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Powerful Partners: Arabidopsis and Chemical Genomics

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Chemical genomics (i.e. genomics scale chemical genetics) approaches capitalize on the ability of low molecular mass molecules to modify biological processes. Such molecules are used to modify the activity of a protein or a pathway in a manner that it is tunable and reversible. Bioactive chemicals resulting from forward or reverse chemical screens can be useful in understanding and dissecting complex biological processes due to the essentially limitless variation in structure and activities inherent in chemical space. A major advantage of this approach as a powerful addition to conventional plant genetics is the fact that chemical genomics can address loss-of-function lethality and redundancy. Furthermore, the ability of chemicals to be added at will and to act quickly can permit the study of processes that are highly dynamic such as endomembrane trafficking. An important aspect of utilizing small molecules effectively is to characterize bioactive chemicals in detail including an understanding of structure-activity relationships and the identification of active and inactive analogs. Bioactive chemicals can be useful as reagents to probe biological pathways directly. However, the identification of cognate targets and their pathways is also informative and can be achieved by screens for genetic resistance or hypersensitivity in *Arabidopsis thaliana* or other organisms from which the results can be translated to plants. In addition, there are approaches utilizing “tagged” chemical libraries that possess reactive moieties permitting the immobilization of active compounds. This opens the possibility for biochemical purification of putative cognate targets. We will review approaches to screen for bioactive chemicals that affect biological processes in Arabidopsis and provide several examples of the power and challenges inherent in this new approach in plant biology.

INTRODUCTION

Descriptions of plant chemical composition have existed for many years (Macey, 1970; Avato, 1987). However, the power of small molecules to address biological questions began to be realized and accelerated in the middle 1990s (Mitchison, 1994; Schreiber, 1998) with the production of new synthetic combinatorial chemistry techniques. The key innovation was the systematic design and synthesis of original molecules and the utilization of these chemicals as probes for biological processes (Stockwell, 2002; Blackwell and Zhao, 2003; Lokey, 2003). Chemical genetics studies have been applied extensively to diverse biological models including bacteria (Eggert et al., 2001), cancer biology (Torrance et al., 2001), vertebrate development (Peterson et al., 2000), and neurobiology (Stockwell, 2002, 2004). The term “chemical genomics” expands the previous term by encompassing the enormous volume of gene and protein sequence that are now available as well as knowledge of gene expression profiles. It also incorporates the powerful tools that are now at our disposal for uncover-

ing cognate targets including reverse genetics and gene mapping. All of these modern genomics approaches have enabled and accelerated the use of small molecules for biological discovery.

Chemical genomics is now poised at the interface of chemistry, biology, and especially bioinformatics (Stumpfe et al., 2007; Vogt et al., 2007) since data mining is required for structural analysis, data sharing and the extraction of other data. Chemical genomics approaches are based on the ability of low molecular mass molecules to modify the activity of proteins or pathways which can overcome important limitations inherent to mutational approaches (Stockwell, 2000; Alaimo et al., 2001; Shogren-Knaak et al., 2001). In Arabidopsis, T-DNA inactivation mutants are a valuable tool but can lead to lethality due to the loss of function or, in many cases, no phenotype due to complete or co-redundancies in gene function. Along these lines, a protein may have several functions in a cell. In the case of a gene deletion mutant all functions are lost, although point mutants can be employed. However, it is possible to find small molecules that perturb only one of several functions of a protein resulting in a level of understanding of protein function

that would be difficult to achieve through gene-based perturbation alone (Kuruvilla et al., 2002). One of the advantages of using small molecules is a high degree of temporal control over the function of protein targets. This is due to the fact that bioactive chemicals can be added at different time points or developmental stages to induce an effect. The chemicals can then be washed away to return a cell to its wild-type state.

In forward chemical genomics screens, thousands or tens of thousands of compounds are tested for their ability to alter a specific pathway resulting in a phenotype. In reverse chemical genomics screens, small molecules are used to specifically inhibit or activate known selected targets in order to study the functional consequences. Ultimately, bioactive compounds targeted to specific pathways can be useful in plants to understand and dissect molecular and biochemical processes.

The power of bioactive molecules in plant biology has been amply illustrated by the use of specific chemical inhibitors of biological processes including Brefeldin A (BFA) which affects the end-membrane system (Dinter and Berger, 1998; Nebenfuhr et al., 2002), Latrunculin B (LAT B) which affects cytoskeleton (Friml et al., 2002), and various auxin transport inhibitors (Geldner et al., 2001).

HOW IS CHEMICAL GENOMICS DIFFERENT THAN DRUG DISCOVERY?

Many synthetic chemists simply view plants as sources of diverse and structurally complex natural products with novel activities. Historically, a major challenge in drug discovery has been the lack of well-characterized targets whose activities may be impacted by chemicals. Thus, many drugs were found either through years of methodical biochemistry and molecular biology or by luck to some extent. That is, some chemicals happened to produce a desirable phenotype. Aspirin is an example; the target was not known before it was used medicinally. An enormous amount of data about modes of action has now been acquired. Additional knowledge is also streamlining drug discovery. For example, most of the genes in a handful of organisms have been identified, and there is extensive data on gene expression for many others. There are large collections of indexed gene knock-out mutants that can provide phenotypic data linked to specific genes. What this knowledge of the genome now permits is a more target-based approach to drug discovery that pharmaceutical companies are capitalizing upon. Thus, much of drug discovery these days is based upon assays that are either highly specific for defined targets, or they are at least focused at the level of understanding what classes of gene products are involved in phenotypes of interest. Such approaches have become much more focused in the last several decades. Target-based approaches, however, require extremely large chemical libraries that are synthesized around specific scaffolds that are likely to provide drug-like properties. For example the so-called Lipinski rule of five accounts for membrane permeability, mass and other properties deemed to be characteristic of successful drugs (Lipinski, 1997). The objective of drug screening is also much more rigorous in many ways compared to chemical genomics. Drugs must meet many criteria such as low toxicity and display relatively short half-lives in the body as well as show a lack of side effects. This contributes to the need for automated high-throughput screens.

Chemical genomics on the other hand employs a different set of criteria. Rather than screening for drugs, researchers look for compounds that have informative biological effects. In this regard, a target-based approach cannot be used typically. Rather, scientists have to use forward chemical genomics of highly diverse libraries, structurally speaking, as opposed to large libraries designed around more defined scaffolds. Only after defining informative chemicals can we then think about screening libraries that are more focused structurally. Furthermore, although plant biologists desire compounds that are membrane permeable and easily transported through the plant vasculature, we do not require the high potencies necessary for drug efficacy. This extends to chemical metabolic turnover. Although it is desirable to have compounds that are reversible in chemical genomics, it is not required in all cases. Perhaps the most critical difference between drug discovery and chemical genomics resides in the fact that drugs must be well-defined in their action which means that their cognate targets must be known and their interaction with the drug understood. For chemical genomics, it is desirable to know the cognate target of a chemical for sure; however in many cases simply understanding the pathway affected may be informative and lead to testable hypotheses. This is not to imply that only drug discovery requires cognate targets and a deep understanding of chemical interactions. For example to understand and enhance herbicide and fungicide action, it is desirable to identify cognate targets. As will be described in the examples to follow, relatively broad phenotypic screens provide a large target space and, thus, far fewer compounds need to be screened to identify those that are informative biologically.

The underlying concept is that most protein function can be altered by chemical binding. There are four major aspects that characterize chemical genomics: 1) chemical library synthesis and assembly, 2) screening for bioactive compounds, 3) identification of protein targets, and 4) using the chemicals as tools to understand target function and discover networks. For an overview, see Figure 1.

SOURCES OF DIVERSE CHEMICAL LIBRARIES

The specific chemical structures needed to bind to different proteins may be extremely diverse: the requisite structure is determined by the contours, charge, hydrophobicity and other factors associated with the available binding pockets on each protein. So, in an 'ideal' chemical library for chemical genomics - one that contains a small-molecule ligand or binding partner for all existing proteins - a vast number of structures would be needed. The potential chemical diversity of organic compounds (defined as the number of unique chemical structures composed of carbon, hydrogen, nitrogen, oxygen, sulfur, phosphorous, and the halogens) of molecular mass 1,000 likely exceeds 10^{60} , whereas there exist in the chemical literature around 10 million pure compounds (Dobson, 2004). The compounds that have thus far been tested for effects on plants are therefore only a miniscule fraction of the structural possibilities. The development of combinatorial and automated techniques for synthesizing novel compounds has significantly enhanced the productivity of chemists and increases the likelihood of synthesizing compound libraries that are of sufficient size to better represent the diversity of "chemical space". In fact, with the ad-

Figure 1. A broad workflow of the chemical genomics approach. Libraries are available as either diversity oriented or focused and can contain both natural and synthetic compounds. Following a high-throughput screen, it is important to characterize bioactive chemicals utilizing a range of physiological and cellular approaches depending upon the pathway being studied. An integral part of the characterization is the examination, when possible, of analogues and substructures. As described in the text, such information can provide clues about the nature of the target(s). In many cases researchers are must deal with more compounds than can be easily characterized; thus, chemical characterization will also enable the prioritization of compounds for later steps. In some cases, it may also be desirable to create libraries that are focused around the scaffold of a particularly interesting compound (dashed line). Compound prioritization can be based on different factors including potency, specificity of phenotype, availability, metabolic stability, and chemical cost. Well-characterized compounds can be valuable as reagents for asking biological questions or are the basis for determining the target(s) via genetics or biochemistry.

vent of combinatorial chemistry (Oliver and Abell, 1999), larger synthetic libraries and high-throughput screening techniques over the past decade, the pace of chemical library screening has escalated dramatically.

In plant biology, even at this relatively early stage of plant chemical genomics, the use of unbiased libraries of diverse small molecules is permitting plant biologists to discover new bioactive molecules that are proving valuable for studying the function of uncharacterized plant genes. In concert with Arabidopsis functional genomics, chemical genomics is facilitating the analysis of regulatory networks for specific biological processes (Raikhel and Pirrung, 2005).

The most widely used probe reagents for chemical genomics studies are small organic molecules which are available from a number of commercial suppliers and include compounds from historical archives, natural sources, or those produced using combinatorial chemistry (Table 1). Typically, commercial suppliers will provide compounds in 96- or 384-well plates and provide structure data (SD) files with electronic chemical structures and quality control data on each compound's purity. Larger, more customized library collections can be assembled from available compounds or synthesized de novo. Beyond these sources is an NIH

initiative known as the NIH Molecular Libraries. The objective of the initiative is to provide a collection of diverse chemicals to researchers via a series of 10 centers that will curate and distribute the chemicals.

