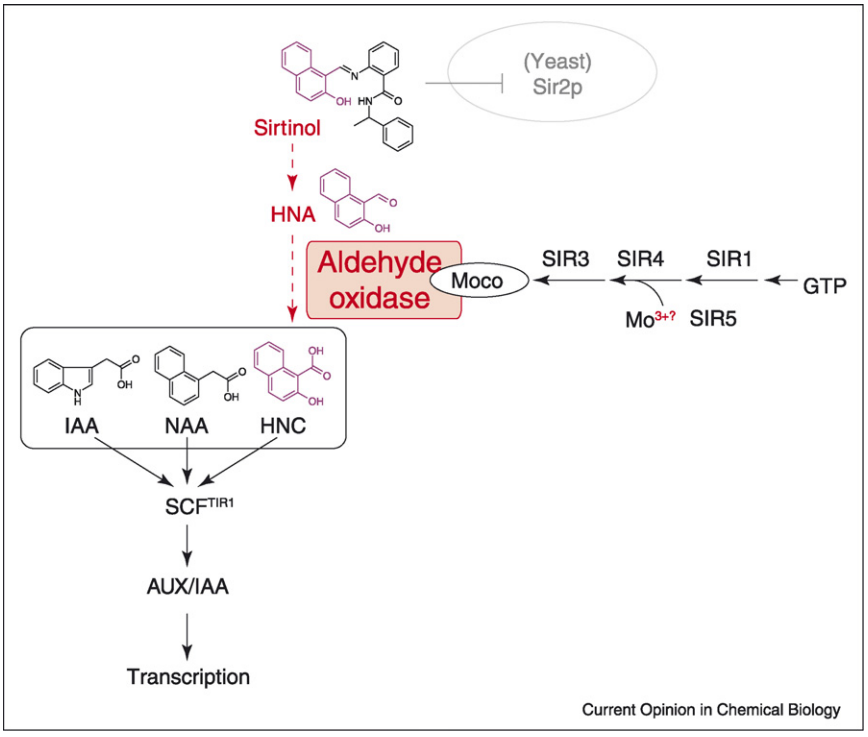
napthol) did not affect histone acetylation or morphology in mammalian cells, but it strongly affected root and vascular tissue development in *Arabidopsis* [27]. This *Arabidopsis* phenotype occurs because sirtinol activates the auxin-signalling pathway [28].

The receptor for auxin was unknown until 2005, so targets of sirtinol were of high interest before that time. *Sir* mutants are sirtinol resistant and sensitive to auxin, indicating that *Sir* genes act upstream in auxin signalling. Zhao *et al.* [28] first cloned *Sir1*. This gene encodes a protein that was annotated as a molybdopterin synthase sulfurylase, but the authors pointed out that parts of the SIR1 protein are homologous to an ubiquitin E1 ligase and a prolyl isomerase [28]. This alternative explanation was appealing — it suggested a role for SIR1 in targeted degradation of the AUX/IAA transcription factors during auxin signalling.

This work was followed up by another publication that shed a different light on the previously drawn conclusions. Dai *et al.* [29••] discovered that also the *Sir3*, *Sir4* and *Sir5* genes all encode enzymes for the biosynthesis pathway of the molybdopterin cofactor (moco). In addition, other moco biosynthesis mutants appeared to be sirtinol insensitive [29••]. The role of moco biosynthesis in auxin signalling was puzzling, but the shocking explanation came from analysis of sirtinol derivatives. The active moiety of sirtinol is 2-hydroxy-1-naphthoic acid (HNC), which has a striking structural similarity to the synthetic auxin NAA (1-naphthaleneacetic acid [Figure 4]), suggesting that a sirtinol degradation product acts as an auxin [29••]. The conversion of sirtinol to HNC is likely to involve the oxidation of an aldehyde intermediate (HNA, 2-hydroxy-1-naphthaldehyde), which requires an aldehyde oxidase and its cofactor moco (Figure 4). Thus, *Sir* genes are required to convert sirtinol into an auxin, and have no role in auxin signalling itself; nor do they represent a deacetylase or any other direct target for sirtinol.

