Meeting Report

Frontiers of Plant Cell Biology: Signals and Pathways, System-Based Approaches 22nd Symposium in Plant Biology (University of California—Riverside)

The 22nd Symposium in Plant Biology (January 15th to 18th, 2003) hosted by the University of California—Riverside (UCR) focused on systems-based approaches to plant cell biology research. The symposium speakers and over 130 posters placed emphasis on recent developments in plant cellular biology and molecular genetics, particularly those employing emerging genomic tools. One of the highlights of the trip was a tour of the new, state-of-the-art Core Instrumentation Facility at UCR, which is part of the Center for Plant Cell Biology (CEPCEB) and under the umbrella of UCR Genomics Institute. This facility possesses many of the high-throughput instrumentation (e.g. visual microscopy) and bioinformatic tools that will lead us into the postgenomic era and beyond. I am sure that the other attendees of the 22nd Symposium will join me in congratulating the Symposium's organizers, CEPCEB's director Natasha V. Raikhel and Zhenbiao Yang, for organizing this most successful meeting.

CELL-TO-CELL COMMUNICATION

An individual plant cell must communicate with other cells composing the plant, so that whole can be integrated into a functioning unit. In addition, cell-to-cell communication is important during the establishment of various types of interactions that plants form with other organisms—both harmful and helpful. Cell-to-cell communication involves the perception of extracellular signals by cell surface receptors. Many of these cell receptors possess intrinsic protein kinase activity and regulate transcription of target genes through phosphorylation events.

Jeanette Nadeau (Sachs Lab, Ohio State University) discussed an interesting example of the involvement of a protein kinase receptor in cell-to-cell communication. Her presentation concerned the Arabidopsis mutant *mustaches* (*mus*) that is defective in guard cell morphogenesis. Guard cells arise from the symmetrical division of a progenitor cell followed by the deposition of a lens-shaped cell wall thickening between the two cells. This "lens" later separates to form the pore opening. At the same time, guard cells expand in a regulated fashion, to attain the kidney shape necessary for stomatal valve action. This shape is probably acquired when microtubules are organized in a radial array focused at the pore, so as to guide radial deposition of cellulose microfibrils. In *mustaches*, the radial array of microtubules is distorted or absent in guard cells, yet these cells still express molecular and cytological markers of guard cell identity. The finding that there are spatial defects in the localized deposition of cell wall material suggests that MUS plays a role in organized wall building, through targeted vesicular deposition and/or controlled deposition of cellulose microfibrils. Furthermore, the loss of symmetry between guard cell pairs in *mus* suggests that it participates in morphogenetic events coordinated between the cell pair. The recent discovery that *MUS* encodes a receptor kinase suggests a direct link between cell signaling and the spatial organization of the specialized cytoskeleton necessary for guard cell morphogenesis.

Philip N. Benfey (Duke University, Durham, NC) provided another example of cell-to-cell communication in plant morphogenesis. In particular, he discussed two Arabidopsis mutants called *scarecrow* (*scr*) and *short-root* (*shr*) that exhibit altered radial patterning in their roots. An analysis of tissue-specific markers indicated that *SCR* is primarily required for the asymmetric division that gives rise to the cortex and endodermis, whereas the *SHR* gene is required for the asymmetric cell division responsible for formation of ground tissue as well as specification of the endodermis. Both SHR and SCR are members of the GRAS family of putative transcription factors. Although SHR apparently acts by regulating the amount of mRNA that is made by the *SCR*, it is surprising that *SHR* is not expressed in the same cells as *SCR*. Two types of protein localization studies, however, revealed that SHR protein is present in the stele and in the cells immediately adjacent to it. These cells include the endodermis, its initial cell as well as the quiescent center, which are precisely where *SCR* is expressed. Benfey proposed that SHR is an important regulator in a "signaling center" located in the internal cell layers of the Arabidopsis root that provides pattern information to the surrounding cell layers. Indeed, the ectopic expression of *SHR* results in supernumerary cell layers and altered cell specification.