In practice, there are two types of chemical libraries that can be synthesized: 'focused libraries' and 'diversity-oriented libraries' (Young and Ge, 2004). Focused libraries are designed around defined scaffolds that may be related to endogenous ligands for a specific class of proteins (Stockwell, 2004). Another type of library is the diversity-oriented libraries composed of diverse compounds (Stockwell, 2004; Zhang et al., 2008). Each of these strategies has its advantages and disadvantages. Compounds in focused libraries are more likely to be bioactive but may only target a single known class of proteins. Diversity-oriented libraries, in contrast, offer the possibility of targeting entirely new classes of proteins, but any given individual compound may display lower probability of activity or potency. From the perspective of biological discovery, an ideal chemical library for chemical genomics would contain highly diverse compounds affecting a diverse range of proteins and phenotypes. The ability to design enhanced libraries would also incorporate information about the bioactivity and structural diversity of existing libraries. This would mean that optimal libraries

for a given purpose could be rationally assembled from members of other libraries. Many such approaches have been reported (Olah et al., 2004) and have been undertaken as well at the Center for Plant Cell Biology (CEPCEB) at the University of California, Riverside. An example of analysis of structural diversity among some commercial libraries are available at an affiliated website known as ChemMine (Girke et al., 2005). ChemMine is a set of comprehensive web-based tools for analysis of chemical structure similarities and also lists more than 55,000 diverse compounds housed at CEPCEB from a combination of commercial and University sources (<http://bioweb.ucr.edu/ChemMineV2/>). As an indication of the importance of chemical biology, the number of commercial sources of chemical libraries has increased steadily in the past few years.

RECENT CHEMICAL GENOMICS SCREENS

We want to introduce readers to important categories of small molecules of interest as well as examples of chemical screens with an emphasis on the endomembrane system which has been the focus of several recent investigations. Later, we will focus more precisely on what has been learned about cognate targets and pathways. For a summary of representative chemical structures to be discussed, see Table 2.

Herbicides Targeted to the Cell Wall

Although perhaps not realized by many academic scientists, the agrochemical industry has utilized combinatorial chemistry and the principles of high throughput screening for many years to discover new herbicides and pesticides and to identify their cognate targets. As an example, the biosynthesis of plant cell walls is highly complex and is understood poorly. Although cellulose is the most abundant component of cell wall, even cellulose synthesis remains an enigmatic process mechanistically (Delmer, 1999). This linear 1,4- β -linked glucan is synthesized in plants by a hexameric protein complex in the plasma membrane referred to as the rosette (Kimura et al., 1999). In addition to mutants, chemical inhibitors can be powerful tools for the molecular dissection of biological processes. Several herbicides inhibiting cellulose synthesis have been described such as dichlorobenzonitrile (DCB), isoxaben (N-[3(1-ethyl-1-methylpropyl)-5-isoxazolyl]), and CGA325_615 (Peng et al., 2001). Isoxaben is a pre-emergence, broad leaf herbicide used primarily on small grains, turf, and ornamentals (Heim et al., 1989, 1990). It is selectively phytotoxic to dicotyledonous plants. With an IC₅₀ value in the nanomolar range, isoxaben is a highly potent chemical that specifically inhibits radioactive Glc incorporation into the acid insoluble cellulosic cell wall fraction. In spite of such knowledge, only within the last decade has the target site of isoxaben been identified using a genetic approach and shown to be a cellulose synthase (Scheible et al., 2001).

Very recently, several forward chemical screens for compounds affecting cell wall synthesis and morphology have been reported. To identify new chemical inhibitors of cellulose synthesis, a recent screen utilized a library of 20,000 compounds from the DIVERSet collection (ChemBridge, San Diego, CA) (DeBolt et al., 2007). Inhibition of cell wall biogenesis can result in an

organ swelling phenotype due to an alteration of cell morphology resulting from changes in cell wall composition or microfibril orientation. Focusing on this phenotype, the chemical Morlin resulted in a swollen root phenotype in Arabidopsis. Interestingly, it caused a defect in cytoskeleton organization and its functional interaction with the cellulose synthase CESA. Thus, Morlin appears to be an excellent probe to better understand cellulose biosynthesis.

A different cell wall-related forward screen was focused on the identification of chemicals that affected the alignment of cortical microtubules that determine the orientation of cellulose microfibrils (Yoneda et al., 2007). This screen utilized tobacco BY-2 cells which grow rapidly and can be easily adapted to high-throughput screening. This screen utilized several libraries (Spectrum, LATCA; Table 1) and focused on cells having a swollen phenotype; very apparent were cells that were highly rounded compared to the normal BY-2 cellular morphology. The outcome of the screen produced compounds in each of three categories: those that affected cortical microtubules (detected with GFP- α tubulin), those that resulted in a reduction in microfibril content (detected with calcofluor staining), and those that affected the alignment of existing cortical microtubules with microfibrils (both probes). The authors were particularly interested in the third category and described the chemical Cobtorin that impacted the alignment of microfibrils. The study also utilized informatics to examine the activity of chemical analogs. Although the target is not yet known, it is possible that the target could be involved in coupling microtubules to the wall synthetic apparatus.

Plant Growth Regulators

Chemical genetics has historically been a powerful tool to better understand plant growth regulation, and this has accelerated with the advent of Arabidopsis genomics. Auxin is probably the best-studied small molecule in plants, and its phenotypic and molecular effects have been examined for many decades. In an attempt to discover small molecule effectors of auxin responses in Arabidopsis, fermentation-derived natural products from the soil microorganism *Streptomyces diastatochromogenes* were screened for the ability to inhibit of auxin-responsive gene expression (Kirst et al., 1995; Hayashi et al., 2001). The researchers used transgenic Arabidopsis harboring the β -glucuronidase (GUS) reporter under the control of an auxin-inducible promoter to screened for compounds that inhibited reporter expression. The screen yielded yokonolides A and B which could serve as bioprobes for understanding auxin signal transduction in Arabidopsis.

Another example is a screen which was done using a small library of biaryl-derived molecules for their effects on germination in Arabidopsis (Spring et al., 2002). Although the chemical library was composed only of 57 compounds, the axially dissymmetric cyclic biaryl motif present is a common structural element of numerous biologically active compounds. One biaryl compound, (P)-4k, was reported to be responsible for stunted development of Arabidopsis, leading to loss of pigmentation by day four after germination, and death by day seven.

In a more target-based screen, Zheng and co-workers recently investigated the mechanism of auxin perception by the auxin receptor and ubiquitin ligase, TIR1. TIR1 is a substrate of the

Table 1. A summary of selected chemical libraries.

Library	Website	Library Type	Number of Compounds
National Cancer Institute	http://cactus.nci.nih.gov/ncidb2/download.html	Diverse	250,250
Aurora Fine Chemicals	http://www.aurorafinechemicals.com/	Diverse/custom	29,519
ChemBridge	http://chembridge.com/chembridge/	Diverse/custom	436,548
Timtec	http://www.timtec.com/	Diverse/custom	~400,000
Vistas-M Laboratory	http://vitasmlab.com/	Diverse/custom	~200,000
Asinex	http://www.asinex.com/support_download_zone.html	Diverse	~350,000
Life Chemicals	http://www.lifechemicals.com/	Diverse/custom/targeted	448,000
NIH Molecular Libraries	http://mlsmr.glpq.com/MLSMR_HomePage/identify.html	Diverse/target/natural products	500,000
Microsource Spectrum	http://www.msdiscovery.com/index.html	Bioactives/Diverse/targeted	~5000
Library of Active Compounds in Arabidopsis (LATCA)	http://www.cepceb.ucr.edu/ (Dr. Sean Cutler, University of California, Riverside)	Chemicals shown to be bioactive in Arabidopsis	2000+
Library of Pharmacologically Active Compounds (LOPAC)	http://www.sigmaaldrich.com/Area_of_Interest/Chemistry/Drug_Discovery/Validation_Libraries/Lopac1280home.html	Pharmacologically active compounds	1280

SCF^{TIR} complex involved in the regulation of auxin response via protein turnover (Tan et al., 2007). Auxin binding enhances the interaction between TIR1 and AUX/IAA repressor proteins promoting their ubiquitination and degradation. Small molecule inhibitors of F-box protein recruitment were identified by the analysis of auxin agonists and antagonists (Hayashi et al., 2008). These molecules were synthesized by the introduction of alkyl chains to the α -position of the native auxin, indole-acetic acid, and their interaction with the TIR1 complex was demonstrated by x-ray crystallography. This study is an excellent example of the power of chemical biology to affect a broad range of interactions including small molecule interactions affecting ubiquitin ligase function which is important in the regulation of eukaryotic cellular processes.

It is known that plant growth regulators have interactive pathways, and Gendron et al. (Gendron, 2008) have explored the interaction of brassinosteroids and ethylene during plant development. Based on a screen for hypocotyl growth under dark conditions, 10,000 small molecules were screened for inhibitors of brassinosteroid response. The screen resulted in the identification of brassinopride (BRP) which inhibits brassinosteroid biosynthesis also resulting in an exaggerated apical hook. Interestingly, BRP also activates ethylene responses providing a useful probe for pathway interactions.

Seed imbibition and germination are developmental processes that are also closely related to plant responses to growth regulators such as abscisic acid and gibberellic acid. In a unique exploration of germination transcriptional responses, several chemical libraries were screened for compounds that inhibited germination

(Bassel et al., 2008). Among the bioactive compounds were chemicals of known activity including methotrexate, cyclohexamide and 2, 4-dinitrophenol. Cutler and co-workers compared transcript profiles of seeds treated with these chemicals to profiles of mutants defective in germination and other published profiles of germinating seeds. By combining data from transcriptional responses produced by diverse mechanisms, the authors were able to identify a basal transcriptional signature that defines germinating seeds. Among the sets of up and down-regulated genes were a significant number with unknown functions indicating that germination is yet to be characterized fully at the molecular level. Such information will provide the basis for hypothesis-driven experiments aimed at identifying new regulatory circuits in development.

Plant Pathogens

Screens for effectors of plant pathogenesis have the potential to identify bioactive compounds for basic biology as well as commercial applications. Such screens are now underway. One of the first screens has been reported by Schreiber et al. (Schreiber et al., 2008) and took advantage of a bleaching phenotype that is evident when Arabidopsis seedlings grown in liquid are challenged with the pathogen *Pseudomonas syringae*. The bleaching was associated with loss of cotyledon chlorophyll which was correlated with virulence. As a validation of the assay, compounds known to induce defense responses such as salicylic acid and flagellin prevented bleaching when applied to seedlings prior to pathogen inoculation. By screening the LATCA library (Table 1), the authors

Table 2. Summary of compounds, mode of action, and target, if known.