Sirtinol has been useful despite these misleading results. Some sirtinol-insensitive mutants were also auxin insensitive, and most were caused by mutations in components of the SCF^{TIR1} ubiquitin protein ligase [29••], which appeared to be one of the bona fide auxin receptors [30•,31•]. One of the sirtinol/auxin-insensitive mutants carries a mutation in *AtCAND*, which encodes a protein

Figure 4



Mode of action of sirtinol. Sirtinol conversion probably involves the conversion of HNA (2-hydroxy-1-naphthaldehyde) into HNC (2-hydroxy-1-naphthoic acid), which is catalyzed by an aldehyde oxidase, requiring a molybdopterin cofactor (moco). Moco is synthesized through the action of *Sir* genes, which were identified from screens for sirtinol insensitivity. HNC is structurally related to the synthetic auxin NAA (1-naphthalene acetic acid), which mimics the action of the endogenous auxin IAA (indole-3-acetic acid). IAA, NAA and HNC probably all bind and activate the SCF^{TIR1} ubiquitin ligase complex, which targets the AUX/IAA transcriptional repressors for proteasome-dependent degradation, resulting in auxin-induced gene induction.

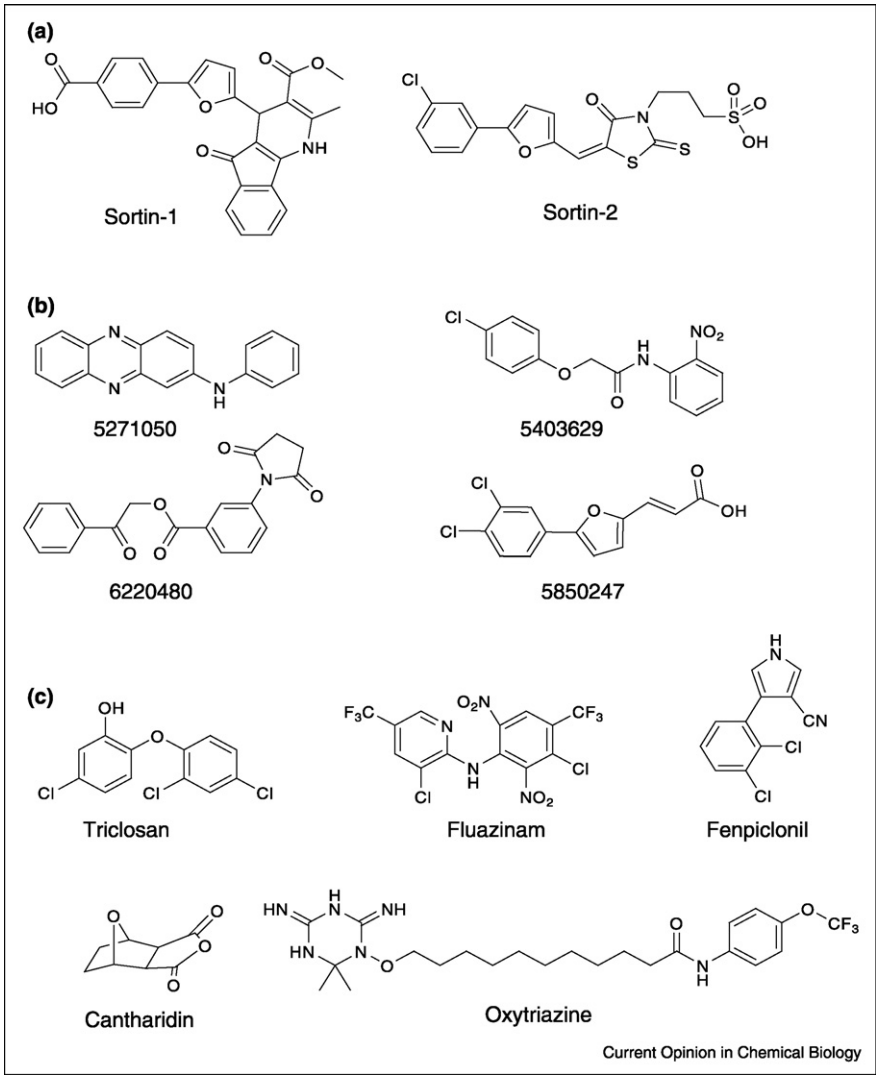
regulating the assembly and disassembly of the SCF protein complex [32]. Another sirtinol/auxin-insensitive mutant carries a mutation in the anticodon of $tRNA^{ala}$, which probably leads to multiple mutations in many downstream auxin effectors [33].

