Opportunities and challenges in plant chemical biology

Glenn R Hicks & Natasha V Raikhel

Chemical biology is beginning to enhance our understanding of diverse cellular processes in plants, including endomembrane trafficking, hormone transport and cell wall biosynthesis. To reach its potential requires the development of a community-wide infrastructure of technology and expertise. We present some of the opportunities and challenges in this emerging branch of plant biology and offer some suggestions for enhancing the approach to the benefit of the community at large.

Although resources devoted to plant biology are modest in comparison to biomedical and other areas of research, plant biologists will play a disproportionate and crucial role in the future of mankind. Basic plant biology and its application will be the major vehicle through which we will improve human health in a cost-effective manner on a global scale. Progress toward meeting the challenges facing the world—improved nutrition, increased crop yield, resistance to pests, sustainable biofuels, raw materials for industry and serious environmental problems—is being fueled by ongoing technical and intellectual advances. One area of advancement is plant genomics, which has resulted in the sequencing of the genomes of *Arabidopsis thaliana*, rice, poplar and many other species. More recently, the introduction of massively parallel next-generation sequencing technologies has spawned the beginnings of a revolution through metagenomic studies[1] and an era of rapid access to genotypic variation between individual genomes[2].

Another critical, but as yet less developed, innovation has been the integration of small-molecule approaches with plant biology. We see plant chemical biology as the application of small bioactive chemicals to interrogate dynamic cellular networks in plants. Mutational genetics has been the primary approach toward understanding such networks through the elucidation of individual gene function. However, even in plants such as *Arabidopsis thaliana* where there are well-established genetic tools, many networks have been recalcitrant to classical genetic strategies owing to a combination of gene redundancy (yielding no observable change in phenotype) and gene lethality.

Plant chemical biology can address these issues by using bioactive chemicals to affect the active sites of individual protein targets or, alternatively, whole classes of protein targets in a manner that is very controllable in terms of dose and treatment time. The application of small-molecule approaches to plant biology requires a new cadre of plant scientists who are using approaches that straddle the interface of chemistry, informatics and biology. They are poised to make important contributions by addressing the inherent challenges of multi-cellular land plants as biological systems. We will discuss some of the enormous opportunities to advance our knowledge of basic plant processes through the use of small bioactive molecules and some of the major hurdles to jump to maximize the potential of plant chemical biology. We will highlight some investigations of small-molecule structure and the identification of cognate targets, but will emphasize the ability of plant-bioactive chemicals to provide a network-level view of complex dynamic processes because this is one of the great opportunities in plant chemical biology. We also offer some suggestions for choosing resources likely to benefit the plant biology community most.

Glenn R. Hicks and Natasha V. Raikhel are at the Center for Plant Cell Biology, Institute for Integrative Genome Biology and Department of Botany and Plant Sciences, University of California, Riverside, California, USA.
e-mail: nraikhel@ucr.edu

Within the past decade, plant chemical biologists have begun to make important contributions to our understanding of cell wall biosynthesis, the cytoskeleton, hormone biosynthesis and signaling, gravitropism, pathogenesis, purine biosynthesis and endomembrane trafficking (reviewed in refs. 3,4). In many cases, plant phenotype–based chemical screens using cell cultures and seedlings have identified small molecules targeting these processes (reviewed in ref. 4). In other cases, important cognate gene targets have been identified[5–10]. Several examples below highlight the recent contributions of chemical biology in uncovering new and useful knowledge of broad potential impact.