Chemical	Model	Mode of Action	Target	Reference	Structure
Yokonolide A and B	Arabidopsis	Inhibition of auxin induction	unknown	Hayashi et al., 2001, Kirst et al., 1995	Yokonolide B
Isoxaben	Arabidopsis	Inhibition of cellulose synthesis	CESA3, CESA6	Desprez et al., 2002, Scheible et al., 2001	Isoxaben
(P)-4k	Arabidopsis	Inhibition of germination	unknown	Spring et al, 2002	(P)-4K
Brassinazole	Arabidopsis	Inhibition of brassinosteroids synthesis	unknown	Asami et al., 2003	Brassinazole
Sirtinol	Arabidopsis	Regulation of auxin inducible genes	Auxin signaling F-box protein5-TIR analog, SIR1	Zhao et al., 2003	Sirtinol
Sortin1	Yeast/Arabidopsis	Endomembrane system	unknown	Zouhar et al.2004	Sortin1
Sortin1 substructure 259	Yeast/Arabidopsis	Endomembrane system	unknown	unpublished	Sortin1 substructure 259
Sortin1 substructure 275	Yeast/Arabidopsis	Endomembrane system	unknown	unpublished	Sortin1 substructure 275
Sortin2	Yeast/Arabidopsis	Endomembrane system	unknown	Zouhar et al.2004	Sortin2
Compound A, B, C, D	Arabidopsis	Inhibition of auxin signaling	unknown	Armstrong et al., 2004
5271050	Arabidopsis	Inhibition of gravitropic response	unknown	Surpin, et al., 2005	5271050
DAS534	Arabidopsis	Inhibition of growth	ABF5	Walsh et al., 2006	DAS534
Gravacin	Arabidopsis	Inhibition of auxin transport	PGP19	Rojas-Pierce et al., 2007 Surpin et al., 2005	Gravacin
Triclosan	Arabidopsis	Early immune response	FASII	Serrano et al., 2007	Triclosan
Compound A	Arabidopsis	Inhibition of auxin signaling	unknown	Sungur et al., 2007
DAS734	Arabidopsis	Inhibition of growth, bleaching herbicide	Phosphorybosylpyrophosphate Amidotransferase	Walsh et al., 2007	DAS734
Hypostatin	Arabidopsis	Inhibition of etiolation in Ler background	HYR1	Zhao et al., 2007	Hypostatin
Sortin2	Yeast	Endomembrane system	unknown	Norambuena et al., 2008	Sortin2
ES1	Arabidopsis / tobacco	Inhibition of early step of endocytosis	unknown	Robert et al., 2008	ES1
Morlin	Arabidopsis	Cytoskeletal organization/ interaction with cellulose synthase	unknown	DeBolt et al., 2007	Morlin
Cobtorin	Arabidopsis/BY-2 cells	Cortical microtubule alignment	unknown	Yoneda et al., 2007	Cobtorin

found a class of sulfanilamide compounds that inhibited the bleaching phenotype apparently without affecting the pathogen significantly indicating that these compounds impact plant resistance. In fact, although the mechanism of resistance is not yet clear, assays of leaves from soil-grown plants showed reduced populations of pathogen.

ENDOMEMBRANE BIOLOGY AS A TARGET FOR CHEMICAL GENOMICS

The formation of the endomembrane compartments (endoplasmic reticulum, Golgi, endosomes, vacuole), plasma membrane (PM) and cell walls relies on vesicular trafficking which involves vesicle formation, transport and fusion with target membranes. In addition to unique organelles such as chloroplasts, plants contain organelles and trafficking components found in animals and fungi. The plant endomembrane system serves many functions including protein and hormone transport, membrane protein recycling, ion and metabolite storage, and wall precursor synthesis (Sanderfoot and Raikhel, 1999). Plant vesicular trafficking is necessary for development, signal transduction, and responses to biotic and abiotic stresses (Surpin and Raikhel, 2004). The limitations of classical genetic approaches to study the endomembrane system and, specifically, vesicular trafficking were illustrated by the isolation of the *vacuoleless 1* (*vcl1*) mutant in Arabidopsis from a screen of over 5,000 T-DNA insertion lines (Rojo et al., 2001). The mutation leads to defective vacuole biogenesis and embryo lethality indicating that vacuoles are essential organelles in plants unlike other organisms such as yeast. In addition to *vcl1*, T-DNA insertions in numerous other components of the endomembrane system result in gametophyte or embryo lethality. It is also well known that, due to gene redundancy, loss-of-function mutations in Arabidopsis frequently fail to produce observable phenotypes. To overcome these limitations, chemical genomics approaches have been initiated to facilitate our understanding of endomembrane trafficking.

Sortins Affect the Secretory System

A suite of compounds known as Sortins were identified as chemical probes to study vacuolar sorting in Arabidopsis (Zouhar et al., 2004). The primary screen for Sortins took advantage of the evolutionary conservation between plants and the budding yeast *Saccharomyces cerevisiae*. A library of 4,800 compounds was screened for those that induced the aberrant secretion of carboxypeptidase Y (CPY), which is normally targeted to the vacuole, in a yeast high throughput 96-well assay. Essentially, the primary screen queried the chemical library for compounds that would mimic the vacuole protein sorting (*vps*) phenotype (Bowers and Stevens, 2005). Of the 4800 diverse chemicals screened in yeast, 14 resulted in CPY secretion in the primary screen. In Arabidopsis seedlings, two of these chemicals (Sortin1 and Sortin2) displayed reversible effects on vacuole biogenesis, root development, and CPY secretion. It was also shown using electron microscopy that beyond the vacuole, other organelles such as the endoplasmic reticulum and Golgi appeared normal following Sortin1 treatment suggesting that this compound did not cause broad disruption of the endomembrane system as shown for other drugs

such as BFA (Zouhar et al., 2004). More recent studies using a range of GFP marker fusions support that conclusion that Sortin1 affects primarily the biogenesis of vacuoles (Hicks, Norambuena and Raikhel, unpublished data). Only two of the 14 Sortins were active in Arabidopsis indicating differences in target space, uptake, activation or metabolism of chemicals between plant and yeast. Nevertheless, the data also demonstrate that for some pathways, in particular those that are evolutionary conserved and cell autonomous, it is possible to take advantage of simpler single-celled eukaryotes such as yeast to study multi-cellular organisms such as Arabidopsis.

A recent study of Sortin2 highlights the value of combining a chemical screen with later genetic characterization to uncover gene networks. As a follow-up to the chemical screen described, a genetic screen for Sortin2 hypersensitivity was done using a yeast haploid deletion library to identify cellular pathways affected by Sortin2 (Norambuena et al., 2008). Interestingly, the yeast mutants hypersensitive to the drug were mainly components of the endomembrane system, for example some of the ESCRT complex components. Using bioinformatics, functional information about the cellular pathways affected by Sortin2 was obtained and indicated that specific proteins, so-called hubs, may be key regulators of endomembrane trafficking. Furthermore, the intracellular location displaying the greatest enrichment was the endosome suggesting that Sortin2 may be affecting primarily these compartments which functionally precede vacuole targeting or secretion. Thus, such approaches can yield testable hypotheses concerning the regulation of trafficking.

Connections Between Endomembrane Trafficking and Other Pathways

More recently, chemicals have been used to begin to understand relationships between pathways leading to sophisticated phenotypes reflective of complex biological processes. The connection between gravitropic responses and the endomembrane system is an excellent example. Mutations in endomembrane components such as the SNARE proteins VTI11 and SYP22 result in defects in shoot or root agravitropism demonstrating a genetic link between the endomembrane system and gravitropism (Schumacher et al., 1999; Kato et al., 2002; Morita et al., 2002; Yano et al., 2003; Silady et al., 2004). A screen of 10,000 diverse compounds for those that affect shoot gravitropism in Arabidopsis led to the identification of four compounds affecting vacuolar targeting and morphology and shoot gravitropism (Surpin et al., 2005). The semi-high-throughput primary screen focused on compounds that effected gravitropic bending. The positives from the primary screen were examined in a secondary screen utilizing an Arabidopsis line expressing the tonoplast reporter δ -TIP-GFP. This secondary level of screening by confocal microscopy identified compounds that also affected vacuolar morphology. The effect of these compounds on gravitropism and endomembrane system morphology is being characterized, and screens for resistant and hypersensitive mutants are underway. One of these screens resulted in the identification of Gravacin as a strong inhibitor of auxin signaling and shoot and root gravitropism; it also affects the delivery of the tonoplast marker δ -TIP-GFP to the tonoplast. Analysis of Gravacin analogs indicated that two independent targets were responsible

for its bioactivity. One target was involved in the inhibitory activity on gravitropism; whereas a second target was responsible for the mistargeting of δ -TIP-GFP from the tonoplast to an endoplasmic reticulum-like compartment. Using a genetic and biochemical approach, the P-glycoprotein (PGP19) (Rojas-Pierce et al., 2007) was identified as the target responsible for inhibition of gravitropism in *Arabidopsis thaliana*. PGPs are ATP-binding cassette transporters that translocate a myriad of molecules across biological membranes. Human P-gp has been implicated in the multi-drug resistance of tumor cells, and inhibitors of this protein are being analyzed for their potential in cancer therapy. In plants, PGP19 transports the plant hormone auxin and participates in physiological changes required for responses to gravitropic stimuli. The interaction of Gravacin and PGP19 results in the inhibition of PGP19 auxin transport activity (Rojas-Pierce et al., 2007). Recently, Gravacin hypersensitive mutants that display endoplasmic reticulum-like localization of δ -TIP-GFP as well as normal sensitivity in gravitropic assays have been isolated. Analyses of these mutants are underway (Rojas-Pierce and Raikhel, unpublished data).

Utilizing Pollen to Identify Affecters of Endocytic Pathways

Screens for mutants defective in embryo patterning have identified vesicular trafficking components that are required for cytokinesis and polar auxin transport (Steinmann et al., 1999; Assaad et al., 2001; Heese et al., 2001). This is another area in which chemicals could clearly help to dissect links between pathways, and it was being addressed recently with a high throughput pollen screen. The pollen tube is a single polarized cell whose tip growth is highly dependent upon vesicle trafficking, especially endocytosis and exocytosis (Zheng and Yang, 2000; Yang, 2002; Cole and Fowler, 2006). Furthermore, the endomembranes are relatively easy to view by confocal microscopy (see for example, (Hicks et al., 2004)). In addition, the organization of endocytic and exocytic vesicles are clearly visible near the growing tip using styryl dyes such as FM4-64 and FM1-43 (Zonia and Munnik, 2008). Genetic approaches for mutants defective in endocytosis using *Arabidopsis* pollen would be problematic in that endomembrane mutations commonly result in gametophyte lethality. However, the pollen system is extremely valuable when combined with chemical genomics because chemicals can act rapidly in a dose-dependent manner; plus the concentration and time-course of treatments are easily controlled.