Screens for inhibitors of trafficking, gravitropism and immune responses

Although the initial chemical genetic studies were aimed to dissect hormone-signalling pathways, publications in the past year demonstrate that also other processes in plants are being explored, for example to find modifiers of membrane trafficking, gravitropism and immune responses.

Endomembrane trafficking in plants appears to be a complex process of which much remains to be uncovered. To develop new tools to study this, the laboratory of Raikhel [34••] used a yeast-based screen to find compounds that cause secretion of the yeast vacuolar marker protein carboxypeptidase Y (CPY). Fourteen bioactive compounds were selected and tested on *Arabidopsis* seedlings. Only sortin-1 and -2 (Figure 5a) caused phenotypes on plants, manifested by a partially fragmentized vacuolar membrane and retarded root development. Sortin-2 showed high toxicity in *Arabidopsis* and tobacco cell cultures, whereas sortin-1 triggered CPY secretion in *Arabidopsis* cell cultures, making sortin-1 a useful tool for further studies on endomembrane trafficking. Although this study was aimed at generating chemical tools to study trafficking in plants, it revealed that small molecule targets in endomembrane trafficking are different between plants and yeast.

Figure 5



Structures of modulators of **(a)** membrane trafficking, **(b)** gravitropism and **(c)** immune responses.

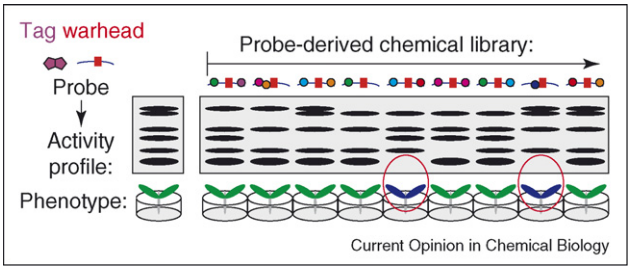
To identify more compounds that interfere in endomembrane trafficking in plants, a 10 000-compound library was screened on *Arabidopsis* seedlings for an altered gravitropism response [35••]. Gravitropism is the process whereby roots grow in the direction of gravity, and it is known that gravitropism mutants often show altered endomembrane trafficking [35••]. Of the 34 compounds that affected gravitropism, four (Figure 5b) also severely changed the morphology of the endomembrane system, monitored with a specific vacuolar membrane marker. These compounds differed from those that Armstrong *et al.* [26••] identified from the same library when screening for inhibitors of auxin signalling. One compound, 5403629, is predicted to act as an auxin; two others, 5271050 and 6220480, affect gravitropic responses and endomembrane trafficking via non-auxin pathways. Compound 5850247 decreases auxin responsiveness possibly via a mechanism that also affects membrane transport. Thus, this study revealed a close link between gravitropism, endomembrane trafficking and auxin signalling.

Chemical genetics has also been used to identify small molecules that interfere in plant immune responses. A chemical library of 120 preselected bioactive compounds has been screened for molecules that interfere in the activation of a reporter gene driven by the *ATL2* promoter [36•]. Endogenous *ATL2* is rapidly induced by cellulysin and flagellin, two different pathogen-associated molecular patterns (PAMPs) that can elicit the plant defence response. Oxytriazine induces *ATL2* expression in the absence of PAMPs, whereas four other small molecules (triclosan, fluazinam, cantharidin and fenpiclonil) reduced PAMP-induced *ATL2* expression (Figure 5c). These four molecules have different effects on the release of reactive oxygen species and on the internalization of the flagellin receptor, which indicates that these molecules interfere via different modes of actions. Triclosan probably inhibits the MOD1 enoyl ACP reductase, suggesting a role for lipid signalling in early immune responses. Although this screen was performed on a small library enriched for bioactive compounds, this work introduces a powerful procedure to screen larger, unbiased libraries that can generate additional chemical tools to study plant immune responses.

