J Plant Growth Regul (2005) 24:46–54
DOI: 10.1007/s00344-005-0039-5

J P G R
Journal of Plant Growth Regulation

MEETING REPORT

Plant Cell Signaling: *In Vivo* and -omics Approaches

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Scientists from around the world converged on Santa Fe, NM, February 1–6, 2005 (Figure 1), for a Keystone Symposium that brought together recent and exciting developments in plant cell signaling. The meeting also provided a platform to lay out and discuss the most modern methodologies and approaches that will govern how the next generation of scientists will propel plant biology into the ''post-genomic'' era. Although the remit of the conference covered Plant Cell Signaling, important developments and discoveries frequently spanned the biology of multiple species, and this was ably demonstrated in a number of talks that discussed experimental systems from different kingdoms. Such versatility was especially evident in the keynote address given by Michael Snyder (Yale University), who provided an overview of information made available by massive sequencing projects (~250 fully sequenced genomes) and efficient use of this sequence information to reach the ultimate goal of understanding the function and regulation of each gene and protein. Within plant biology, it is clear that *Arabidopsis thaliana* largely remains the model system of choice, as its fully sequenced genome is being successfully exploited to fuel concepts within systems biology, large scale and high throughput screening. However, it is important to note that, in contrast to this, both novel methodology and exciting new developments were presented at this meeting using tobacco, maize, cotton, barley, tomato, and rice, illustrating continued diversity in experimental plant systems.

On the technical side, advances in microscopy such as developments in Förster/fluorescence resonance energy transfer (FRET) techniques, and multi-mode fluorescence microscopy have allowed the maturation of practical methods not only to visualize fluorescently tagged proteins or subcellular compartments but also to analyze *in vivo* the dynamics of protein–protein interactions and intracellular trafficking in living cells. To this end, a specific workshop on FRET-related techniques and a session dedicated to development of single-cell techniques were also held at the meeting.

Additionally, the power of the ''*in silico*'' biology became apparent with the introduction of a number of new Web-based tools allowing dissection of the ever-growing amount of data from high-throughput screens and analyses. In this report we focus on presentations that provided direction to the plant community and that summarized the most recent developments in the field.

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Received: 7 April 2005; accepted: 11 April 2005; Online publication: 28 July 2005

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Figure 1. Historic Santa Fe, New Mexico. Location of the 2005 Keystone Symposia on Plant Cell Signaling: *In Vivo* and -omics Approaches. (Photograph courtesy of Dr. Jutta Ludwig–Müller.)

Hormones and Signal Transduction

Recent advances in understanding plant hormonal signaling have resulted in the identification of a variety of signaling components, including cytokinin receptors, components of auxin signal transduction, and abscisic acid (ABA) regulators. In addition, the existence of cross-talk between pathways in plants indicates a role for these signaling cascades in the relay of exogenous signals into the nucleus. Emerging components of signaling pathways of plant hormones were presented during the meeting.

Tatsuo Kakimoto (Osaka University) described the role of phosphorylation in cytokinin signaling in *Arabidopsis.* Using a molecular genetic approach, he and his colleagues isolated triple cytokinin receptor mutants, *cre1-12 ahk2-2 ahk3-3*, which have no responses to cytokinin during callus formation and which also show different phenotypes in the root (Higuchi and others 2004). Kakimoto also reported that suppressors of the cytokinin receptor cytokinin response 1 (CRE1) were identified. Among them, $CRE1^{wol}$ does not bind cytokinin, suggesting that $CRE1^{wol}$ may be locked in the cytokinin-unbound form of CRE1. When expressed in heterologous systems (insect cells, yeast), CRE1 catalyzes cytokinin-dependent phototransfer. That phosphotransfer can be bidirectional; for example, CRE1 can phosphorylate histidine phosphotransfer (HPt) proteins in the presence of cytokinin and dephosphorylate HPt proteins independently of cytokinins.