Some impacts of plant chemical biology

Cellulose is the primary polymeric constituent of plant cell walls and is thus an important source of biomass for biofuels and industrial products. Understanding the mechanisms of cellulose synthesis and deposition are important basic and practical goals. Investigations of resistance to the cellulose biosynthesis inhibitor isoxaben have provided insight into two genes now known to encode the cellulose synthases CESA3 and CESA6 (refs. 10,11). These proteins are two of at least ten CESA cellulose synthases that are involved in a macromolecular complex known as the particle rosette or terminal complex that coordinates the incorporation of glucan chains into cellulose microfibrils of the nascent cell wall[12] (**Fig. 1**). The selectivity of isoxaben for two CESA proteins was essential for overcoming gene redundancy, which is a common feature in plants including *Arabidopsis*. The chemicals morlin[13], which affects cortical

microtubules, and 2,6-dichlorobenzonitrile[14], whose precise mode of action is unknown, both inhibit rosette motility. This and other evidence indicates that CESA-containing rosettes interact with cortical microtubules as part of a potential guidance mechanism that determines the orientation of cellulose microfibrils[12]. This orientation is crucial in determining the directionality of cell growth and ultimately plant morphology. Thus, these small bioactive molecules have contributed to the functional dissection of CESA complexes as well as their interaction with another macromolecular complex, microtubules. Given the selectivity of isoxaben, it is probable that other chemicals could prove valuable in further defining the roles of the *CES* gene family in development.

Another recent example is gravacin[6], identified in a screen of a diverse chemical library as an inhibitor of root and shoot gravitropism and protein targeting to the tonoplast (that is, vacuole membrane) in *Arabidopsis* seedlings. Gravitropism is the bending either toward (roots) or away from (stems) the gravitational force; the orientation is sensed by root tips and shoots and requires the cell-to-cell transport of the growth-modulating hormone auxin. This transport is strongly directional and controlled by a family of plasma membrane efflux transporters known as the PINS[15], along with transporters of the large ATP-binding cassette sub-family B (ABCB)/P-glycoprotein (PGP) family. The ABCBs, or multidrug-resistance transporters, may function by stabilizing PIN localization at distinct domains in the plasma membrane[16]. The demonstration that ABCB19/PGP19 is one of the cognate targets of gravacin[6] indicates not only gravacin's utility as a chemical probe for ABCB function, but suggests that it could help in the search for drugs to enhance the effectiveness of cancer therapeutics. Interestingly, whereas the *pgp19* mutant displayed resistance to the inhibitory effects of gravacin on gravitropism, the mutant was not resistant to a second chemically induced phenotype, which resulted in the mistargeting of tonoplast proteins to the endoplasmic reticulum[6]. Because endomembrane trafficking relies on different machinery than gravitropism, this suggests that there is a genetically distinct second target site for the compound in a separate pathway (**Fig. 2a**). This kind of cross-talk can be viewed as positive in uncovering new pathways, or as a hindrance in genetic screens for resistance mutations. Bioactive compounds, such as endosidin 1, discussed below, can also help us to define points of interaction within a network of pathways (**Fig. 2b**).

The contributions of these and other chemical biology studies rely on the availability of community-wide tools established in a few model systems, especially *Arabidopsis*, which has a small, fully sequenced genome. These tools include (i) well-developed reverse genetics, including the availability of extensive collections of T-DNA insertion and activation-tagged lines[17], (ii) molecular markers that facilitate the fine mapping of mutations[18], (iii) structurally diverse chemical collections (reviewed in ref. 4), which in some cases were pre-screened for plant active compounds[7,19], and (iv) structural databases of chemicals incorporating plant phenotype data[20,21]. Combined, these features make plants a valuable research platform for fundamental discovery.

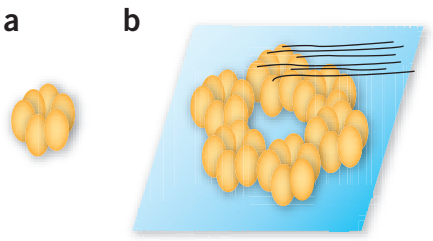

Figure 1 Cellulose microfibrils are one of the primary components of plant cell walls and are composed of 36 hydrogen-bonded β-1,4-linked glucose residues, each produced from a single CESA subunit. (**a**) Six CESA subunits assemble into a CESA complex. (**b**) Each CESA complex is in turn assembled in the Golgi into a particle rosette composed of six CESA complexes, which is targeted to the plasma membrane. Each of the 36 CESA subunits contributes to the nascent cellulose microfibril. For clarity, cellulose (black lines) is shown emerging from only a single CESA complex of a particle rosette in the plasma membrane. There is a family of ten CESA proteins in *Arabidopsis*, of which five appear to be involved in the synthesis of primary cell-wall cellulose microfibrils. Among these genes, mutations in CESA3 and CESA6 confer resistance to isoxaben. Thus, a bioactive small molecule was used to overcome gene redundancy.