Guided reverse chemical genetics (chemetics)

As discussed above, forward chemical genetic screens (from phenotype — via chemical — to protein) have the disadvantage that it is a challenge to identify the mode of action and the targeted proteins. By contrast, targets of reverse chemical genetic screens (from protein — via chemicals — to phenotype) are known, but these screens have not yet been applied in plants. A bottleneck in reverse chemical genetic screens is that they are usually performed *in vitro* and only selected small molecules are subsequently tested *in vivo* to investigate any phenotypic effects by chemically knocking out the target proteins.

Figure 6



'Chemetics' – reverse chemical genetics guided by activity-based profiling with probe-derived libraries. Activity-dependent probes (e.g. biotinylated inhibitors, top left) are used to display activity profiles for entire enzyme classes (left). Profiling in the presence of a probe-derived chemical library (top) will generate inhibition profiles for every enzyme (large panel). Screening the same probe-derived chemical library for phenotypic effects (bottom) results in phenotypic data that can be compared with the enzyme inhibition profiles. A correlation between the activity of a particular enzyme and phenotypes (red circles) indicates a causal relationship between the enzyme activity and the phenotype.

Obviously, bioavailability and potential 'off-target' effects of small molecules considerably influence these assays. It is therefore preferable to design specific *in vivo* assays for the target proteins.

To facilitate *in vivo* reverse chemical genetics, these screens can be guided by *in vivo* activity-based profiling, a technology that can verify specific inactivation of target enzymes. Activity-based profiling is based on the use of labelled, mechanism-based inhibitors that react with classes of enzymes in an activity-dependent manner. This technology was successfully applied to display the activities of multiple papain-like cysteine proteases in plant extracts [37•]. More probes are available for other enzyme classes [38,39], and *in vivo* profiling with these probes is possible through a two-step labelling procedure [40•]. In the first step proteins are labelled with a small, tag-free membrane-permeable probe *in vivo*. The second step involves a chemistry reaction ('click chemistry') of the labelled proteome to add a fluorescent or affinity tag onto the labelled proteins. When combined with phenotypic assays, *in vivo* activity-based profiling can be used to screen probe-derived chemical libraries to make correlations between phenotypes and enzyme activity (Figure 6).

Conclusion

Chemical genetic screens have generated novel modulators of plant growth, development and defence. The small molecule screens varied in the diversity, content and size of the chemical library and the specificity of the assays. Most of the bioactive molecules were identified from forward chemical genetic screens, except for the cytochrome P450 inhibitors, which were identified from a focussed reverse chemical genetic screen. Sirtinol was identified by a true reverse chemical genetic screen, but that was done in yeast. Genetic screening for

chemical-insensitive mutants is sometimes also called chemical genetics [41,28], but this terminology is not consistent with most of the reports on chemical genetics.

Auxin signalling appears to be a favourite subject for chemical genetics. This is probably because chemical genetics is a good approach to study auxin signalling in the absence of pleiotropic effects often encountered in auxin-signalling mutants. Assays in the auxin research field were also already adapted to the use of small molecules. In addition, auxin-mimicking molecules also appear in other chemical genetic screens, underlining the crucial role for auxin in multiple plant processes.

Bioactive molecules can cause highly specific effects on plants. Transcriptome analysis has proven to be a strong technology to investigate the effects of small molecules, and also phenotypic comparisons with genetic mutants have provided valuable information, for example on the specificity of Brz and sirtinol.

The fate of small molecules in plants is still largely unknown. It is generally assumed that small molecules have an easy access through roots. However, some sirtinol derivatives were effective on seedlings in liquid cell cultures, but not on solid medium [29••], indicating that bioavailability is higher through other tissues. Apart from uptake, the conversion of small molecules should also be considered.

The true challenge for chemical genetic approaches lies in the identification of the targets. So far, this problem has mainly been tackled by screening for chemical-insensitive mutants, but this approach is limited to genes that are not redundant or lethal. Moreover, genes that have a role in transport and conversion of bioactive molecules can also be identified using this approach. Other approaches to identify targets are offered by yeast three hybrid technology and affinity purification.