Jutta Ludwig-Müller (Technische Universität Dresden) described the novel roles and interaction of different hormone signaling pathways during club root infection caused by *Plasmodiphora brassicae* in *Arabidopsis.* By transcriptome analysis, she showed that cross-talk between auxin and cytokinin is involved during pathogenesis. Additional involvement of ethylene and jasmonate (JA) during this process was also discussed. Mark A. Estelle (Indiana University) described characterization of transport inhibitor response 1 (TIR1) proteins as nuclear auxin receptors and focused his talk on protein turnover in auxin signaling. Using a series of pull-down assays, he showed that auxin can promote interaction between Aux/IAA proteins and the F-box protein TIR1. Introduction of purified TIR1 protein in insect cells makes them auxin responsive, confirming that TIR1 is an auxin receptor. However the *tir1* mutant has a weak phenotype, suggesting that auxin response is mediated by multiple proteins with overlapping function. Indeed, there are five TIR1-related proteins in *Arabidopsis* (AFB1-5). Three of them, AFB1-3, can interact with Aux/IAA. Phenotypic analysis of quadruple mutants lacking TIR1 and AFB1-3 suggested that AFB1-3 may also function as auxin receptors.

Sarah M. Assmann (Pennsylvania State University) described the role of heterotrimeric G proteins in different ABA signaling pathways in *Arabidopsis.* With the help of mutants lacking one or more subunits of the G protein complex, she showed that in ABA-inhibition of stomatal opening, a G protein–coupled receptor 1 (GCR1) functions in the same pathway as the G protein α subunit 1 (GPA1) and G protein β subunit 1 (AGB1), but as a negative regulator (Pandey and Assmann 2004). However, for ABA-inhibition of seed germination, all the components act in the same direction. These data suggest that signals in pathways mediated by G protein components are tissue and cell specific in response to ABA. Alan M. Jones (University of North Carolina at Chapel Hill) focused on the role of the *Arabidopsis* heterotrimeric G protein in sugar sensing. He described that the regulator of G protein–signaling protein 1 (RGS1) interacts with GPA1 and negatively regulates G protein signaling. Additionally, D-glucose induces rapid internalization of RGSl-GFP. For example, *rgs1* null seedlings are less sensitive to glucose (Chen *and* others 2004), suggesting that RGS1 might be a D-glucose sensor. He also described identification and characterization of a new *G*PA1 *i*nteracting *p*rotein, GIP1; in this case, *gip1* null seedlings are hypersensitive to D-glucose, whereas GIP1 overexpressing plants are less sensitive, suggesting that GIP1 may also have a role in sugar sensing along with RGS1. Hak Soo Seo (Rockefeller University) assessed the role of constitutively photomorphogenic 1 protein (COP1) in sumoylation and ubiquitination during abiotic

stresses. SIZ is an *Arabidopsis* SUMO E3 ligase that can interact with COP1 protein by binding to its WD40 domain. Further analysis of *cop1* and *siz1* mutants showed that COP1 is an E3 ubiquitin ligase for SIZ1 and can negatively regulate SIZ1 to control SUMO-protein conjugate levels under drought stress. A number of new SUMO- conjugates were identified under drought stress using mass spectrometry. All these proteins have the conserved sumoylation motif ψKXE/D, and transcription levels of these genes were increased by abiotic stresses (Seo and others 2004).

Cell Dynamics

The session on cell dynamics was dedicated to the study of signaling between intracellular compartments. Jennifer A. Lippincott-Schwartz (National Institutes of Health) described different models of intra-Golgi trafficking: maturation model, vectorial model, and rapid equilibrium model. Using an inverse fluorescence recovery after photobleaching (iFRAP) technique, and with the example of human VSVG (vesicular stomatitis virus G protein) as a marker for cargo moving through the secretory pathway, she showed that most of the available data favor the rapid equilibrium model of intracellular trafficking (Altan-Bonnet and others 2004). Federica Brandizzi (University of Saskatchewan) described protein cargo export at the endoplasmic reticulum/Golgi surface in tobacco epidermal cells. *In vivo* confocal laser scanning microscopy showed that the Golgi-to-endoplasmic-reticulum transfer of proteins is a highly dynamic process and that protein cargo continuously recycles between these two compartments (daSilva and others 2004).