Cell-to-cell communication is also important in several steps of the pollination process. Elizabeth M. Lord (University of California—Riverside) discussed the adhesion and guidance of lily (*Lilium* spp.) pollen tubes during pollination. In lily, pollen grains germinate on the stigma surface and are guided into the style through openings in the stigma. A transmitting tract epidermis that is continuous with the stigmatic surface and the placenta lines the hollow style. The pollen tubes adhere not only to the extracellular ma-

www.plantphysiol.org/cgi/doi/10.1104/pp.900080.

trix (ECM) of this stylar epidermis, but also to one another. At least two molecules were found to be necessary for adhesion; one is a low esterified pectic polysaccharide, the other a peptide called SCA (stigma/stylar Cys rich adhesin). The two bind to each other and form an adhesive ECM on the surface of the transmitting tract epidermis. Pollen tubes in liquid growth medium adhere to and grow on this matrix in vitro. Treatment of the pectic polysaccharide with endopolygalacturonase before preparation of the assay, results in a non-adhesive matrix, as does treatment of the SCA preparation with a protease. Antibodies made to both SCA and low esterified pectins, show co-localization on the transmitting tract of the style. On the stigma, SCA occurs both bound to the wall and free, so it may be capable of forming a gradient to guide pollen tubes into the style.

June Nasrallah (Cornell University, Ithaca, NY) discussed receptor-ligand interactions in the self-incompatibility (SI) response of crucifers. In this family, the arrest of self-pollen occurs at the surface of the epidermal cells of the stigma and is manifested by inhibition of pollen hydration, germination, or pollen tube invasion of the stigma epidermis. The S locus, which consists of two highly polymorphic and co-evolving genes, is the genetic determinant of SI specificity. One gene encodes a receptor Ser-threonine kinase in the stigma epidermis, whereas the second gene encodes a small Cys rich peptide that is localized in the pollen coat and is the ligand for the stigma receptor. Recognition of self-pollen is based on allele-specific interactions between the stigma receptor and its pollen ligand. These interactions result in activation of the receptor and presumably trigger a signaling cascade within the stigma epidermal cell that leads ultimately to the inhibition of pollen tube emergence or growth.

Cell-to-cell communication is also involved in determining how plant cells respond to pathogen invasion. Pamela C. Ronald (University of California—Davis) discussed the *Xa21* gene of rice (*Oryza sativa*) that encodes for a receptor kinase with Leu rich repeats (LRRs) in its extracellular domain. XA21 is a key recognition and signaling determinant in the immune response of rice to infection by *Xanthomonas oryzae*. The extracellular LRR domain of XA21 is apparently responsible for specific recognition of the *Xoo* strains carrying avr*Xa21* activity. Three of the six *Xoo* genes (*raxA*, *raxB*, *raxC*) that encode for proteins required for avrXa21 activity share similarity with components of Type I secretion systems of gram-negative bacteria. In bacterial Type I systems, effectors are secreted into the extracellular environment where they can be recognized by cell surface receptors. Three avirulence determinants encoding proteins with similarity to *Rhizobium meliloti* host specificity genes have also been identified. These include *raxP* and *raxQ* that encode both ATP sulfurylase and adenosine 5′-phosphosulfate kinase activities as well as *raxST* that shares similarity with mammalian and bacterial genes encoding for sulfotransferases. The *raxQ* and *raxP* encoded proteins function in concert to produce phosphoadenosine phosphosulfate (PAPS), an active form of sulfate. Ronald hypothesizes that the avrXa2l effector molecule is sulfated by *RaxST* and then secreted by a Type I secretion system making it available for race specific interactions with the rice receptor kinase XA21.

Shou-Wei Deng (University of California—Riverside) discussed evidence that a viral protein that confers virulence in Arabidopsis independently of viral infection actually plays a dual role in pathogenesis. The viral protein serves not only to suppress the host's RNA-silencing antiviral defense but also enhances the accumulation of six endogenous microRNAs (miRNA). These miRNAs apparently direct the cleavage of three transcriptional factors that are critical for normal plant development. These findings suggest that miRNAs may play a pathogenic role in the induction of viral diseases.