A continuous intimate collaboration between small molecule chemists and plant biologists is essential to further pursue chemical genetic approaches. The mode of action and the identification and validation of the targets require the design of novel synthesized derivatives of bioactive molecules. Also, an increased diversity of the chemical libraries and further development of guided reverse chemical genetics depends on chemistry. Considering the tremendous potential of bioactive small molecules in plants, these collaborations will prove highly rewarding. There is a magnitude of stunning bioactive molecules in the unexplored chemical space, waiting to be discovered.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. D'Auria JC, Gershenzon J: **The secondary metabolism of *Arabidopsis thaliana*: growing like a weed**. *Curr Opin Plant Biol* 2005, **8**:308-316.

2. • Miles GP, Samuel MA, Jones AM, Ellis BE: **Mastoparan rapidly activates plant MAP kinase signalling independent of heterotrimeric G proteins**. *Plant Physiol* 2004, **134**:1332-1336.
This paper illustrates that targets of small molecules are not necessarily the same in animals and plants.

3. • Hatsugai N, Kuroyanagi M, Yamada K, Meshi T, Tsuda S, Kondo M, Nishimura M, Hara-Nishimura I: **A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death**. *Science* 2004, **305**:855-858.
Important finding in the quest for 'plant caspases': VPEs are essential for the hypersensitive cell death and react with caspase probes. VPEs also share structural homologies with caspases, but their subcellular location and substrate specificity is clearly different.

4. • Coffeen WC, Wolpert TJ: **Purification and characterisation of serine proteases that exhibit caspase-like activity and are associated with programmed cell death in *Avena sativa***. *Plant Cell* 2004, **16**:857-873.
Proteolysis of RuBisCo during victorin-induced programmed cell death can be inhibited with various protease inhibitors, including the caspase inhibitor VAD-cmk. Purification of the corresponding proteases uncovered that subtilase-like serine proteases can also react with the caspase probe VAD-cmk, illustrating the low specificity of this small molecule.

5. Scheible WR, Eshed R, Richmond T, Delmer D, Somerville C: **Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *Arabidopsis Ixr1* mutants**. *Proc Natl Acad Sci USA* 2001, **98**:10079-10084.

6. Desprez T, Vernhettes S, Fagard M, Refregier G, Desnos T, Aletti E, Py N, Pelletier S, Höfte H: **Resistance against herbicide Isoxaben and cellulose deficiency caused by distinct mutations in same cellulose synthase isoform CESA6**. *Plant Physiol* 2002, **128**:482-490.

7. Van Hulten M, Pelser M, Van Loon LC, Pieterse CM, Ton J: **Costs and benefits of priming for defense in *Arabidopsis***. *Proc Natl Acad Sci USA* 2006, **103**:5602-5607.

8. Izumi K, Yamaguchi I, Wada A, Oshio H, Takahashi N: **Effects of a new plant growth retardant (E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-0l (S-3307) on the growth and gibberellin content of rice plants**. *Plant Cell Physiol* 1984, **25**:611-617.

9. Choe S, Dilkes BP, Hujioka S, Tkasuto S, Sakurai A, Feldmann KA: **The *DWF4* gene of *Arabidopsis* encodes a cytochrome P450 that mediates multiple 22α-hydroxylation steps in brassinosteroid biosynthesis**. *Plant Cell* 1998, **10**:231-243.

10. Min YK, Asami T, Fujioka S, Murofushi N, Yamaguchi I, Yoshida S: **New lead compounds for brassinosteroid biosynthesis inhibitors**. *Bioorg Med Chem Lett* 1999, **9**:425-430.

11. Asami T, Mizutani M, Fujioka S, Goda H, Min YK, Shimada Y, Nakano T, Takatsuto S, Matsuyama T, Nagata N *et al.*: **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* 2001, **276**:25687-25691.

12. Goda H, Shimada Y, Sami T, Fujioka S, Yoshida S: **Microarray analysis of brassinosteriod-regulated genes in *Arabidopsis***. *Plant Physiol* 2002, **130**:1319-1334.

13. Nagata N, Min YK, Nakano T, Asami T, Yoshida S: **Treatment of dark-grown *Arabidopsis thaliana* with a brassinosteroid-biosynthesis inhibitor, brassinazole, induces some characteristics of light-grown plants**. *Planta* 2000, **211**:781-790.

14. Nagata N, Asami T, Yoshida S: **Brassinazole, an inhibitor of brassinosteroid biosysnthesis, inhibits development of**

secondary xylem in cress plants (*Lepidium sativum*). *Plant Cell Physiol* 2001, **42**:1006-1011.