Dorus Gadella (University of Amsterdam) explained the use of green fluorescent protein (GFP) based multimode fluorescence microscopy to study microtubule dynamics and endomembrane trafficking during spindle formation and cytokinesis in transformed tobacco cells (Dhonukshe and Gadella 2003). He focused on the use of modern techniques such as multichannel confocal microscopy, FSPIM (fluorescence spectral imaging microscopy), FLIM (fluorescence lifetime imaging microscopy) and FRET-FLIM (a combination of FRET and lifetime imaging), and use of these techniques to study various processes in living plant cells, including homo-and heterodimerization of signaling proteins. He proposed that endocytic material is the main source for generation of the cell plate rather than Golgi-derived material (Vermeer and others 2004). Tian Jin (National Institutes of Health) used the model organism *Dictyostelium* to visualize spatiotemporal patterns of G protein activation during chemotaxis via the FRET technique. He showed that these cells can sense the stimulus gradient by differential activation of G proteins on the cell surface. A uniformly applied stimulus, however, leads to a biphasic signal response and amplification but monophasic G protein activation (Jin and others 2000).

Maureen R. Hanson (Cornell University) used fluorescence labeling to revise a prevailing concept of plastid morphology. She described the small appendage-like structures present on chloroplasts termed ''stromules''. These are stroma-filled structures that may be involved in increasing plastid surface area and that may serve as channels through which materials move through cells. FRAP studies indicate that these structures may also be involved in protein trafficking (Kwok and Hanson 2004).

Intracellular Signaling

The portion of the meeting dedicated to intracellular signaling focused on the coordinated regulation of cytoplasmic and nuclear events within cells. More specifically, the talks addressed both how intracellular events such as meiotic recombination are regulated and how intracellular organelles communicate with each other. The session opened with a talk by Gareth Jones (University of Birmingham), who introduced meiosis with an emphasis on the role of molecular genetics and molecular cell biological techniques to follow meiosis in real time. He described the isolation and characterization of a number of meiotic genes in *Arabidopsis* that were originally identified in yeast— for example, *ASY1*, which is required for normal recombination and synapsis, and *RAD51*, which is required for recombination. He also discussed that there are at least two different pathways that control crossover events; a major pathway controlled by genes such as *MSH4* (*Arabidopsis* homolog of *MSH4*, Higgins and others 2004), and about 15% of events that are independent of this pathway.

Gregory Copenhaver (University of North Carolina at Chapel Hill) extended Jones's discussion with a description of different models of crossover interference during meiosis. He used the *quartet* mutant of *Arabidopsis*, where the tetrads do not separate after meiosis, and he discussed a chromosome-wide analysis he had performed to develop a statistical model showing that *Arabidopsis* has a highly complex crossover interference system similar to Drosophila and humans. To dissect the molecular

basis of these pathways, he described the use of pollen-specific promoters tagged with different fluorescent markers that will enable the performance of large-scale visual screens (reviewed in Copenhaver and others 2000).

Joanne Chory (Salk Institute) focused her talk on retrograde signaling between plastids and the nucleus in plant cells. She described isolation of different *genomes uncoupled (gun)* mutants where these signaling pathways are compromised. With this screen, at least two redundant retrograde plastid tetrapyrrole biosynthetic pathways have been identified. Because accumulation of Mg-protoporphyrin (Mg-ProtoIX) abolishes the *gun* phenotype, it is proposed that Mg-ProtoIX acts as a signaling molecule between plastid and nucleus. GUN4 is a novel chloroplast protein required for chlorophyll synthesis (Lakin and others 2003). Depending on GUN4 localization within the chloroplast (envelope, stroma, or thylacoid), GUN4 associates with specific proteins to form different complexes. For instance, GUN4 co-purified with GUN5 in thylakoid complexes. To better understand this complex formation, reconstitution of Mg-chelatase activity with recombinant *Synechocystis* proteins was carried out (Verdecia and others 2005).

Cellomics

In a session on cellomics three talks focused on signaling components of one specific cell type using combinations of ''omic'' techniques. Julian Schroeder (University of California) described a microarray-based cloning strategy to identify ion accumulation or guard-cell ABA signaling mutants that are otherwise difficult to phenotype (Gong and others 2004; Leonhardt and others 2004). As a result of this single cell-type genomic approach, he described the role of NADPH oxidase (Kwak and others 2003) and the role of two Ca^{2+}-dependent protein kinases, CPK3 and CPK6, in ABA signaling in guard cells with the help of molecular genetics and electrophysiological approaches. He also presented data showing new CO_2 signaling mechanisms in guard cells.