Challenges faced by plant biologists

Of course, there is little value in developing such an extensive genomics infrastructure in plants, and at least the foundations of a community-wide infrastructure in chemical genomics, without the goal of insightful, interesting biology and the development of useful applications. In this regard, plants are poised to excel. Their lives are characterized by relative immobility, resulting in the evolution of adaptive responses to environmental challenges that are highly flexible, permitting plants to survive extensive drought, flood and biotic extremes. At the cellular level, lack of mobility has resulted in vegetative development that is dependent upon specific stem cells that give rise to differentiated organs derived from the shoot and root apical meristems. The uniqueness of plant development is obvious during embryogenesis, which is achieved without cell migration as is necessary in animals. As more plant genomes are sequenced, other evolutionary features of plants have been highlighted that present both inherent challenges to unraveling the biology of these interesting species and important opportunities for chemical biologists to significantly contribute to the field.

Lethality and redundancy limits the usefulness of mutational genetics. The high degree of redundancy among plant genes is well established[17]. As a result of this redundancy, mutations resulting in loss of function frequently yield phenotypes not observably different from wild type, which limits the effectiveness of the existing strategies. In other instances, loss-of-function mutations in essential genes result in lethality. Both redundancy and lethality have been found among genes encoding components of endomembrane trafficking in plants[22]. These issues are being addressed effectively by chemical genomics. Given the enormous structural variations possible among small molecules, they can be used to perturb protein function in a highly specific manner, as in the case of essential genes. Furthermore, because chemical dosages can be modulated, the products of essential genes can be studied under nonlethal conditions. Redundancy can be addressed through the use of chemicals that are more broadly targeted to protein families sharing common features or activities that can be perturbed. The cellulose synthases and ABCBs underscore the ability of small bioactive chemicals to identify functionally one or a small number of members of redundant families.

***In vivo* approaches are needed to dissect cellular networks.** Plant stem cells differentiate into a wide range of cell types and organs, and plant structures are composed of distinct layers of cells, each with its own identity. Because of this high degree of complexity, it is difficult, with few exceptions, to isolate and culture plant cells derived exclusively from a single organ or cell lineage and, if isolated, to maintain normal cell function under the conditions necessary to establish the cultures. Thus, despite the apparent ease and scalability of obtaining non-pure cultured plant cells for *in vitro* chemical screens, the approach should be considered cautiously in terms of biological discovery. An *in vitro* approach may work for basic cell-autonomous processes perhaps, but will be of limited value in studying development, organism responses to environment or even cellular processes, such as the establishment of cellular polarity. Thus,

in the absence of pure cell cultures, plants must be studied *in vivo*.

An *in vivo* approach utilized the endocytosis inhibitor endosidin 1 (ES1)[19] to define endosome/trans-Golgi network compartments involved in the sorting of plasma membrane proteins, which are either recycled between endosomes and the plasma membrane or are sent to the vacuole for turnover. The compound was found by means of a high-throughput *in vitro* screen for chemical inhibitors of pollen germination. Among other factors, pollen germination and pollen tube elongation are dependent upon proper endocytosis and exocytosis at the tip of the growing pollen tube, which is a single cell displaying highly polarized tip growth. *In vivo* studies were done in *Arabidopsis* roots expressing cycling plasma membrane markers including the auxin transporters PIN2 and AUX1 and the plant steroid hormone receptor brassinosteroid-insensitive1 (BRI1). ES1 was discovered to block a step in endocytosis at an early endosome/trans-Golgi network compartment containing the syntaxin SYP61, which is involved in the endocytosis specifically of PIN2, AUX1 and BRI1, and allowed visualization of the colocalization of these proteins with SYP61 (**Fig. 3**). ES1 also perturbs steroid signaling by BRI1, providing important support for previous suggestions that BRI1 resides in-part in an endosome compartment from which it is involved in signal transduction leading to the regulation of steroid-responsive genes[23].