15. Sun Y, Veerabomma S, Abdel-Mageed HA, Fokar M, Asami T,
• Yoshida S, Allen RD: **Brassinosteroid regulates fiber development on cultures cotton ovules**. *Plant Cell Physiol* 2005, **46**:1384-1391.
Good example of using chemical interference to study processes in non-model organisms. Together with earlier work [12–14], these data illustrate the specificity and applicability of Brz.

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

17. He JX, Gendron JM, Sun Y, Gampala SSL, Gendron N, Sun CQ,
• Wang ZY: **BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses**. *Science* 2005, **307**:1634-1638.
BZR1 was originally identified from a screen for mutants that were insensitive for brassinazole [16], a clear example of how chemical tools can be used to address biological questions.

18. Borevitz JO, Maloof JN, lutes J, Dabi T, Redfern JL, Trainer GT, Werner JD, Asami T, Berry CC, Weigel D, Chory J: **Quantitative trait loci controlling light and hormone response in two accessions of *Arabidopsis thaliana***. *Genetics* 2002, **160**:683-696.

19. Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S, Ueguchi-Tanaka M, Mizutani M, Sakata K, Takatsuto S, Yoshida S *et al.*: **Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice**. *Nat Biotechnol* 2006, **24**:105-109.

20. Saito S, Okamoto M, Shinoda S, Kushiro T, Koshiba T, Kamiya Y,
• Hirai N, Todoroki Y, Sakata K, Nambara E, Mizutani M: **A plant growth retardant, uniconazole, is a potent inhibitor of ABA catabolism in *Arabidopsis***. *Biosci Biotechnol Biochem* 2006, **70**:1731-1739.
See annotation to [21•].

21. Kitahata N, Saito S, Miyazawa Y, Umezawa T, Shimada Y, Min YK,
• Mizutani M, Hirai N, Shinozaki K, Yoshida S, Asami T: **Chemical regulation of abscisic acid catabolism in plants by cytochrome P450 inhibitors**. *Bioorg Med Chem* 2005, **13**:4491-4498.
This is a very nice demonstration that some triazole-type cytochrome P450 inhibitors specifically inhibit the degradation of the drought stress hormone abscisic acid. Triazole treatment increases drought tolerance. This study was extended by Saito *et al.* [20•].

22. Spring DR, Krishnan S, Blackwell HE, Schreiber SL: **Diversity-oriented synthesis of biaryl-containing medium rings using a one bead/one stock solution platform**. *J Am Chem Soc* 2002, **124**:1354-1363.

23. Blackwell HE, Zhao Y: **Chemical genetic approaches to plant biology**. *Plant Physiol* 2003, **133**:448-455.

24. Hayashi K, Ogino K, Oono Y, Uchimiya H, Nozaki H: **Yokonolide A, a new inhibitor of auxin signal transduction, from *Streptomyces diastatochromogenes* B59**. *J Antibiot* 2001, **54**:573-581.

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

26. Armstrong JI, Yuan S, Dale JM, Tanner VN, Theologis A:
•• **Identification of inhibitors of auxin transcriptional activation by means of chemical genetics in *Arabidopsis***. *Proc Natl Acad Sci USA* 2004, **101**:14978-14983.
One of the few real chemical genetic screens on plants. Of the 10 000 diverse small molecules, 30 interfere in auxin signalling and four were further studied. This study illustrates the potential of nonbiased chemical genetic screens.

27. Grozinger CM, Chao ED, Blackwell HE, Moazed D, Schreiber SL: **Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening**. *J Biol Chem* 2001, **276**:38837-38843.

28. Zhao Y, Dai X, Blackwell HE, Schreiber SL, Chory J: **SIR1, an upstream component in auxin signalling identified by chemical genetics**. *Science* 2003, **301**:1107-1110.

29. Dai X, Hayashi K, Nozaki H, Cheng Y, Zhao Y: **Genetic and**
•• **chemical analysis of the action mechanisms of sirtinol in *Arabidopsis***. *Proc Natl Acad Sci USA* 2005, **102**:3129-3134.
Remarkable discoveries are described concerning the deceiving action of sirtinol, uncovered by collaboration with organic chemists, who produced sirtinol derivatives that act as an auxin. This work, together with the identification of sirtinol [27] and the identification of Sir1 [28], illustrates the difficulties that can be encountered by chemical genetic approaches.