Daphne Preuss (University of Chicago) focused on the pollen components of cell–cell signaling that regulate mate choice during pollen adhesion and pollen tube growth. Taking an integrated approach, she described isolation of specific pollen coat proteins, glycine-rich pollen surface proteins (GRPs), and extracellular lipase proteins (EXLs) and mutants that lack these proteins. She also compared these genes in an evolutionary perspective across different species from the *Brassicaceae* family and found that GRPs have a higher rate of substitutions and deletions and have evolved more rapidly than other proteins, thus providing the species-specificity to the pollen/stigma interaction (Fiebig and others 2004). Finally, Pierre Broun (University of York) used glandular trichomes of Solanaceous species as a model system to study the terpenoid metabolic pathway that offers attractive targets for genetic and metabolic engineering.

Whole Plant Functional Genomics and Proteomics

Talks presented in a session on whole plant genomics and proteomics dealt with different aspects of signaling in response to biotic stress in plants. alph Panstruga (Max-Planck-Institut für Züychtungsforschung, Cologne) described the role of Mlo (powdery mildew resistance locus O) proteins in pathogen resistance in barley. These seven trans-membrane span proteins can function independently of G proteins, similar to the CCR5 class of chemokine receptors (GPCR) in humans. Mlo is required for host cell invasion upon attack by the ascomycete powdery mildew fungus *Blumeria graminis*. Mlo, as well as another plasma membrane syntaxin protein, ROR2 (required for mlo-specified resistance 2 isolated as a suppressor of *mlo*), focally accumulate at the site of fungal penetration. FRET–FLIM analysis showed that these two proteins interact at defined places, showing a dynamic interaction (Bhat and others 2005).

Ko Shimamoto (Nara Institute of Science and Technology) described the role of a small G protein, Rac1, in rice disease resistance. Rac proteins interact with NADPH oxidase (Rboh) proteins, as shown by yeast two-hybrid and FRET analysis. Cinnamoyl CoA reductase (CCR), the enzyme catalyzing the first step of lignin monomer synthesis, also interacts with OsRacl in a GTP-dependent manner. With affinity chromatography and immunoprecipitation, OsRac1 was found to form a large multi-protein complex consisting of other proteins involved in disease resistance signaling in rice.

Scott Peck (Sainsbury Laboratory) described the role of protein phosphorylation during plant–microbe interaction using large-scale proteomics approaches. He showed that with a combination of sub-fractionation, immobilized metal affinity chromatography (IMAC), and different chromatography techniques he and his colleagues were able to purify up to 90% pure phosphoproteins (Nuhse and others 2004). Almost 340 phosphorylation sites were

mapped on 200 membrane proteins with wide ranges of molecular mass and pI. They also used the iTRAQ technique for quantitative comparison of *in planta* samples. By comparison of these phosphoproteins inter and intra-species, it was found that the phosphorylation sites are usually not conserved in paralogs but are conserved in orthologs. Identification of new substrates for MAP kinases was also discussed.

Frederick M. Ausubel (Harvard Medical School) compared immune responses in vertebrates (adaptive immunity) versus plants (innate immunity) at the levels of receptors, downstream signaling pathways, and the final response. It appears that *Arabidopsis* response to PAMPs (pathogen-associated molecular patterns), for example Flg22, a peptide representing the elicitor-active epitope of flagellin, and oligogalacturonides (OGs) represents a highly conserved evolutionary pathway most similar to Toll-like receptor pathways of humans. Ausubel described how both the Flg22 and OG signaling pathways activate overlapping MAPK signaling cascades. He also explained that plants possibly respond to pathogen specific signals by activating synthesis of a number of antimicrobial compounds and showed involvement of type III secretion systems in these processes. Also, how a plant's response to a pathogen is integrated by pathways mediated by salicylic acid, jasmonate, ethylene, and PAMPs was discussed.

Other talks included in this section focused on ''omics'' studies at the whole plant level, rather than on a single cell type or process. Joseph R. Ecker (Salk Institute) described the accurate determination of gene structure and completeness of gene inventories as an essential task for the new phase of post-genomic studies with the ''reference'' plant *Arabidopsis.* He described the use of Affymetrix whole genome tiling arrays and computational and empirical methods for construction of an *Arabidopsis*-ORFeome.