THE CELL WALL AND EXTRACELLULAR MATRIX

The spatial and temporal control of cell wall deposition plays a critical role during growth and development in plants, yet little is known about the molecular mechanisms by which cell wall polysaccharides are synthesized and secreted.

Herman Höfte's (INRA, Paris) laboratory has isolated a large collection of Arabidopsis mutants with hypocotyl growth defects. They adapted Fourier Transform InfraRed (FTIR) microspectroscopy to identify which of these mutants have cell wall defects. A large class of mutants with defects in the deposition of cellulose microfibrils in primary walls, corresponding to at least 6 complementation groups, was identified. The genes encode, respectively; 3 cellulose synthase catalytic subunits, a membrane-bound endo-β-1,4-glucanase, a member of the basic chitinase gene family and a novel plasma membrane-bound protein. Höfte's group has also been investigating the role of cellulose synthesis in the control of growth. It is generally thought that the orientation of microfibrils is not critical for the extent of cell expansion but that it controls growth anisotropy. Surprisingly, Höfte's group has observed reduced cell expansion and increased radial expansion in several cellulose deficient mutants, without a change in the orientation of the microfibrils.

Antony Bacic (University of Melbourne, Australia) discussed how the composition of cell walls in higher plants differs between species and developmental stages. Cellulose is the main load-bearing polysaccharide in all cell wall types. Grasses and their close relatives, however, employ polysaccharides such as glucuronoarabinoxylans and (1–3, 1–4) beta-D glucans to form a matrix to hold the cellulose microfi-

brils together. Some of the genes (CesAs) responsible for the synthesis of cellulose in higher plants have been identified, but the search for the genes involved in the synthesis of the noncellulosic cell wall polysaccharides continues. The CesA superfamily, currently classified into 8 cellulose synthase-like (CsIA-CsIH) families plus the CesA family, contains candidate genes for the synthesis of polysaccharides such as cellulose, heteroxylans and (1–3, 1–4) beta-D glucan. One way to deduce the functions of cell wall polysaccharides is to correlate gene expression patterns with changes in cell wall composition. Bacic's laboratory has developed a technique for the functional analysis of polysaccharide synthase genes by adapting a double-stranded RNA interference (dsRNAi) procedure to enable transiently induced gene silencing of putative *CesA/CsI* genes in barley via particle bombardment into the coleoptiles of immature barley embryos.

Chris Somerville (Carnegie Institution, Stanford, CA) discussed mutants of Arabidopsis that have defects in cell wall synthesis and assembly. These mutants have been identified by a variety of screening methods, including forward screens for embryo-lethal mutants, screens for altered sensitivity to inhibitors, screens for altered mucilage composition and by reverse genetics. Characterization of the various genes involved has implicated *N*-linked glycosylation and glycosylphosphatidyl inositol (GPI)-anchoring in cellulose synthesis. Somewhat surprisingly, GPI anchoring appears to be required for multicellular development but not for growth of plant cells in culture.

The laboratory of Tamara L. Western (Haughn Lab, University of British Columbia, Canada) has been engaged in studying pectin biosynthesis in the mucilage secretory cells of the Arabidopsis seed coat. Two novel genes, *MUCILAGE MODIFIED4* (*MUM4*) and *PRAIRIE*, appear to be involved in the regulation of pectin biosynthesis in these cells. Detailed characterization of both the cell biology of wild type cells and the defects of the mutants has been undertaken using high pressure frozen samples and transmission electron microscopy. Due to their seed specific epidermal alterations, MUM4 and PRAIRIE may act downstream of the known transcription-regulating, epidermal regulators TTG1, TTG2, GL2 and MYB61.