A high throughput screen using a 384-well microtiter plate format was developed that is applicable to germinating pollen or cultured cells (Robert et al., 2008). For germinating pollen, the screen involves several tiers. In the primary screen, compounds are scored for inhibition of tobacco pollen germination which is dependent upon tip-focused vesicle targeting and recycling. Tobacco pollen was used because it germinates with high frequency *in vitro*. Among the possibilities, such inhibitors could affect exocytosis, endocytosis, protein targeting, interacting ROP GTPases, calcium signaling pathways, or cytoskeleton. More than 90% of diverse chemicals did not affect pollen germination; however, a small proportion were strong inhibitors. In a secondary screen, the inhibitors were applied to germinating pollen expressing a PM associated tip-focused marker, but other marker systems are possible. The tip marker utilized in this screen was ROP1-interacting partner 1 (RIP1 or ICR1 (Lavy et

Figure 2. Compounds that result defects in PM targeting and endocytosis. Tobacco pollen was germinated for 3 to 4 hours in the absence (A), or presence (B–D) of each of several different chemicals discovered utilizing a high-throughput automated screen. B. Pollen germinated in the presence of 6.6 μ M ES1 displays isodiametric growth (loss of growth polarity) and mis-localization of the pollen tip marker GFP-RIP1. C. The marker displays little or no tip localization when pollen was germinated in the presence of 129 μ M erysolin, a less potent natural isothiocyanate compound. D. Similar results were obtained in the presence of 127 μ M cantheridin, a PP2A phosphatase inhibitor. Pollen exine displays autofluorescence unrelated to expression of RIP1-GFP marker as indicated by asterisk (B, C, D). *Arabidopsis* root tips expressing PIN2-GFP, a marker that is known to cycle between the PM and endosomes. In the absence of ES1 (E) PIN2-GFP localization is strongly PM. However after 2 hours incubation in the presence of 33 μ M ES1 the compound results in intracellular agglomerations that we term endosidin bodies. These bodies coincide with markers for the TGN/early endosomes (see text). Scale bars as indicated.

al., 2007)) which is localized to and recruits the GTPase ROP1 to the pollen tube tip (Li and Yang, unpublished data). Germination inhibitors at a sub-inhibitory concentration were scored against the tip marker for mis-localization or other signs of polarity loss such as isodiametric growth (tip swelling). From an initial screen of 2016 chemicals, four resulted in mis-localization of GFP-RIP1 and tip swelling. One of the chemicals was a

known phosphatase inhibitor, whereas the other three were novel. Examples of three such compounds are shown (Figure 2 A-D) in which the tip marker is localized inappropriately.

One of the bioactive compounds termed endosidin1 (ES1) was examined using markers expressed in pollen from transgenic tobacco and Arabidopsis (Hicks, Chary and Raikhel, unpublished data). The fact that the PM marker ACA9-YFP (Schiott et al., 2004) and the secreted marker INV-GFP (Cheung et al., 2002) were not mis-localized suggests that ES1 is selective for RIP1-GFP in pollen and does not affect secretion. Chemicals identified in the screen that are known to inhibit pollen germination included cytochalasins, cyclohexamide, and several ionophores; however, none resulted in mis-localization of GFP-RIP1 in pollen. Interestingly, an acetate derivative of ES1 caused no phenotype providing data for examining structure activity relationships (SAR).

Beyond pollen, ES1 has been examined in detail in Arabidopsis roots, where markers for PM proteins known to recycle between the PM and endomembrane compartments are available (Robert et al., 2008). When seedlings are exposed to ES1 for 2 hrs to detect primary effects, the auxin transporter markers PIN2-GFP (Xu and Scheres, 2005) and AUX1-YFP (Swarup et al., 2004) and the brassinosteroid (BR) receptor marker BRI1-GFP (Geldner et al., 2007) formed intracellular agglomerations termed “endosidin bodies” (Figure 2 E, F). The markers PIN1-GFP (Benkova et al., 2003) and PIN7-GFP (Blakeslee et al., 2007) were unaffected indicating that the ES1 is selective. ES1 also disrupted a TGN/endosome compartment defined by the syntaxin SYP61 and the ATPase subunit VHA-a1. A range of cell biology experiments finally led to the conclusion that ES1 affects the endocytosis and sorting of PIN2, AUX1 and BRI1 by impacting a pool of early endosomes defined by SYP61. Endosomes are thought to be a site of intracellular signaling, and there evidence that an endosome pool of the BRI1 receptor is essential for BR signaling (Geldner et al., 2007). To determine if endosidin bodies incorporating BRI1 impacted BR signaling, the output of a BL-specific transcription factor (Nemhauser et al., 2006) was examined using quantitative PCR. The results indicated that ES1 antagonized BR-signaling. Moreover, when grown in the presence of ES1 in the dark seedlings displayed a light-grown phenotype (short hypocotyls, open cotyledons) similar to that of the brassinosteroid receptor mutant *bri1-116* (Li and Chory, 1997). ES1 is a powerful example of how a bioactive chemical can be used to study a highly dynamic process that has not been readily accessible by classical genetics. The possession of a suite of such compounds affecting endosome sorting pathways could lead to the eventual dissection of these processes.

STRUCTURAL ANALYSIS OF BIOACTIVE CHEMICALS

Biologists who utilize chemical genomics quickly realize that there is a wealth of information to be gained by interrogating the structures of bioactive compounds. Such information can provide important details such as:

- one or more regions of the molecule that are important for activity
- specific chemical moieties such as carboxyls, sulfhydryls, hydroxyls, or halogens that may contribute to activity in terms of charge or steric features
- hydrophobicity and other properties that influence cellular uptake and transport

By investigating structural analogs and substructures, such details can be further defined. Ultimately such information can potentially inform a researcher about the nature of the binding site or even the target. At a minimum, the possession of active and inactive structural analogs and substructures can greatly facilitate genetic screens or biochemical approaches to identify cognate targets. One of the wonderful things about this approach is that it is interdisciplinary; in this case biologists can benefit greatly from collaborations with chemists and informaticians. What follows are several examples of such structural analyses. For structures and references of selected compounds identified via chemical screens, see Table 2.

Growth Regulator Analogs

Of course, there are many synthetic auxins available. However, novel auxin-related compounds obtained through chemical screens represent a useful tool to elucidate additional components or provide insights into the role of auxin. Several small molecules affecting auxin-related processes during plant growth and development have been either synthesized or purified from microbial sources (Hayashi et al., 2001; Hayashi, 2003; Dai et al., 2005; Yamazoe et al., 2005). Additional inhibitory compounds have been identified by large-scale screens of combinatorial libraries (Armstrong et al., 2004; Surpin et al., 2005). Defining the active core moieties of a bioactive compound can assist in describing its mode of action (MOA) as well as identifying cellular components that are targeted. For example, Sungur and co-workers synthesized various auxinic analogs of compound A, a furyl acrylate ester of a thiadiazole heterocycle (Sungur et al., 2007). The activity of these compounds was initially assessed for their ability to inhibit the expression of the BA3-GUS reporter gene and indicated that 2-furylacrylic acid (2-FAA) is the active portion of the molecule. Both root and hypocotyl elongation are inhibited by 2-FAA treatment in the micromolar range. This also results in the inhibition of the auxin-inducible expression of IAA5. Thus, 2-FAA antagonizes auxin responses. However, 2-FAA fails to alter the interaction of IAA7/AXR2 with the SCF^{TR} complex which is involved in the rapid ubiquitin-dependent proteolysis of negative regulators of auxin-induced transcription. Based on the structure-function analysis, the authors thus concluded that 2-FAA is liberated by hydrolysis of an ester linkage. The identification of 2-FAA as the bioactive region of compound A provides an avenue for biochemistry and genetics aimed at identifying its cellular target. As previously described, auxin analogs have also proven powerful for examining ubiquitin ligase interactions (Hayashi et al., 2008).

In their recent work, Gendron and co-workers (Gendron, 2008) examined the SAR of the brassinosteroid biosynthesis inhibitor BRP. The compound also affected ethylene responses. Of 12 analogs investigated, one compound, a6, appeared to be more specific for the ethylene pathway and less active at inhibition of brassinosteroid biosynthesis than BRP indicating that the chemical could partially uncouple the effects of the two growth regulators. Such specificity of chemicals points to the potential to investigate the intersection of developmental pathways influenced by multiple growth regulators.

Substructures to Study a Kinase Target

In most cases, the cognate target of a compound identified in a chemical screen would be unknown. However, this is dependent upon the nature of the screen. It is conceivable that a researcher is interested in identifying a novel effector of a known target. As an example of how chemicals can be used *in vivo* to study a known target, Bohmer and co-workers combined chemical analogues of ATP with an ATP-analogue sensitive (as) kinase variant and molecular fingerprinting techniques to study members of the plant calcium-dependent protein kinase (CDPK) family *in vivo* (Bohmer and Romeis, 2007). Plant CDPKs carrying the as-mutation did not show altered phosphorylation kinetics utilizing ATP as a substrate, but were able to use ATP analogues as phosphate donors or as kinase inhibitors. The authors established the use of analogues as a tool for the investigation of plant-specific protein kinases *in planta*. This facilitated the identification of rapid changes of molecular biomarkers in kinase-mediated signaling networks.

Sortin Analogues Can Define the Properties of the Target Site

Recent efforts looking at the SAR of Sortin2 demonstrates what can be learned from analogues and substructures of compounds with as yet unknown targets (Norambuena et al., 2008). In order to understand the structural determinants of Sortin2 necessary for its activity in disrupting vacuole targeting and morphology, a panel of analogues and substructures were examined. The results showed that a sulfite substitution and a benzoic acid group play key roles in Sortin2 activity. The analysis also showed that Sortin2 interaction with its target would probably require opposite ends of the molecule suggesting a binding pocket that recognizes one face of Sortin2. Although the possibility of multiple binding pockets or targets cannot be ruled out, these types of analyses can provide clues about the nature of such sites.