Many cellular processes occur rapidly *in vivo*. Many cellular processes are highly dynamic in nature. Endocytosis is known to occur in a matter of minutes. Whereas mutants are clearly valuable in the study of such processes, unless the mutations are conditional, one has to accept that the state of the cell is at equilibrium with the mutation. In the case of ES1, the compound is added and the plant cells respond in less than an hour. When the chemical is removed from the growth medium, the cells return to the state before treatment within a few hours. In other words, the addition of bioactive chemicals permits the study of dynamic processes on a biological time scale.

Chemical biology presents inherent challenges

Although chemical biology addresses many of the inherent difficulties of plants as biological systems, the discipline has yet to gain broad acceptance among plant scientists and biologists at large. For example, biologists often find it bothersome when a bioactive chemical is promiscuous, as the overriding feeling is that, to be informative, chemicals should display strong activity against a single cognate target. Although this is helpful in forward genetic screens to identify resistance genes, it does not negate the value of compounds with well-characterized cellular effects, which as discussed, can provide many biological insights. An excellent example of this concept is the toxin Brefeldin A (BFA)[24], which is now known to target the SEC7 family of ARF/GEFs (ADP-ribosylation factor/GDP/GTP exchange factor) of which the best known in plants is *GNOM*[25]. Even before its target sites were known, BFA was the most widely used drug for studying the endomembrane system in plants and still is today. The impact of BFA on its multiple targets results in the disruption of Golgi-based secretion and has secondary effects that include the disruption of endocytosis and exocytosis and their associated compartments such as endosomes. In the case of BFA and ES1, which is more specific for endosomes[26], their value as reagents resides in their well-characterized cellular effects. For example, starting with ES1, one could envision that a suite of chemicals having different specificities for markers of dynamic cycling plasma membrane would permit the definition of a network of functionally overlapping compartments necessary for endocytosis (**Fig. 4**). Such a systems-based approach toward the screening and application of chemicals would take maximum advantage of the rapid and conditional nature of bioactive chemicals, especially for the direct study of highly dynamic processes. This is not to reduce the importance of identifying cognate targets, but rather to acknowledge the conceptual complementarity of thinking more broadly than targets as the predominant endpoint of chemical biology to dissect networks[27]. In other words, although targets are important, we should not fixate on them to the exclusion of the highly informative biology that is achievable with chemicals that have well-characterized effects.

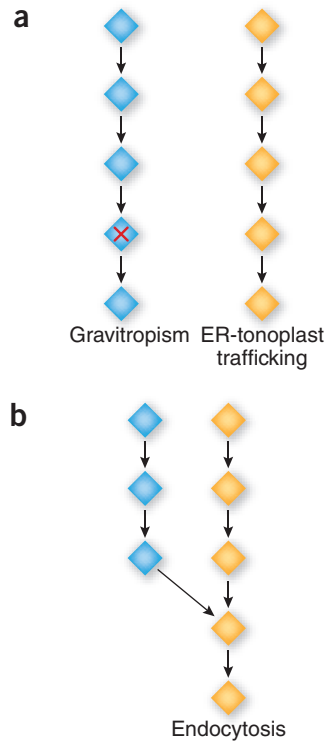

Figure 2 Bioactive chemicals can perturb distinct or intersecting biological pathways. (**a**) Two independent pathways leading to distinct responses can be perturbed by a single chemical. This could occur via conserved functional domains shared by the two protein targets or by interaction of each target site with a distinct domain of the small molecule. For example, the auxin transporter ABCB19 (✕) is the protein target of gravacin within the pathway for gravitropic response (blue). The means by which gravacin causes defects in ER-to-tonoplast protein targeting (orange) is unknown. (**b**) Bioactive chemicals can also identify points where multiple pathways intersect. For example, the chemical endosidin 1 (ES1), whose target is not known, identified a SYP61 endocytic compartment used by a subset of plasma membrane proteins involved in distinct response pathways in plants (see text). Pathways depicted are hypothetical.