Mark Stitt (Max-Planck-Institut für Molekulare Pflanzenphysiologie, Golm) discussed carbon sensing and nitrogen sensing and their interaction and role in plant growth and development. A large-scale phenotypic analysis was performed with *Arabidopsis* in response to varying carbon/nitrogen ratios, and results were analyzed based on bioinformatics tools such as MapMan (Thimm and others 2004), which displays large data sets (for example, gene expression data from *Arabidopsis* Affymetrix arrays) onto diagrams of metabolic pathways or other processes. Bernhard O. Palsson (University of California) described the use of 1D (with the help of sequence information), 2D (network construction (with the help of metabolic, regulatory signaling), 3D (with the help of genome location, pattern strength, codon affinity index, and G/C content), and 4D (changes in genome with time and space) models of genome annotations, their complexities and uses (Mahadevan and Palsson 2005; Papin and others 2005).

New Techniques

Workshop on FRET–based Techniques

One of the main themes of the meeting was science based on FRET techniques. Indeed, several talks contained FRET data of one type or another. To complement this, a key component of the meeting was a workshop designed to not only inform the community of the power and scope of energy transfer–based methods, but also to create awareness of potential problems and pitfalls in attempting FRET. This provided an opportunity for investigators to discuss their methods for FRET and to elaborate on data presented throughout the meeting.

Several key points arose in the session. J. Philip Taylor (University of North Carolina at Chapel Hill) began by describing the essential prerequisites for FRET, choice of donor/acceptor pairing, and how the use of plant cells can complicate recordings. Tian Jin elaborated on this potential problem by applying FRET recording to 32-channel spectrally resolved confocal microscopy. Specific applications of FRET were discussed by Simon Gilroy (Pennsylvania State University) and Raymond Zielinski (University of Illinois). Simon Gilroy described use of the FRET-based Ca^{2+} sensor cameleon, which can be calibrated as a function of calcium concentration. He emphasized the use of FRET in combination with more biochemical/physical data to get the most useful information, and he provided the most important take-home message of the session: ''FRET is all about the numbers'' that is, accurate quantitation. The cameleon, a FRET-based nanosensor uses the ability of the calcium-bound form of calmodulin (CaM) to interact with calmodulin-binding polypeptides to turn the corresponding dramatic conformational change into a change in resonance energy transfer between two fluorescent proteins attached to the fusion protein. The cameleon and its derivatives were successfully used to follow calcium changes in real time, not only in isolated cells but also in whole organs. Zielinski described the use of recombinant fluorescence indicator proteins (FIPs), which are CaM-binding peptides, to report CaM binding to protein substrates *in vitro* (CNGC-FIP). Although attempts to use these proteins *in vivo*, with

stronger 35S promoters have been unsuccessful because of frequent gene silencing, with the use of weaker or restricted expression promoters, the conditions could be optimized for sensitive detection of fluorescence changes in FIPs.

Two novel alternatives to FRET were discussed. Albrecht von Arnim (University of Tennessee) described advances in optimizing bioluminescence resonance energy transfer (BRET), where the fluorescent donor is replaced by a bioluminescent molecule such as RLUC from *Renilla Luciferase* (Subramanian and others 2004). The advantages of BRET as a rapid and robust technique for *in vivo* analysis of protein–protein interactions were presented, with the caveat that at this time, it is less suitable for imaging (as opposed to luminometer measurements) than FRET. Also, a new technique was presented that relies on the complementation of two domains of the yellow fluorescent protein (YFP) individually expressed as fusions to two proteins of interest. Karin Schumacher (University of Tübingen) showed how bimolecular fluorescence complementation (BiFC) can be used to study protein–protein interactions, describing the different types of vectors available for testing such interactions with examples of homodimerization of the basic leucine zipper (bZIP) transcription factor bZIP63 and the zinc finger protein lesion simulating disease 1 (LSD1) from *Arabidopsis*, as well as dimer formation of tobacco 14-3-3 proteins (Walter and others 2005). She also discussed how the technique was limited in that it relies explicitly on the orientation of the two YFP components being complementary and that once formed, the complex is stable, making it unsuitable for the study of transient interactions.