The case of Sortin1 provides another example of informative chemical substructures. Like Sortin2, Sortin1 disrupts vacuole biogenesis and trafficking of vacuolar proteins. To understand the molecular site of Sortin1 bioactivity, Arabidopsis lines expressing endomembrane-specific markers were examined, and it was found that micromolar Sortin1 was highly specific in disrupting vacuole biogenesis but did not affect other endomembrane compartments. Analogs and substructures were found using the ChemMine database (Girke et al., 2005) and obtained from commercial sources. In fact, a more advanced version of this database will be available soon (Cao and Girke, unpublished). These substructures revealed important SAR information. For example, the furan and carboxyl moieties were key features of a bioactive substructure representing roughly half of Sortin1 (Chemical 259, Table 2). Surprisingly, a substructure representing approximately the other half of Sortin1 (Chemical 275, Table 2) was bioactive indicating the possibility of two binding sites. To define the number of interaction sites, mutants were identified that were hypersensitive for root growth, a Sortin1 phenotype (Hicks, Norambuena and Raikhel, unpublished). The seven mutants examined were up to 50-fold hypersensitive to Sortin1 for defective vacuole morphology. The vacuole phenotype of mutants on distinct Sortin1 substructures indicated that four mutants were hypersensitive for the furan-containing half-molecule (259), whereas one was hy-

persensitive for the non-furan substructure (275). One mutant was hypersensitive to Sortin1. The results indicate that there is a primary site of Sortin1 binding effecting vacuole biogenesis and trafficking. However, a genetically distinct second site indicates that the target of Sortin1 may possess a bipartite binding site recognizing the two regions of Sortin1 as observed for some known drug targets. For this compound it will be extremely interesting to identify the cognate target.

METABOLISM OF COMPOUNDS *IN VIVO*

Understanding the complexities of xenobiotic metabolism in plants and animals is challenging. In animals there are three phases of metabolism that have been described (reviewed in (Sandermann, 1992)) for the liver. In Phase I xenobiotics are oxidized, reduced or hydrolyzed. In Phase II, reactive groups can be conjugated to glutathione or other substituents followed by excretion of the resulting hydrophilic conjugates. Plants also display multiple steps in metabolism. These include transformation chemically via enzymes such as P450 monooxygenases and conjugation via relatively well-characterized enzymes such as glutathione transferases. In plants, O-glucosyl and O-malonyl transferases have been described to be involved in xenobiotic metabolism (Sandermann, 1992). More recently, genome sequencing has revealed the presence in Arabidopsis of large gene families (120 members) of glycosyltransferases. This has greatly complicated efforts to understand their biological roles which clearly go beyond xenobiotic metabolism (Gachon et al., 2005). As well, flavin-containing monooxygenases have been recently examined functionally in plants. Not only are they involved in hydroxylation of xenobiotics rendering them more hydrophilic, but these monooxygenases are involved in other processes such as auxin biosynthesis and metabolism of glucosinolates (Schlaich, 2007). Again, they are found to be members of a large gene family in Arabidopsis. Classically, chemically modified xenobiotics are ultimately compartmentalized into the vacuole. Alternatively, they may be secreted into the apoplast or immobilized in the cell wall.

With the identification of any novel bioactive chemical resulting from a screen, metabolism is a general concern. It is not atypical for compounds to be chemically transformed due to inherent reactivity of moieties such as sulfhydryls, carboxyls, esters, thiophenes, furans, or azide groups to name a few or the activity of enzymes. For most chemical screens, compounds that are degraded to inactive products, compartmentalized or immobilized will not be detected. Thus, for the typical chemical screen in plants, our primary concern would be cleavage or other degradation to bioactive products or catabolism which results in chemical activation. In a recent study, Cutler and coworkers (Zhao et al., 2007) used the natural variation of Arabidopsis to identify molecules that induce accession-selective phenotypes or polymorphic hits. The authors screened for growth inhibitors of etiolated hypocotyls because of the ease of scoring and their interest in growth regulators. They focused their effort on a growth inhibitor known as hypostatin which appears to be plant specific. Interestingly, the Columbia and Landsberg erecta ecotypes display polymorphism in terms of hypostatin sensitivity. By mapping the gene responsible for the resistance in Columbia background, the authors found that hypostatin requires glycosylation in order to be active. This is likely

the first example in plants of chemical glycoactivation to bioactivity. Perhaps as important, the study shows that Arabidopsis accessions display natural variation in sensitivity to small molecules and that this variation can be exploited to provide insights into the activity of new compounds.

IDENTIFICATION OF TARGET PATHWAYS

As we have discussed, bioactive chemicals are valuable, themselves, as reagents for the direct examination of biological pathways. In some cases, they may be the best tool for generating hypotheses about processes such as endosome sorting. However, potent compounds associated with easily-scored phenotypes also provide a route to determine the pathway or cognate target of a chemical. Herbicide chemists and physiologists have been involved in cognate target identification for decades before chemical genomics emerged, and their experiences can provide valuable lessons. Herbicides and other compounds that intervene in primary metabolism can be of special interest for chemical genomic studies because they modulate plant biosynthetic pathways and their downstream events. Many herbicides have potent and specific action within these pathways and are instructive chemical probes of plant biosynthetic processes. Much has been learned about the individual target enzymes, the pathways, and their role in plant metabolism via detailed understanding of the effects of herbicides. Targets of commercial herbicides include enzymes in branched chain and aromatic amino acid, fatty acid, cellulose, and plastoquinone biosynthesis (Wakabayashi and Boger, 2002). The available suite of chemical probes includes experimental herbicides that for a variety of reasons did not achieve commercialization. Nevertheless, from the plant biologists viewpoint, such compounds serve as informative inhibitors of pathways.

Isoxaben Target

Cell wall components are a really valuable target for herbicides because the cell wall is essential for plant survival. Isoxaben is one of the major herbicides that are known to inhibit cellulose biosynthesis. In recent years, two isoxaben-resistance loci (IXR1 and IXR2) have been described in Arabidopsis. IXR1 and IXR2 were cloned and shown to encode the cellulose synthases CESA3 and CESA6, respectively (Scheible et al., 2001; Desprez et al., 2002). The recent identification of the isoxaben target CESA6 provides further knowledge of the complex machinery of cellulose synthesis which includes the 10 cellulose synthase isoforms in Arabidopsis. The results of Desprez and co-authors (Desprez et al., 2002) are consistent with the idea that multiple copies of CESA6 and/or CESA3 constitute a rosette complex that functions in the assembly of cellulose microfibrils. The rosette architecture would aid assembly by coordinating the different catalytic subunits (Scheible et al., 2001; Desprez et al., 2002). There is indirect evidence for complex formation between CESA3 and CESA6 and partial redundancy between CESA6 and other CESA isoforms, possibly CESA2, CESA5, or both. With the targets characterized, isoxaben has become an excellent tool for the study of cellulose synthesis in Arabidopsis, and also in other plant species (Desprez et al., 2002). This same concept would apply to novel

compounds from chemical genomics screens; once the target is known, a reagent is in hand to examine target function *in planta*. It has been shown recently that there is an effect of isoxaben on other proteins involved in cellulose synthesis in terms of their localization and function (Robert et al., 2005; Paredes et al., 2006). Thus, the herbicide may now help to elucidate how CESA function relates to the regulation of other components in the cellulose synthetic pathway.

DAS734 Target

A novel phenyltriazole acetic acid compound (DAS734) has been shown to be a potent inhibitor of Arabidopsis growth and produces a bleaching of new growth on a variety of dicotyledon weeds (Walsh et al., 2007). Such a bleaching phenotype is typically associated with herbicides that affect purine biosynthesis. In fact, the effects could be reversed by purine addition. A genetic screen was developed in order to identify the cognate target of DAS734. Walsh and co-workers (Walsh et al., 2007) isolated seven lines of allelic mutants displaying strong resistance to DAS734. Multiple mutations responsible for the resistance were mapped positionally and found to be in a gene encoding an isoform of glutamine phosphoribosylamidotransferase (AtGPRAT2). AtGPRAT2 is a key enzyme in purine biosynthesis, but was not a known herbicide target. The study included the expression of the gene in *E. coli* and the demonstration of direct herbicide binding and inhibition of activity *in vitro*. Furthermore, the mutation sites were mapped structurally to equivalent sites in the *B. subtilis* GPRAT. This combination of chemical, genetic, and biochemical evidence indicates that the phytotoxicity of DAS734 arises from direct inhibition of GPRAT and establishes its utility as a new and specific chemical genetic probe of plant purine biosynthesis.

DAS534 Target

Although a wide range of structurally diverse small molecules can act as auxins, it is not clear whether all these compounds act via the same mechanisms. Walsh and co-workers also produced an interesting study testing a novel class of picolinate auxins, which includes picloram, for selective resistance on Arabidopsis mutants (Walsh et al., 2006). They identified seven alleles at two distinct genetic loci that confer significant resistance to DAS534, but not 2,4D or IAA. These loci were mapped and found to be components involved in auxin signaling. One of the loci has been shown to be a new component in auxin signaling, an auxin-signaling F-box protein 5 (ABF5) which is one of five homologs of TIR1 in Arabidopsis. TIR1 is part of the recognition complex involved in the ubiquitin-proteasome pathway controlled by auxin and has been shown to be a receptor for 2,4D and IAA. The second allele was the tetratricopeptide protein SGT1b that is also associated with the ubiquitin-proteasome during auxin signaling. The interesting point of this study is that due to the specificity of the target for DAS534, the identification of a new member of the TIR1 family of receptors was identified functionally. Screening for 2,4D or IAA resistance may not have identified ABF5. This demonstrates the utility of genetic screens using structurally diverse chemistries to uncover novel pathway components.

Gravacin Target

As mentioned above, Gravacin was isolated as a strong inhibitor of root and shoot gravitropism, auxin responsiveness, and protein trafficking to the tonoplast (Surpin et al., 2005). Rojas-Pierce and co-workers (Rojas-Pierce et al., 2007) demonstrated elegantly that PGP19, a key efflux transporter of the phytohormone auxin, was one of the targets of Gravacin (Rojas-Pierce et al., 2007). It was shown that a mutation in PGP19 confers resistance to the effect of Gravacin. The mutation also resulted in reduced binding of Gravacin to Hela cell microsomal fractions expressing the cloned mutant gene compared to wild type. Furthermore, the direct binding of Gravacin to microsomal fractions was monitored. This constitutes strong evidence that PGP19 is one of the cognate targets of the chemical. One of the main interests was the specificity of the compound and the fact that it could be used as a valuable tool in auxin transport studies. Indeed, this study showed in a very convincing way that Gravacin is a highly specific tool for dissecting auxin transport mechanisms because unlike NPA it does not appear to affect PIN auxin efflux transporters.