Identifying cognate targets efficiently is also important for discovering mechanistic details of new gene functions and networks. At this point, the main approach to target identification is forward genetic screening using ethane methyl sulfonate for chemical resistance and hypersensitivity. This requires the establishment of large mapping populations to define a physical region within reach of Sanger sequencing methods. However, with the advent of the new generation of massively parallel sequencing platforms, it is becoming feasible to sequence individual genomes to identify mutations[2,28] associated with microscopy-based intracellular phenotypes that might otherwise be too difficult and impractical to score and map using conventional fine-mapping approaches. As scientists become more familiar with the complexities of plant cells, biochemistry-based approaches to identify protein targets, such as specialized tagged libraries that permit the direct identification of protein targets[29], will complement the genetic tools developed thus far.

How do we take full advantage of plant systems?

To more fully capitalize on the potential of chemical biology to uncover new pathways and networks there are several key areas that need attention.

Automation. There is an overall need for increased automation to save time and labor. Typical plant-based screens utilize seedlings

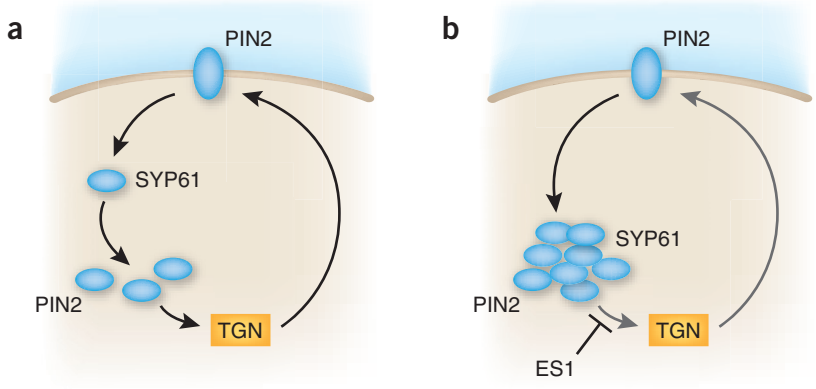

Figure 3 Bioactive small molecules can dissect rapid cellular processes. (**a**) PIN2 (shown in blue), and other endocytosed proteins, transit through a continuum of compartments known as endosomes or the trans-Golgi network (TGN) in plants where they are sorted for recycling back to the plasma membrane or turnover in the vacuole. During early endocytosis, PIN2 transits through a compartment containing the syntaxin SYP61; however, due to the rapidity of endocytosis, fluorescent protein markers for PIN2 and SYP61 co-localize only rarely. (**b**) Endosidin 1 (ES1) inhibits an early step in endocytosis resulting in the accumulation of PIN2 and other specific cargo proteins as well as SYP61 into highly specific endosome agglomerations known as endosidin bodies[21], demonstrating that PIN2 transits through the SYP61 compartment. Thus, bioactive chemicals can perturb dynamic processes and begin to define, in this example, endosome compartments and pathways associated with specific plasma membrane proteins.

germinated in medium containing chemicals of interest. Much of the effort is manual and can be laborious. The development of automated plate preparation, seed plating and imaging are critical to screen large, diverse, chemical libraries effectively. This need for automation is particularly acute in determining the phenotypes resulting from chemical treatment. What may seem as simple as recording root length and curvature becomes a daunting task when attempted on a large scale. Hardware and software solutions for image-based plant phenotyping are required that can simultaneously collect large statistically significant data on plant morphology and responses to stress or environmental challenges (**Fig. 5**). Some imaging tools are being developed to address these issues, for example, by measuring curvature angles dynamically during gravitropism[30]. However, additional support to develop other phenotyping tools would greatly benefit plant biologists.