Fluorescence-based Techniques. Current fluorescence-based approaches are limited in their ability to examine the real-time dynamics of signaling activity (proteins, metabolites, secondary messengers) in living cells. Some of the recent advances in the field of plant nano-sensors and dye-based markers were presented at the meeting. The goal is to develop sensors that display rapid, selective, sensitive and quantitative responses to particular targets *in vivo.*

Wolf B. Frommer (Carnegie Institution) described the development and potential applications of genetically encoded ''sensors'' to study cell-omics with the specific example of sucrose transport in different species. He described the criteria for development of an efficient metabolite sensor, and its use to dissect the spatial and temporal profiles of metabolite levels at the cellular and subcellular levels. The metabolite nanosensors consist of two variants of the GFP fused to bacterial periplasmic binding proteins. A conformational change in the binding protein region can be directly detected as a change in FRET efficiency. The prototypes are able to detect various carbohydrates such as ribose, glucose, and maltose *in vitro.* The nanosensors can be expressed in yeast and in mammalian cell cultures, and they have been used to determine carbohydrate homeostasis in living cells with subcellular resolution (Fehr and others 2005).

Klaus M. Hahn (University of North Carolina at Chapel Hill) talked about new methods derived from FRET ''domain biosensors'' to quantify the spatiotemporal dynamics of protein activation thanks to dyes specifically designed to report protein conformational changes and protein interactions in living cells. He presented the development of custom dyes capable of visualizing the changing activation of an endogenous member of the Rho family in fibroblast living cells (Nalbant and others 2004) and also the FLAIR (fluorescence activation indicator for Rho proteins) method, which was specifically developed to study real-time dynamics of the Racl nucleotide state in living cells from the amoeboid protozoan *Dictyostelium discoideum* (Chamberlain and others 2000; Kraynov and others 2000).

Simon Gilroy (Pennsylvania State University) described the role of pH and Ca^{2+} in gravity sensing in root columella cells, as revealed by tools such as caged calcium ions and ratiometric imaging using confocal 2-photon microscopy. He showed that the touch signal in the root cap produces Ca^{2+} waves that switch on an increase in pH that triggers growth response. The same sequence of events is found in another part of the root. Soren Friis (The Royal Veterinary and Agricultural University, Denmark) described the use of a GFP-based sensor for monitoring H^+ ATPase activity in root hair cells. As GFP fluorescence depends on pH, and there are different ranges of pH inside a cell, he described the development of modified GFP, BFP, and RFPs to record small pH changes (Gao and other 2004). Biosensor development will clearly open up new avenues of research, although much work remains, largely with respect to improvements in device sensitivity and selectivity and the appropriate expression of these nanosensors in plants.

Chemical Genomics. In recent years, investigation of intracellular processes has increasingly benefited from the use of small molecules to perturb complex cellular pathways. Two talks focused on this linkage of chemistry and cell biology by developing methods for the high-throughput screening of small organic molecules. Natasha V. Raikhel (University

Table 1. A Selection of the Internets-Based Resources, Tools, and Databases Available to the Research Community That Were Presented and Discussed at the Keystone Symposia on Plant Cell Signaling: *In Vivo* and -omics Approaches

Participant	Web site	URL
Michael Snyder	Global analysis of protein activities using proteome chips	http://bioinfo.mbb.yale.edu/proteinchip
Albrecht G. Von Arnim	BRET	http://fp.bio.utk.edu/vonarnim
Natasha V. Raikhel	Chemical genomics	http:bioweb.ucr.edu/ChemMine/search.php
Maureen R. Hanson	Chloroplast stromule movies	http://www.mba.cornell.edu/Kohler_Trends.cfm
Patrick S. Schnable	Laser microdissection protocols	http://schnablelab.plantsenomics.iastate.edu/resources
Julian I. Schroeder	*Arabidopsis* ICP mutant screen data - Guard Cell and Mesophyll Cell Affymetrix Expression Arrays	http://www-biology.ucsd.edu/labs/schroeder
Berhard Palsson	Models of genome annotations	http://systemsbiology.ucsd.edu
Marcus Heisler	The computable plant	http://www.computableplant.org
Mark Stitt	Mapman: user-driven tool that displays large datasets onto diagrams of metabolic pathways or other processes	http://gabi.rzpd.de/projects/MapMan
Scott C. Peck	Searchable data base for plant phosphorylation sites maintained by PlantsP	http://plantsp.sdsc.edu