30. Kepinski S, Leyser O: **The *Arabidopsis* F-box protein TIR1 is an**
• **auxin receptor**. *Nature* 2005, **435**:446-451.
See annotation to [31•].

31. Dharmasiri N, Dharmasiri S, Estelle M: **The F-box protein TIR1 is**
• **an auxin receptor**. *Nature* 2005, **435**:441-445.
The receptor of the intensively studied small molecule hormone auxin is finally revealed: auxin directly modulates the activity of the TIR1 ubiquitin ligase complex that targets auxin-responsive transcription factors for degradation. See also [30•].

32. Cheng Y, Dai X, Zhao Y: **AtCAND1, a HEAT-repeat protein that participates in auxin signalling in *Arabidopsis***. *Plant Physiol* 2004, **135**:1020-1026.

33. Perry J, Dai X, Zhao Y: **A mutation in the anticodon of a single rRNAala is sufficient to confer auxin resistance in *Arabidopsis***. *Plant Physiol* 2005, **139**:1284-1290.

34. Zouhar J, Hicks GR, Raikhel NV: **Sorting inhibitors (sortins):**
•• **chemical compounds to study vacuolar sorting in *Arabidopsis***. *Proc Natl Acad Sci USA* 2004, **101**:9497-9501.
One of the earliest true chemical genetic screens of large chemical libraries to find modulators that disturb vacuolar trafficking in yeast. Only a few of the 14 bioactive compounds had similar effects in plants.

35. Surpin M, Rojas-Pierce M, Carter C, Hicks GR, Vasquez J,
•• Raikhel NV: **The power of chemical genomics to study the link between endomembrane system components and the gravitropic response**. *Proc Natl Acad Sci USA* 2005, **102**:4902-4907.
One of the few true forward chemical genetic screens using large diverse libraries is described. A simple assay, based on rotation of plates with seedlings, was used to identify small molecules that interfere in the gravitropic response.

36. Serrano M, Robatzek S, Torres M, Kombrink E, Somssich IE,
• Robinson M, Schulze-Lefert P: **Chemical interference of PAMP-triggered immune responses in *Arabidopsis* reveals a potential role for FAS II complex-derived lipids**. *J Biol Chem*, in press.
Arabidopsis seedlings carrying an elicitor-responsive reporter gene were used to identify diverse modifiers of immune responses with different modes of action.

37. Van der Hoorn RAL, Leeuwenburgh MA, Bogyo M, Joosten MHAJ,
• Peck S: **Activity profiling of papain-like cysteine proteases in plants**. *Plant Physiol* 2004, **135**:1170-1178.
A first step towards guided reverse chemical genetics in plants: a E64-derived probe displays the activities of at least six different papain-like cysteine proteases.

38. Saghatelian A, Cravatt BF: **Assignment of protein function in the postgenomic era**. *Nat Chem Biol* 2005, **1**:130-142.

39. Verhelst SHL, Bogyo M: **Dissecting protein function using chemical proteomic methods**. *QSAR Comb Sci* 2005, **24**:261-269.

40. Speers AE, Cravatt BF: **Profiling enzyme activities in vivo using**
• **click chemistry methods**. *Chem Biol* 2004, **11**:535-546.
A thorough report on the introduction of click chemistry in activity-based proteomics, which facilitates *in vivo* activity-based profiling of various enzymes using chemically tagged, membrane-permeable probes.

41. Zheng W, Zhai Q, Sun J, Li CB, Zhang L, Li H, Zhang X, Li S, Xu Y, Jiang H *et al.*: **Bestatin, an inhibitor of aminopeptidases, provides a chemical genetics approach to dissect jasmonate signaling in *Arabidopsis***. *Plant Physiol* 2006, **141**:1400-1413.
In this case, 'chemical genetics' does not refer to screening of chemical libraries, but to the generation and characterization of chemical-insensitive mutants.

42. Zhang A, Jiang M, Zhang J, Tan M, Hu X: **Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants**. *Plant Physiol* 2006, **141**:475-487.