Phenotyping tools at the cellular level. One of the most powerful applications of chemical biology is the dissection of events at the cellular level that underlie plant development and morphological responses to biotic and abiotic cues. The ability to detect small molecule–induced changes in cellular morphology will provide new insights. But this requires improvements in microscopy and probes. Owing to the complexity of plant organs, it is critical to develop new microscopy approaches to examine cells *in vivo* at cell layers that are deeper than the uppermost layers now accessible. This would allow the collection of data at multiple scales and sites. New instrumentation also should incorporate the analytical and computational tools needed to reduce the data to meaningful quantitative measurements.

In addition to examining deep tissue layers cell by cell, the opposite tact is also critical. Microscopy for the most part is still done sample-by-sample rather than by automation, and the automated microscopes that are available were mostly designed to image animal cells that are grown as monolayers rather than the complex multi-layers in plant tissues. High-throughput chemical screens demand the ability to image thousands of samples rapidly at the cellular level and to analyze this enormous volume of image data to detect subcellular phenotypes of interest. Imaging in great detail and at great depth and imaging thousands of samples in an automated and quantifiable manner are necessary to provide the range of scales required to truly merge chemical biology with cell and developmental biology. In all cases, there is a clear need for the application of image recognition, processing and analysis to analyze organelle position, diameter, velocity and morphology in a manner that is meaningful and quantifiable. Such data must be compiled and made available to the scientific community through the development of databases and web portals. Image analysis tools are becoming available to cell biologists, but as with existing automated microscopes, they are designed mostly with cultured animal cells in mind and not targeted toward the unique challenges faced by plant chemical biologists. Addressing the challenges of microscopy and image processing that plant chemical biologists face will benefit the plant community as a whole.

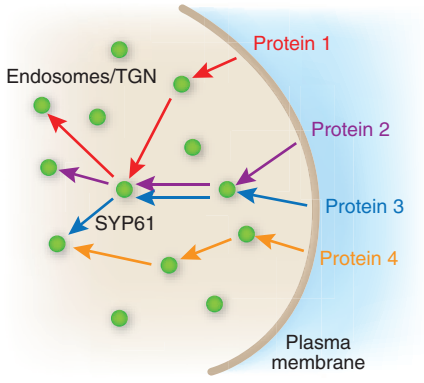

Figure 4 Small molecules can help to define complex cellular networks. Depicted is a conceptual model of interconnected and distinct pathways of the endocytosis of plasma membrane proteins in *Arabidopsis*. The model shows four hypothetical proteins that traffic through endosomes/trans-Golgi network (TGN) during endocytosis. Spheres (green) represent distinct endosome/TGN endomembrane compartments involved in endocytosis. Endocytosis may involve a network of pathways, each pathway being shared by one of more plasma membrane proteins (proteins 1, 2, 3). However, as for PIN2, AUX1 and BRI, the pathways may share specific endosome compartments (in this case marked as SYP61 to provide clarity). Alternatively, the endocytic pathway may be completely distinct (protein 4). The targeting of proteins to the plasma membrane is not part of the model but can be expected to show equivalent complexity. Conceptually and using ES1 as an example, a suite of compounds affecting the endocytosis of diverse plasma membrane proteins could be used to dissect the complex network of dynamic compartments underlying protein cycling at the plasma membrane.

Investment in infrastructure. Thus far, significant discoveries have been made by the efforts of a relatively small number of pioneering

laboratories that have worked to establish chemical biology in plants. But the field is now at the point where it is crucial to develop a shared infrastructure to permit wider accessibility. This infrastructure needs to include not only the technologies already discussed but also new chemical collections that are pre-screened on plants for bioactivity. This will permit nonexpert laboratories to bypass some of the most laborious initial screening that is necessary to find chemicals of biological interest. Few such libraries exist[4,7] and should be expanded greatly. The housing of such resources should, at least in part, be within one or a few centralized facilities that can offer instrumentation (fluids robotics, automated microscopy), informatics (database management, image analysis) and technical expertise (plant-based screens, library curation, analytical and synthetic chemistry). This arrangement will facilitate the rapid adoption of chemical biology by the plant community.