of California–Riverside) talked about the power of chemical genomics to study pathways where traditional knock-out approaches have been unsuccessful. She explained that the use of small molecules to control/perturb biological functions is useful as these effects are reversible; they address redundancy, they require less time, and they can be used at a large scale. She described a high-throughput screen for compounds that cause a vacuolar protein to be secreted in yeast. Two of these compounds, sortin1 and sortin2, were also found to cause defects in vacuole morphology and protein trafficking in *Arabidopsis* (Zouhar and others 2004).

Helen E. Blackwell (University of Wisconsin) discussed the use of small molecules in plant microbe interaction with the example of quorum sensing in bacteria. By using computational modeling, docking studies, and biosynthesis, she explained the use of new modified, synthetic ligands for quorum sensing and discussed how these compounds can be used for therapeutics. She also discussed modified solid–phase chemistry techniques for effective synthesis of up to 500 new compounds within hours and their use for more genome–wide screening approaches.

Other Techniques. Patrick S. Schnable (Iowa State University) described a laser microdissection–based technique for cutting single-cell plant tissues and their use in deciphering cell/tissue-specific transcriptome profiling (Nakazono and others 2003). Waltraud Schulze (University of Southern Denmark) described a proteomic peptide-protein screen approach for detection of phosphorylation that has been optimized for plant samples (Schulze and Mann 2004). Finally, Marcus Heisler (California Institute of Technology) compiled imaging instrumentation, applied biomathematics, and computing to create and apply computational modeling to integrate multidisciplinary approaches and different types of biological data in studying development. This project is called ''*The Computable Plant.*''

Conclusions

Attendees of the meeting were as diverse scientifically as they were geographically, which led to highly interesting discussions and the generation of new ideas and collaborations. There was a high level of interaction between scientists of all levels and a free flow of information. For instance, listed in Table 1 are some of the Web-based tools, databases, and information sources that were discussed by some of the speakers and made available to the community.

The meeting appropriately ended with concluding remarks from Daphne Preuss, who discussed future directions for plant biology research, problems we should focus on, future challenges, and the need to

set priorities. Indeed, genome sequence data allied to the identification of protein function and the increasingly sophisticated construction of transgenic plants have opened new ways to address questions of fundamental biological importance. However, mainly because of the high cost of large-scale techniques, she pointed out the necessity to set priorities on what is ''worth knowing,'' and the need for researchers to function together as a community.

ACKNOWLEDGMENTS

On behalf of the participants and organizers of ''Plant Cell Signaling: *In Vivo* and omics Approaches,'' we extend our gratitude to all the speakers for sharing their insights and unpublished data. Additionally, we are indebted to the organizers, our supervisors, Alan M. Jones (J.P.T.) and Sarah M. Assmann (S.P., L. P-B., and Z.Z.), both for this opportunity and for their continued guidance and support. We apologize to any participant whose contribution has not been included due to space constraints.The Session on Hormones and Signal Transduction was dedicated to the memory of Tony Bleecker, who passed away shortly before the meeting commenced. A memorial book was signed by meeting participants in his honor and later presented to his wife, Sara Patterson. Additionally, we offer congratulations to The Keystone Symposia Education Fund Scholarship Winner, Alessandra Devoto (University of East Anglia); Keystone Symposia Scholarship Recipients Soren Friis (The Royal Veterinary and Agricultural University), Monika Kalde (John Innes Centre), Montrell Seay (Yale University), and J. Philip Taylor (University of North Carolina at Chapel Hill); and the Pfizer education fund scholarship winner, Tomoe Kamada-Nobusada (National Institute for Basic Biology).

Note Added in Proof: Since the meeting, two highly important publications (Dharmasiri N, Dharmasiri S, Estelle M. 2005. The F-box protein TIR1 is an auxin receptor. Nature. 435: 441–445; Kepinski S, Leyser O. 2005. the Arabidopsis F-box protein TIR1 is an auxin receptor. Nature 435:446–451) have emerged that have confirmed the role of transport inhibitor response 1 (TIR1) as an auxin receptor, described by Mark Estelle at the meeting.

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