43. Dai Y, Wang H, Li B, Huang J, Liu X, Zhou Y, Mou Z, Li J: **Increased expression of MAP kinase kinase7 causes deficiency in polar auxin transport and leads to plant architectural abnormality in *Arabidopsis***. *Plant Cell* 2006, **18**:308-320.

44. Robatzek S, Chinchilla D, Boller T: **Ligand-induced endocytosis of the pattern recognition receptor FLS2 in *Arabidopsis***. *Genes Dev* 2006, **20**:537-542.

45. Luan S: **Protein phosphatases in plants**. *Annu Rev Plant Biol* 2003, **54**:63-92.

46. Pak C, Van Doorn WG: **Delay of Iris flower senescence by protease inhibitors**. *New Phytol* 2005, **165**:473-480.

47. Davies DR, Bindschedler LV, Strickland TS, Bolwell GP: **Production of reactive oxygen species in *Arabidopsis thaliana* cell suspension cultures in response to an elicitor from *Fusarium oxysporum*: implications for basal resistance**. *J Exp Bot* 2006, **57**:1817-1827.

48. Nomura K, Debroy S, Lee YH, Pumplin N, Jones J, He SY: **A bacterial virulence protein suppresses host innate immunity to cause plant disease**. *Science* 2006, **313**:220-223.

49. Fiorani F, Beemster GT: **Quantitative analyses of cell division in plants**. *Plant Mol Biol* 2006, **60**:963-979.

50. Dong A, Liu Z, Zhu Y, Yu F, Li Z, Cao K, Shen WH: **Interacting proteins and differences in nuclear transport reveal specific functions for the NAP1 family proteins in plants**. *Plant Physiol* 2005, **138**:1446-1456.

51. Hetherington AM, Brownlee C: **The generation of Ca(2+) signals in plants**. *Annu Rev Plant Biol* 2004, **55**:401-427.

52. Hoat TX, Nakayashiki H, Tosa Y, Mayama S: **Specific cleavage of ribosomal RNA and mRNA during victorin-induced apoptotic cell death in oat**. *Plant J* 2006, **46**:922-933.

53. Loyola-Vargas V, Broeckling C, Badri D, Vivanco J: **Effect of transporters on the secretion of phytochemicals by the roots of *Arabidopsis***. *Planta* 2006, Epub ahead of print, DOI: 10.1007/s00425-006-0349-2.

54. Igamberdiev AU, Shen T, Gardestrom P: **Function of mitochondria during the transition of barley protoplasts from low light to high light**. *Planta* 2006, **224**:196-204.

55. Martinez IM, Chrispeels MJ: **Genomic analysis of the unfolded protein response in *Arabidopsis* shows its connection to important cellular processes**. *Plant Cell* 2003, **15**:561-576.

56. Koo AJ, Fulda M, Browse J, Ohlrogge JB: **Identification of a plastid acyl-acyl carrier protein synthetase in *Arabidopsis* and its role in the activation and elongation of exogenous fatty acids**. *Plant J* 2005, **44**:620-632.

57. Dreher KA, Brown J, Saw RE, Callis J: **The *Arabidopsis* Aux/IAA protein family has diversified in degradation and auxin responsiveness**. *Plant Cell* 2006, **18**:699-714.

58. Rodriguez-Concepcion M, Fores O, Martinez-Garcia JF, Gonzalez V, Phillips MA, Ferrer A, Boronat A: **Distinct light-mediated pathways regulate the biosynthesis and exchange of isoprenoid precursors during *Arabidopsis* seedling development**. *Plant Cell* 2004, **16**:144-156.

59. Dettmer J, Hong-Hermesdorf A, Stierhof YD, Schumacher K: **Vacuolar H^+-ATPase activity is required for endocytic and secretory trafficking in *Arabidopsis***. *Plant Cell* 2006, **18**:715-730.

60. Paredez AR, Somerville CR, Ehrhardt DW: **Visualization of cellulose synthase demonstrates functional association with microtubules**. *Science* 2006, **312**:1491-1495.

61. Zhang L, Tamura K, Shin-ya K, Takahashi H: **The telomerase inhibitor telomestatin induces telomere shortening and cell death in *Arabidopsis***. *Biochim Biophys Acta* 2006, **1763**:39-44.