Of course, such a focused effort requires resources beyond that of individual laboratories that have endeavored to establish chemical biology within the plant community. Now that some of the fundamentals are in place, broader funding is needed to develop existing resources to their full potential. The approaches and instrumentation used by plant cell biologists provides much of the foundation necessary for chemical biology, and increased funding of basic research in plant cell biology will act synergistically with more focused efforts at infrastructure development. In the current economic climate, funding for new initiatives will be another challenge faced by plant biologists.

Overall, plant chemical biology has moved rapidly in the past decade and is beginning to make genuine contributions to basic knowledge. Further advancement will require us to take advantage of bioactive small molecules as system-oriented network dissection tools as well as a means to a target. It will also require us to take a more focused tact to overcome technical problems and to fund these efforts on behalf of the plant community. The benefit technically will be new tools to delve more deeply into the gene networks than current approaches permit. Ultimately, our fellow citizens will benefit through improved health and environment. Attaining these goals will be well worth the effort.

ACKNOWLEDGMENTS

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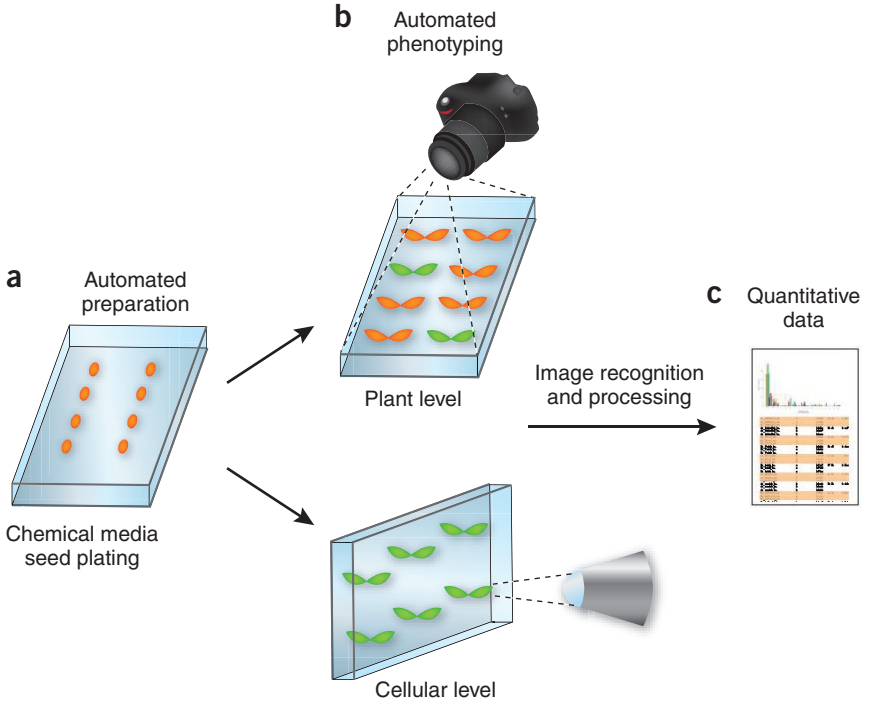

Figure 5 Enhanced microscopy, increased automation and quantification of seedling and cellular-level phenotypic data will drive plant chemical biology toward a more sophisticated, network-oriented view of development by providing access to more quantifiable phenotypes. (**a**) Robotics can greatly speed not only the preparation of chemical media but the rate of seed sterilization and physical placement on media plates. (**b**) Automated phenotyping of leaf area, stem angle or root hair number should be combined with automated microscopy of complex intracellular phenotypes such as organelle diameter, number or velocity in many cell layers to facilitate analysis. (**c**) The raw image data would then be processed using image recognition and analysis software resulting in data that are quantified to the maximum extent possible. Quantifiable phenotypes, especially at the cellular level, will effectively expand the range of networks that can be dissected by chemical biologists.