Interestingly however, there is a second phenotype associated with Gravacin which is the mislocalization of δ -TIP-GFP from the tonoplast to the endoplasmic reticulum. This cellular phenotype is not rescued by the mutation indicating that there is a second target associated with this endomembrane defect. This target is still unknown but is under investigation (Rojas-Pierce and Raikhel, unpublished).

Sirtinol Target

The non-natural small molecule sirtinol was utilized to try and identify components in auxin signaling (Zhao et al., 2003). The authors carried out forward chemical screens for compounds that altered the expression pattern or levels of an auxin reporter line, DR5-GUS (Sabatini et al., 1999). They also scored for suppression/enhancement of phenotypes associated with the auxin overproduction mutant, *yucca* (Zhao et al., 2001). Sirtinol was originally identified as an inhibitor of the Sirtuin family of NAD-dependent deacetylases in *Saccharomyces cerevisiae* and later found to affect root and vascular tissue development in Arabidopsis suggesting that sirtinol affected an auxin-related process (Grozinger et al., 2001). When initially cloned, SIR1 (which possessed a ubiquitin-activating enzyme E1 domain as well as a Rhodanese-like domain) was hypothesized to be an upstream auxin signaling component (Zhao et al., 2003). But subsequent work using a combination of sirtinol substructures and genetics demonstrated that SIR1 and other SIR genes identified genetically were part of a pathway that metabolized sirtinol to an active auxin, 2-hydroxy-1-naphthoic acid (Dai et al., 2005). Thus, the genetic screen in this case yielded a pathway for the bioactivation of sirtinol itself.

The Function and Mechanism of Action of BR Using Biosynthesis Inhibitors

Exploring small molecules that induce a BR deficient-like phenotype in Arabidopsis led to the identification of brassinazole as the first candidate for a BR biosynthesis inhibitor (Asami and Yoshida,

1999; Asami et al., 2000; Nagata et al., 2000; Asami et al., 2003). Investigation of target site(s) of brassinazole revealed that the compound binds directly to the DWF4 protein, a cytochrome P450 monooxygenase that catalyzes 22-hydroxylation of the side chain of BR. Thus, genetic screens against BR biosynthesis inhibitors can identify new components in BR signal transduction. These studies demonstrate that the development of chemicals that induce defined phenotypes are useful for the study of biological systems in plants (Asami et al., 2003).

Targets in Plant Defense Responses

Recently, the use of submerged cultures of Arabidopsis seedlings was shown to permit the intervention of rapid-elicitor-mediated immune responses using chemicals (Serrano et al., 2007). A modest screen of a 120 small molecules led to the isolation of four compounds that reduced cellulysin or flg22-activated gene expression of ATL2, an early pathogen-associated molecular patterns (PAMP)-responsive gene. The activities of four compounds were tested for their capacity to interfere with the production of elicitor-induced reactive oxygen species and the endocytosis of the FLS2 activated PM immune receptor. Enoyl-ACP reductase (a subunit of the FASII complex) was validated as one of the targets by the use of structure variants and enoyl-ACP reductase activity assays. The results indicate a possible role for signaling lipids in PAMP-triggered immune responses and provide the basis for more in-depth screening of natural compound libraries.

Another example of identification of new loci in specific pathways is the discovery of three phenotypic groups of mutants able to resist Bestatin, a potent inhibitor of some aminopeptidases involved in jasmonate wound response (Zheng et al., 2006).

FUTURE OF CHEMICAL GENOMICS

Chemical genomics is a powerful approach. Our efficiency in obtaining chemical libraries and screening for phenotypes of interest has increased greatly as we have learned to devise effective plant-based approaches. However, our ability to obtain targets rapidly is a rate limiting step. Critical to the approach is the ability to screen for mutants that are hypersensitive or resistant to bioactive chemicals and to identify the corresponding genes using genomic-based methods (Scheible et al., 2001; Desprez et al., 2002; Zhao et al., 2003; Walsh et al., 2006; Rojas-Pierce et al., 2007). Reverse-genetics is easily accessible with Arabidopsis because of the large mutant database available worldwide. Mutations conferring resistance or hypersensitivity can affect either the target of the drug, or at least part of a pathway. Importantly, mutations in a component upstream or downstream of the cognate target protein will not only give insights into the function of target genes but should also identify new genes in the corresponding network. In other words, chemical genomics will enhance the power of existing genomic tools.

Beyond the use of genetics, other approaches are being examined for rapid target identification. One of the promising approaches is to use specialized libraries that incorporate reactive groups that permit the simple immobilization of bioactive compounds for affinity purification of targets. These are also known as "tagged libraries". One difficulty with immobilization of active com-

pounds to an affinity matrix is preventing a loss of activity. Having used substructures and analogues to generate some SAR data is helpful in this regard as discussed. To facilitate this process, Chang et al have developed a tagged triazine library containing a linker that provides a straightforward transition from phenotypic screening to target identification (Khersonsky et al., 2003; Williams et al., 2004; Jung et al., 2005; Ni-Komatsu et al., 2005; Kim et al., 2006; Ahn and Chang, 2007; Min et al., 2007). In essence, bioactive compounds from the library will already possess a tag for immobilization. A triazine scaffold was highly successful for the introduction of the intrinsic tag with the diversification of library members. The tagged library was synthesized through combinatorial methods and screened in the presence of the tag in phenotypic assays.

This tagged triazine library has been applied to a series of model systems, which has resulted in the identification of several small molecule regulators. The selected compounds were easily utilized for the isolation of their target proteins. Although such an approach will not work in every instance due to differences in target binding affinity and abundance, examples of successful target identification are highly encouraging. Thus, a tagged library and related approaches will probably be part of the chemical biologists toolbox for target identification.

New chemical libraries are being marketed by commercial sources. In addition, as collaborations between biologists and chemists increase the production of more customized chemical libraries should become more widespread. In addition, new innovations are being reported. For example, a recent approach identified immunostimulatory R-galactosylceramides using synthetic combinatorial libraries (Li et al., 2007). Two 60+-membered libraries of R-galactosylceramides were prepared by reactions between activated ester resins and two core, fully deprotected galactosylated sphingoid bases. Because no product purification was required, this approach is particularly attractive as a method for rapid synthesis of large libraries of potential immunomodulatory glycosylceramides.

Chemical genomics databases for sharing this community resource are a critical aspect of the approach. Simply reporting the nature of chemical screens and the details of the results may save effort and duplication. In some instances, a group may screen for a phenotype of interest while bypassing other phenotypes. But having a collection of images of primary hits from that screen posted via web resources would permit others to view the data and obtain materials of interest collaboratively. A web-based system such as ChemMine (<http://bioweb.ucr.edu/ChemMineV2>) should provide the plant community with one such valuable resource. Related to this is the inherent assumption that compounds sharing structural similarities to existing ligands will probably display the same or related biological activities. It is therefore important to annotate chemical libraries with biological information. The ChemMine portal allows users to query compound collections by chemical property, structure, substructure, and activity. An integrated drug-informatics workbench provides access to online analysis tools that facilitate basic QSAR analyses of hits using approaches like structure-based clustering (hierarchical, binning) and predictions of chemical properties and drug-likeness. It also allows the release of a public screening and phenotype database that efficiently manages and disseminates the screening data from different projects. It allows scientists to

upload their bioactivity data to ChemMine and share their results with the community. The development of the screening component of the database involved the implementation of several enabling resources that include: (1) a screening annotation and scoring scheme for complex forward genetics and microscope screens; (2) image upload, annotation and viewing capabilities; (3) fast full-text search utilities and (4) support for gene, plant structure, chemical and other ontologies.

Chemical genomics is just beginning to attract the attention of plant biologists. It has taken time for founder laboratories to begin to publish their data. And as more meaningful biology is derived from the approach it will gain in popularity. Like other advances, one approach will not answer all questions. However the approach is a powerful addition to the genomics we have at our disposal in Arabidopsis. As a final thought, one especially critical aspect of bioactive chemicals is that they can be translated to non-model crop species easily, which can also have practical benefits to agriculture.

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REFERENCES

- Ahn, Y.H., and Chang, Y.T. (2007). Tagged small molecule library approach for facilitated chemical genetics. *Acc. Chem. Res.* **40**: 1025-1033.
- Alaimo, P.J., Shogren-Knaak, M.A., and Shokat, K.M. (2001). Chemical genetic approaches for the elucidation of signaling pathways. *Curr. Opin. Chem. Biol.* **5**: 360-367.
- 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. USA* **101**: 14978-14983.
- Asami, T., and Yoshida, S. (1999). Brassinosteroid biosynthesis inhibitors. *Trends Plant Sci.* **4**: 348-353.
- Asami, T., Nakano, T., Nakashita, H., Sekimata, K., Shimada, Y., and Yoshida, S. (2003). The Influence of Chemical Genetics on Plant Science: Shedding Light on Functions and Mechanism of Action of Brassinosteroids Using Biosynthesis Inhibitors. *J. Plant Growth Regul.* **22**: 336-349.
- Asami, T., Min, Y.K., Nagata, N., Yamagishi, K., Takatsuto, S., Fujioka, S., Murofushi, N., Yamaguchi, I., and Yoshida, S. (2000). Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. *Plant Physiol.* **123**: 93-100.
- Assaad, F.F., Huet, Y., Mayer, U., and Jurgens, G. (2001). The cytokinesis gene KEULE encodes a Sec1 protein that binds the syntaxin KNOLLE. *J. Cell. Biol.* **152**: 531-543.
- Avato, P. (1987). Chemical genetics of epicuticular wax formation in maize. *Plant Physiol. Biochem.* **25**: 179-180.
- Bassel, G.W., Fung, P., Chow, T.F., Foong, J.A., Provart, N.J., and Cutler, S.R. (2008). Elucidating the germination transcriptional program using small molecules. *Plant Physiol.* **147**: 143-155.

- Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G., and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**: 591-602.
- Blackwell, H.E., and Zhao, Y. (2003). Chemical genetic approaches to plant biology. *Plant Physiol.* **133**: 448-455.
- Blakeslee, J.J., Bandyopadhyay, A., Lee, O.R., Mravec, J., Titapiwatanakun, B., Sauer, M., Makam, S.N., Cheng, Y., Bouchard, R., Adamec, J., Geisler, M., Nagashima, A., Sakai, T., Martinoia, E., Friml, J., Peer, W.A., and Murphy, A.S. (2007). Interactions among PIN-FORMED and P-Glycoprotein Auxin Transporters in Arabidopsis. *Plant Cell* **19**: 131-147.
- Bohmer, M., and Romeis, T. (2007). A chemical-genetic approach to elucidate protein kinase function in planta. *Plant Mol. Biol.* **65**: 817-827.
- Bowers, K., and Stevens, T.H. (2005). Protein transport from the late Golgi to the vacuole in the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta* **1744**: 438-454.
- Cheung, A.Y., Chen, C.Y., Glaven, R.H., de Graaf, B.H., Vidali, L., Hepler, P.K., and Wu, H.M. (2002). Rab2 GTPase regulates vesicle trafficking between the endoplasmic reticulum and the Golgi bodies and is important to pollen tube growth. *Plant Cell* **14**: 945-962.
- Cole, R.A., and Fowler, J.E. (2006). Polarized growth: maintaining focus on the tip. *Curr. Opin. Plant Biol.* **9**: 579-588.
- 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. USA* **102**: 3129-3134.
- DeBolt, S., Gutierrez, R., Ehrhardt, D.W., Melo, C.V., Ross, L., Cutler, S.R., Somerville, C., and Bonetta, D. (2007). Morlin, an inhibitor of cortical microtubule dynamics and cellulose synthase movement. *Proc. Natl. Acad. Sci. USA* **104**: 5854-5859.
- Delmer, D.P. (1999). CELLULOSE BIOSYNTHESIS: Exciting Times for A Difficult Field of Study. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**: 245-276.
- Desprez, T., Vernhettes, S., Fagard, M., Refregier, G., Desnos, T., Aletti, E., Py, N., Pelletier, S., and Hofte, H. (2002). Resistance against herbicide isoxaben and cellulose deficiency caused by distinct mutations in same cellulose synthase isoform CESA6. *Plant Physiol.* **128**: 482-490.
- Dinter, A., and Berger, E.G. (1998). Golgi-disturbing agents. *Histochem Cell Biol.* **109**: 571-590.
- Dobson, C.M. (2004). Chemical space and biology. *Nature* **432**: 824-828.
- Eggert, U.S., Ruiz, N., Falcone, B.V., Branstrom, A.A., Goldman, R.C., Silhavy, T.J., and Kahne, D. (2001). Genetic basis for activity differences between vancomycin and glycolipid derivatives of vancomycin. *Science* **294**: 361-364.
- Friml, J., Wisniewska, J., Benkova, E., Mendgen, K., and Palme, K. (2002). Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. *Nature* **415**: 806-809.
- Gachon, C.M., Langlois-Meurinne, M., and Saindrenan, P. (2005). Plant secondary metabolism glycosyltransferases: the emerging functional analysis. *Trends Plant Sci.* **10**: 542-549.
- Geldner, N., Friml, J., Stierhof, Y.D., Jurgens, G., and Palme, K. (2001). Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413**: 425-428.
- Geldner, N., Hyman, D.L., Wang, X., Schumacher, K., and Chory, J. (2007). Endosomal signaling of plant steroid receptor kinase BRI1. *Genes Dev.* **21**: 1598-1602.
- Gendron, J.M., Haque, A., Gedron, N., Chang, T., Asami, T., and Wang, Z.-Y. (2008). Chemical genetic dissection of brassinosteroid-ethylene interaction. *Mol. Plant* **1**: 368-379.
- Girke, T., Cheng, L.C., and Raikhel, N. (2005). ChemMine. A compound mining database for chemical genomics. *Plant Physiol.* **138**: 573-577.
- Grozier, 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 sir-tuin family of NAD-dependent deacetylases by phenotypic screening. *J. Biol. Chem.* **276**: 38837-38843.
- Hayashi, K., Ogino, K., Oono, Y., Uchimiya, H., and Nozaki, H. (2001). Yokonolide A, a new inhibitor of auxin signal transduction, from *Streptomyces diastatochromogenes* B59. *J. Antibiot. (Tokyo)* **54**: 573-581.
- 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. USA* **105**: 5632-5637.
- Hayashi, K., Jones A.M., Ogino, K., Yamazoe, A., Oono, Y., Inoguchi, M., Kondo, H., mozaki, H. (2003). Yokonolide B, a novel inhibitor of auxin action, blocks degradation of AUX/IAA factors. *J. Biol. Chem.* **278**: 23797-23806.
- Heese, M., Gansel, X., Sticher, L., Wick, P., Grebe, M., Granier, F., and Jurgens, G. (2001). Functional characterization of the KNOLLE-interacting t-SNARE AtSNAP33 and its role in plant cytokinesis. *J. Cell Biol.* **155**: 239-249. Epub 2001 Oct 2008.
- Heim, D.R., Roberts, J.L., Pike, P.D., and Larrinua, I.M. (1989). Mutation of a Locus of *Arabidopsis thaliana* Confers Resistance to the Herbicide Isoxaben. *Plant Physiol.* **90**: 146-150.
- Heim, D.R., Roberts, J.L., Pike, P.D., and Larrinua, I.M. (1990). A Second Locus, *lrx B1* in *Arabidopsis thaliana*, that Confers Resistance to the Herbicide Isoxaben. *Plant Physiol.* **92**: 858-861.
- Hicks, G.R., Rojo, E., Hong, S., Carter, D.G., and Raikhel, N.V. (2004). Germinating pollen has tubular vacuoles, displays highly dynamic vacuole biogenesis, and requires VACUOLESS1 for proper function. *Plant Physiol.* **134**: 1227-1239.
- Jung, D.W., Williams, D., Khersonsky, S.M., Kang, T.W., Heidary, N., Chang, Y.T., and Orlow, S.J. (2005). Identification of the F1F0 mitochondrial ATPase as a target for modulating skin pigmentation by screening a tagged triazine library in zebrafish. *Mol. Biosyst.* **1**: 85-92.
- Kato, T., Morita, M.T., Fukaki, H., Yamauchi, Y., Uehara, M., Niihama, M., and Tasaka, M. (2002). SGR2, a phospholipase-like protein, and ZIG/SGR4, a SNARE, are involved in the shoot gravitropism of Arabidopsis. *Plant Cell* **14**: 33-46.
- Khersonsky, S.M., Jung, D.W., Kang, T.W., Walsh, D.P., Moon, H.S., Jo, H., Jacobson, E.M., Shetty, V., Neubert, T.A., and Chang, Y.T. (2003). Facilitated forward chemical genetics using a tagged triazine library and zebrafish embryo screening. *J. Am. Chem. Soc.* **125**: 11804-11805.
- Kim, W., Kim, Y., Min, J., Kim, D.J., Chang, Y.T., and Hecht, M.H. (2006). A high-throughput screen for compounds that inhibit aggregation of the Alzheimer's peptide. *ACS Chem. Biol.* **1**: 461-469.
- Kimura, S., Laosinchai, W., Itoh, T., Cui, X., Linder, C.R., and Brown, R.M., Jr. (1999). Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angularis*. *Plant Cell* **11**: 2075-2086.
- Kirst, H.A., Larsen, S.H., Paschal, J.W., Oocolowitz, J.L., Creemer, L.C., Steiner, J.L., Lobkovsky, E., and Clardy, J. (1995). Structure of the new spiroketal-macrolide A82548A. *J. Antibiot. (Tokyo)* **48**: 990-996.
- Kuruvilla, F.G., Shamji, A.F., Sternson, S.M., Hergenrother, P.J., and Schreiber, S.L. (2002). Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. *Nature* **416**: 653-657.
- Lavy, M., Bloch, D., Hazak, O., Gutman, I., Poraty, L., Sorek, N., Sternberg, H., and Yalovsky, S. (2007). A Novel ROP/RAC effector links cell polarity, root-meristem maintenance, and vesicle trafficking. *Curr. Biol.* **17**: 947-952.
- Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* **90**: 929-938.
- Li, Q., Ndonye, R.M., Illarionov, P.A., Yu, K.O., Jerud, E.S., Diaz, K., Bricard, G., Porcelli, S.A., Besra, G.S., Chang, Y.T., and Howell, A.R. (2007). Rapid identification of immunostimulatory alpha-galactosylceramides using synthetic combinatorial libraries. *J. Comb. Chem.* **9**: 1084-1093.

- Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J.** (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Discovery Rev.* **23**: 3-25.
- Lokey, R.S.** (2003). Forward chemical genetics: progress and obstacles on the path to a new pharmacopoeia. *Curr. Opin. Chem. Biol.* **7**: 91-96.
- Macey, M.J.K., Barber, H.N.** (1970). Chemical genetics of wax formation on leaves of *Pisum sativum*. *Phytochemistry* **9**: 5-12.
- Min, J., Kyung Kim, Y., Cipriani, P.G., Kang, M., Khersonsky, S.M., Walsh, D.P., Lee, J.Y., Niessen, S., Yates, J.R., 3rd, Gunsalus, K., Piano, F., and Chang, Y.T.** (2007). Forward chemical genetic approach identifies new role for GAPDH in insulin signaling. *Nat. Chem. Biol.* **3**: 55-59.
- Mitchison, T.J.** (1994). Towards a pharmacological genetics. *Chem. Biol.* **1**: 3-6.
- Morita, M.T., Kato, T., Nagafusa, K., Saito, C., Ueda, T., Nakano, A., and Tasaka, M.** (2002). Involvement of the vacuoles of the endodermis in the early process of shoot gravitropism in *Arabidopsis*. *Plant Cell* **14**: 47-56.
- 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.
- Nebenfuhr, A., Ritzenthaler, C., and Robinson, D.G.** (2002). Brefeldin A: deciphering an enigmatic inhibitor of secretion. *Plant Physiol.* **130**: 1102-1108.
- Nemhauser, J.L., Hong, F., and Chory, J.** (2006). Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell* **126**: 467-475.
- Ni-Komatsu, L., Leung, J.K., Williams, D., Min, J., Khersonsky, S.M., Chang, Y.T., and Orlow, S.J.** (2005). Triazine-based tyrosinase inhibitors identified by chemical genetic screening. *Pigment Cell Res.* **18**: 447-453.
- Norambuena, L., Zouhar, J., Hicks, G.R., and Raikhel, N.V.** (2008). Identification of cellular pathways affected by Sortin2, a synthetic compound that affects protein targeting to the vacuole in *Saccharomyces cerevisiae*. *BMC Chem. Biol.* **8**: 1.
- Olah, M.M., Bologa, C.G., and Oprea, T.I.** (2004). Strategies for compound selection. *Curr. Drug Discov. Technol.* **1**: 211-220.
- Oliver, S.F., and Abell, C.** (1999). Combinatorial synthetic design. *Curr. Opin. Chem. Biol.* **3**: 299-306.
- Paredes, A.R., Somerville, C.R., and Ehrhardt, D.W.** (2006). Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312**: 1491-1495.
- Peng, L., Xiang, F., Roberts, E., Kawagoe, Y., Greve, L.C., Kreuz, K., and Delmer, D.P.** (2001). The experimental herbicide CGA 325'615 inhibits synthesis of crystalline cellulose and causes accumulation of non-crystalline beta-1,4-glucan associated with CesA protein. *Plant Physiol.* **126**: 981-992.
- Peterson, R.T., Link, B.A., Dowling, J.E., and Schreiber, S.L.** (2000). Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proc. Natl. Acad. Sci. USA* **97**: 12965-12969.
- Raikhel, N., and Pirrung, M.** (2005). Adding precision tools to the plant biologists' toolbox with chemical genomics. *Plant Physiol.* **138**: 563-564.
- 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. USA* **105**: 8464-8469.
- Robert, S., Bichet, A., Grandjean, O., Kierzkowski, D., Satiat-Jeunemaitre, B., Pelletier, S., Hauser, M.T., Hofte, H., and Vernhettes, S.** (2005). An *Arabidopsis* endo-1,4-beta-D-glucanase involved in cellulose synthesis undergoes regulated intracellular cycling. *Plant Cell* **17**: 3378-3389.
- Rojas-Pierce, M., Titapiwatanakun, B., Sohn, E.J., Fang, F., Larive, C.K., Blakeslee, J., Cheng, Y., Cuttler, S., 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.
- Rojo, E., Gillmor, C.S., Kovaleva, V., Somerville, C.R., and Raikhel, N.V.** (2001). VACUOLELESS1 is an essential gene required for vacuole formation and morphogenesis in *Arabidopsis*. *Dev. Cell* **1**: 303-310.
- Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P., and Scheres, B.** (1999). An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Cell* **99**: 463-472.
- Sanderfoot, A.A., and Raikhel, N.V.** (1999). The specificity of vesicle trafficking: coat proteins and SNAREs. *Plant Cell* **11**: 629-642.
- Sandermann, H., Jr.** (1992). Plant metabolism of xenobiotics. *Trends Biochem. Sci.* **17**: 82-84.
- Scheible, W.R., Eshed, R., Richmond, T., Delmer, D., and Somerville, C.** (2001). Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *Arabidopsis* *lxr1* mutants. *Proc. Natl. Acad. Sci. USA* **98**: 10079-10084.
- Schiott, M., Romanowsky, S.M., Baekgaard, L., Jakobsen, M.K., Palmgren, M.G., and Harper, J.F.** (2004). A plant plasma membrane Ca²⁺ pump is required for normal pollen tube growth and fertilization. *Proc. Natl. Acad. Sci. USA* **101**: 9502-9507.
- Schlaich, N.L.** (2007). Flavin-containing monooxygenases in plants: looking beyond detox. *Trends Plant Sci.* **12**: 412-418.
- Schreiber, K., Ckurshumova, W., Peek, J., and Desveaux, D.** (2008). A high-throughput chemical screen for resistance to *Pseudomonas syringae* in *Arabidopsis*. *Plant J.* **54**: 522-531.
- Schreiber, S.L.** (1998). Chemical genetics resulting from a passion for synthetic organic chemistry. *Bioorg. Med. Chem.* **6**: 1127-1152.
- Schumacher, K., Vafeados, D., McCarthy, M., Sze, H., Wilkins, T., and Chory, J.** (1999). The *Arabidopsis* *det3* mutant reveals a central role for the vacuolar H⁺-ATPase in plant growth and development. *Genes Dev.* **13**: 3259-3270.
- Serrano, M., Robatzek, S., Torres, M., Kombrink, E., Somssich, I.E., Robinson, M., and Schulze-Lefert, P.** (2007). Chemical interference of pathogen-associated molecular pattern-triggered immune responses in *Arabidopsis* reveals a potential role for fatty-acid synthase type II complex-derived lipid signals. *J. Biol. Chem.* **282**: 6803-6811.
- Shogren-Knaak, M.A., Alaimo, P.J., and Shokat, K.M.** (2001). Recent advances in chemical approaches to the study of biological systems. *Annu. Rev. Cell Dev. Biol.* **17**: 405-433.
- Silady, R.A., Kato, T., Lukowitz, W., Sieber, P., Tasaka, M., and Somerville, C.R.** (2004). The gravitropism defective 2 mutants of *Arabidopsis* are deficient in a protein implicated in endocytosis in *Caenorhabditis elegans*. *Plant Physiol.* **136**: 3095-3103; discussion 3002.
- Spring, D.R., Krishnan, S., Blackwell, H.E., and Schreiber, S.L.** (2002). Diversity-oriented synthesis of biaryl-containing medium rings using a one bead/one stock solution platform. *J. Am. Chem. Soc.* **124**: 1354-1363.
- Steinmann, T., Geldner, N., Grebe, M., Mangold, S., Jackson, C.L., Paris, S., Galweiler, L., Palme, K., and Jurgens, G.** (1999). Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. *Science* **286**: 316-318.
- Stockwell, B.R.** (2000). Chemical genetics: ligand-based discovery of gene function. *Nat. Rev. Genet.* **1**: 116-125.
- Stockwell, B.R.** (2002). Chemical genetic screening approaches to neurobiology. *Neuron* **36**: 559-562.
- Stockwell, B.R.** (2004). Exploring biology with small organic molecules. *Nature* **432**: 846-854.

- Stumpfe, D., Ahmed, H.E., Vogt, I., and Bajorath, J.** (2007). Methods for computer-aided chemical biology. Part 1: Design of a benchmark system for the evaluation of compound selectivity. *Chem. Biol. Drug Des.* **70**: 182-194.
- 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.
- Surpin, M., and Raikhel, N.** (2004). Traffic jams affect plant development and signal transduction. *Nat. Rev. Mol. Cell Biol.* **5**: 100-109.
- 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. USA* **102**: 4902-4907.
- Swarup, R., Kargul, J., Marchant, A., Zadik, D., Rahman, A., Mills, R., Yemm, A., May, S., Williams, L., Millner, P., Tsurumi, S., Moore, I., Napier, R., Kerr, I.D., and Bennett, M.J.** (2004). Structure-function analysis of the presumptive Arabidopsis auxin permease AUX1. *Plant Cell* **16**: 3069-3083.
- 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.
- Torrance, C.J., Agrawal, V., Vogelstein, B., and Kinzler, K.W.** (2001). Use of isogenic human cancer cells for high-throughput screening and drug discovery. *Nat. Biotechnol.* **19**: 940-945.
- Vogt, I., Stumpfe, D., Ahmed, H.E., and Bajorath, J.** (2007). Methods for computer-aided chemical biology. Part 2: Evaluation of compound selectivity using 2D molecular fingerprints. *Chem. Biol. Drug Des.* **70**: 195-205.
- Wakabayashi, K., and Boger, P.** (2002). Target sites for herbicides: entering the 21st century. *Pest Manag. Sci.* **58**: 1149-1154.
- 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.
- Walsh, T.A., Bauer, T., Neal, R., Merlo, A.O., Schmitzer, P.R., Hicks, G.R., Honma, M., Matsumura, W., Wolff, K., and Davies, J.P.** (2007). Chemical genetic identification of glutamine phosphoribosylpyrophosphate amidotransferase as the target for a novel bleaching herbicide in Arabidopsis. *Plant Physiol.* **144**: 1292-1304.
- Williams, D., Jung, D.W., Khersonsky, S.M., Heidary, N., Chang, Y.T., and Orlow, S.J.** (2004). Identification of compounds that bind mitochondrial F1F0 ATPase by screening a triazine library for correction of albinism. *Chem. Biol.* **11**: 1251-1259.
- Xu, J., and Scheres, B.** (2005). Dissection of Arabidopsis ADP-RIBOSYLATION FACTOR 1 function in epidermal cell polarity. *Plant Cell* **17**: 525-536.
- 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.
- Yang, Z.** (2002). Small GTPases: versatile signaling switches in plants. *Plant Cell* **14 Suppl.**: S375-388.
- Yano, D., Sato, M., Saito, C., Sato, M.H., Morita, M.T., and Tasaka, M.** (2003). A SNARE complex containing SGR3/AtVAM3 and ZIG/VT111 in gravity-sensing cells is important for Arabidopsis shoot gravitropism. *Proc. Natl. Acad. Sci. USA* **100**: 8589-8594.
- Yoneda, A., Higaki, T., Kutsuna, N., Kondo, Y., Osada, H., Hasezawa, S., and Matsui, M.** (2007). Chemical genetic screening identifies a novel inhibitor of parallel alignment of cortical microtubules and cellulose microfibrils. *Plant Cell Physiol.* **48**: 1393-1403.
- Young, S.S., and Ge, N.** (2004). Design of diversity and focused combinatorial libraries in drug discovery. *Curr. Opin. Drug Discov. Devel.* **7**: 318-324.
- Zhang, J., Garrossian, M., Gardner, D., Garrossian, A., Chang, Y.T., Kim, Y.K., and Chang, C.W.** (2008). Synthesis and anticancer activity studies of cycloamine derivatives. *Bioorg. Med. Chem. Lett.* **18**: 1359-1363.
- 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.
- Zhao, Y., Christensen, S.K., Fankhauser, C., Cashman, J.R., Cohen, J.D., Weigel, D., and Chory, J.** (2001). A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* **291**: 306-309.
- Zhao, Y., Chow, T.F., Puckrin, R.S., Alfred, S.E., Korir, A.K., Larive, C.K., and Cutler, S.R.** (2007). Chemical genetic interrogation of natural variation uncovers a molecule that is glycoactivated. *Nat. Chem. Biol.* **3**: 716-721.
- Zheng, W., Zhai, Q., Sun, J., Li, C.B., Zhang, L., Li, H., Zhang, X., Li, S., Xu, Y., Jiang, H., Wu, X., and Li, C.** (2006). Bestatin, an inhibitor of aminopeptidases, provides a chemical genetics approach to dissect jasmonate signaling in Arabidopsis. *Plant Physiol.* **141**: 1400-1413.
- Zheng, Z.L., and Yang, Z.** (2000). The Rrop GTPase switch turns on polar growth in pollen. *Trends Plant Sci.* **5**: 298-303.
- Zonia, L., and Munnik, T.** (2008). Vesicle trafficking dynamics and visualization of zones of exocytosis and endocytosis in tobacco pollen tubes. *J. Exp. Bot.* **59**: 861-873.
- Zouhar, J., Hicks, G.R., and Raikhel, N.V.** (2004). Sorting inhibitors (Sortins): Chemical compounds to study vacuolar sorting in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **101**: 9497-9501.