Michael Mølhøj (Reiter Lab, University of Connecticut, Storrs) provided insights into the biosynthesis of D-galacturonate and L-Ara, two monosaccharide components that are abundant in plant cell wall material and glycoproteins but absent from other eukaryotes. The activated forms of these monosaccharides (UDP-GalA and UDP-Ara) are formed by the 4-epimerization of UDP-GlcA and UDP-Xyl, respectively, but the genes encoding the interconversion enzymes have not previously been cloned and characterized in plants. Positional cloning of the *MUR4* gene of Arabidopsis, and its functional expression in yeast (*Pichia pastoris*) revealed that it encodes a UDP-Xyl 4-epimerase with a predicted transmembrane domain at the N terminus. Subcellular localization of the MUR4 protein indicated targeting to the Golgi apparatus. Using bioinformatics approaches, Mølhøj identified six potential UDPGlcA 4-epimerases (GAEs) in the Arabidopsis genome, and confirmed the predicted function of two of them by expression in *Pichia*. Like MUR4, all six GAE isoforms contain an N-terminal transmembrane domain, which suggests that these enzymes are also targeted to the Golgi.

PROTEIN TRAFFICKING

Natasha Raikhel (University of California—Riverside) discussed genetic and biochemical approaches toward elucidating secretory pathways in plants. Cargo proteins destined for plant vacuoles contain positive sorting signals that lead to their segregation from the default secretory pathway to the cell surface. Two of these sorting signals, an N-terminal propeptide (NTPP) and a C-terminal propeptide (CTPP) are directed to the vacuole by separate and distinct pathways. Raikhel's group has isolated 15 putative mutant lines in the CTPP pathway, which appears to be unique to plants. Proteins that carry NTPPs are sorted by NTPP receptors at the trans-Golgi network (TGN) and packaged into clathrin-coated transport vesicles. The vesicles are transported to the prevacuolar compartment (PVC), which subsequently fuses with the vacuole. This process is similar but not identical to protein trafficking to the mammalian lysosome and the yeast vacuole. Correct targeting of NTPP vesicles to the PVC most likely involves SNARE proteins. In order to understand the vacuolar trafficking step between the TGN and PVC, Raikhel's laboratory has isolated two related SNARE genes from Arabidopsis: *AtVTI11* and *AtVTI12.* Although closely related, *AtVTI11* and *AtVT112* complement different yeast *vtil* mutants and in Arabidopsis, are located on different organelles in the endomembrane system and form complexes with different SYPs (syntaxins).

Klaus Pälme's (University of Freiburg, Germany) laboratory is studying a 8-member family of genes encoding auxin efflux carrier proteins (PINs) in Arabidopsis. Arabidopsis PIN1 is localized at the basal end of elongated parenchymatous xylem and cambial cells of inflorescence axes. In roots, AtPINI is expressed in phloem parenchyma cells, and located at the apical cell ends. During lateral root formation, the polarly oriented AtPINI protein becomes reoriented to the new basipetal axis. AtPIN2 is involved in root growth and gravitropism. AtPIN3 has a predominantly lateral localization in shoot hypocotyls and stems suggesting a role in lateral auxin distribution and in mediation of tropic responses. Gravity induced root curvature has long been considered to be regulated by differential distribution of the plant hor-

mone auxin. However, the cells establishing these gradients, and the transport mechanisms involved, remain to be identified. A green fluorescent protein (GFP)-based auxin biosensor with cellular resolution was developed to monitor auxin during Arabidopsis root gravitropism. These studies suggest that elevated auxin levels occur at the root apex in columella cells, the site of gravity perception, and that an asymmetric auxin flux occurs from these cells to the lateral root cap and toward the elongation zone, upon gravistimulation. Live cell auxin imaging provides unprecedented insights into gravity regulated auxin flux at cellular resolution, and strongly suggests that this flux is a prerequisite for root gravitropism.