1. Cokus, S.J. *et al. Nature* **452**, 215–219 (2008).
2. Ossowski, S. *et al. Genome Res.* **18**, 2024–2033 (2008).
3. Blackwell, H.E. & Zhao, Y. *Plant Physiol.* **133**, 448–455 (2003).
4. Robert, S., Raikhel, N.V. & Hicks, G.R. in *The Arabidopsis Book* (American Society of Plant Biologists, Rockville, Maryland, USA, 2009).
5. Dai, X., Hayashi, K., Nozaki, H., Cheng, Y. & Zhao, Y. *Proc. Natl. Acad. Sci. USA* **102**, 3129–3134 (2005).
6. Rojas-Pierce, M. *et al. Chem. Biol.* **14**, 1366–1376 (2007).
7. Zhao, Y. *et al. Nat. Chem. Biol.* **3**, 716–721 (2007).
8. Walsh, T.A. *et al. Plant Physiol.* **144**, 1292–1304 (2007).
9. Walsh, T.A. *et al. Plant Physiol.* **142**, 542–552 (2006).
10. Desprez, T. *et al. Plant Physiol.* **128**, 482–490 (2002).
11. Scheible, W.R., Eshed, R., Richmond, T., Delmer, D. & Somerville, C. *Proc. Natl. Acad. Sci. USA* **98**, 10079–10084 (2001).
12. Somerville, C. *Annu. Rev. Cell Dev. Biol.* **22**, 53–78 (2006).
13. DeBolt, S. *et al. Proc. Natl. Acad. Sci. USA* **104**, 5854–5859 (2007).
14. DeBolt, S., Gutierrez, R., Ehrhardt, D.W. & Somerville, C. *Plant Physiol.* **145**, 334–338 (2007).
15. Feraru, E. & Friml, J. *Plant Physiol.* **147**, 1553–1559 (2008).
16. Titapiwatanakun, B. *et al. Plant J.* **57**, 27–44 (2009).
17. Borevitz, J.O. & Ecker, J.R. *Annu. Rev. Genomics Hum. Genet.* **5**, 443–477 (2004).
18. Lukowitz, W., Gillmor, C.S. & Scheible, W.R. *Plant Physiol.* **123**, 795–806 (2000).
19. Robert, S. *et al. Proc. Natl. Acad. Sci. USA* **105**, 8464–8469 (2008).
20. Girke, T., Cheng, L.C. & Raikhel, N. *Plant Physiol.* **138**, 573–577 (2005).
21. Cao, Y. *et al. Bioinformatics* **24**, 1733–1734 (2008).
22. Surpin, M. & Raikhel, N. *Nat. Rev. Mol. Cell Biol.* **5**, 100–109 (2004).
23. Geldner, N., Hyman, D.L., Wang, X., Schumacher, K. & Chory, J. *Genes Dev.* **21**, 1598–1602 (2007).
24. Nebenfuhr, A., Ritzenthaler, C. & Robinson, D.G. *Plant Physiol.* **130**, 1102–1108 (2002).
25. Geldner, N. *et al. Cell* **112**, 219–230 (2003).
26. Drakakaki, G., Robert, S., Raikhel, N.V. & Hicks, G.R. *Plant Signal. Behav.* **4**, 57–62 (2009).
27. Peterson, R.T. *Nat. Chem. Biol.* **4**, 635–638 (2008).
28. Sarin, S., Prabhu, S., O'Meara, M.M., Pe'er, I. & Hobert, O. *Nat. Methods* **5**, 865–867 (2008).
29. Mitsopoulos, G., Walsh, D.P. & Chang, Y.T. *Curr. Opin. Chem. Biol.* **8**, 26–32 (2004).
30. Lewis, D.R., Miller, N.D., Splitt, B.L., Wu, G. & Spalding, E.P. *Plant Cell* **19**, 1838–1850 (2007).