Ranjan Swarup (Bennett Lab, University of Nottingham, UK) also reported on studies of auxin transport auxin influx (AUX1) and efflux transporters (PINI). In contrast to PIN1 that is localized to the lower PM face of root stele cells, AUX1 is asymmetrically localized to the upper plasma membrane (PM) face of protophloem cells but exhibits a nonpolar distribution in the PM of lateral root cap cells. Colocalization of AUX1 and PIN1 has revealed that PIN1 adopts an apical distribution in protophloem cells in contrast to the basal localization of AUXI. Although PIN1 is localized normally in *axr4* mutants, the polar targeting of AUX1 to the upper PM face of protophloem cells is selectively disrupted. An epitope-tagged version of AUX1 was apparently mistargeted and accumulated intracellularly in unidentified compartments.

Gerd Jürgens (Universität Tübingen, Germany) updated the audience on the *gnom* mutant of Arabidopsis that exhibits a loss of cell-to-cell alignment along the embryonic axis, lacks embryonic roots, and often displays a fusion or deletion of cotyledons. These phenotypic abnormalities are reminiscent of the effects elicited by high doses of auxins or inhibitors of auxin transport. GNOM encodes a GDP/GTP exchange factor for small G proteins of the ARF class (ARF-GEF) that plays an important role in the recruitment of vesicle coats for vesicle budding and cargo selection. Jürgens presented evidence that GNOM is a brefeldin A-sensitive ARF-GEF that is required for the proper localization of PIN1. Both PIN1 localization and auxin transport are also sensitive to BFA. When an engineered, BFA-resistant version of GNOM was transgenically introduced into Arabidopsis, PIN1 localization and auxin transport were no longer sensitive to BFA, whereas the trafficking of other proteins remained sensitive to BFA. These results indicate that GNOM is required for the recycling of auxin transport components and suggest that ARF-GEFs regulate specific endosomal trafficking pathways.

The laboratory of Erik Nielsen (Danforth Plant Science Center, St. Louis) has focused on Rab GTPases, a class of regulatory molecules that control membrane trafficking in eukaryotes, as a mechanism to identify endomembrane compartments that are involved in cell wall biogenesis in Arabidopsis. To this end, they have examined the function of a plant Rab GTPase (AtRabA4b) during polarized expansion in developing root hair cells. When AtRabA4b is fused with yellow fluorescent protein, the resulting fluorescent complex localizes specifically to the tips of growing root hair. Interestingly, this localization disappears in mature root hair cells that have stopped expanding. Similarly, tip localization of the complex is abolished if expansion is inhibited by disruption of the Ca^{2+} gradient in the tube or by the depolymerization of actin cytoskeletal elements. Nielsen proposes that AtRabA4b is localized to a post-Golgi compartment involved in the polarized deposition of new cell wall during tip growth in root hair cells.

SIGNAL TRANSDUCTION

Plants must respond appropriately to a wide range of continuously changing internal and external signals. The numerous and interacting signal transduction mechanisms that plants employ in various process were the subject of many presentations.

Zhenbiao Yang (University of California—Riverside) discussed Rop GTPase signaling networks in Arabidopsis. Rop is a plant-specific subfamily of Ras-related Rho GTPases conserved in all eukaryotic cells. Consistent with the lack of Ras and the scarcity of heterotrimeric G proteins in plants, the Rop subfamily, which consists of 11 members in Arabidopsis has emerged as a pivotal master switch that regulates signaling in a wide range of processes ranging from cell polarity development and cell growth through hormone responses to responses to biotic and abiotic stresses in plants. The ability of ROP GTPases to control various signaling pathways in part lies in a class of structurally divergent ROP target proteins named RICs. Yang's talk focused on a signaling network mediated by the pollen-specific ROP1 GTPase. ROP1 controls polarized growth by modulating F-actin dynamics in the tube tip by two downstream pathways that are respectively controlled by RIC3 and RIC4 and which interact with each other in a spatially and temporally coordinated manner.

The laboratory of Qingxi J. Shen (University of Nevada, Las Vegas) is using bioinformatic approaches to explore how GA and ABA signals are transduced in rice aleurone cells. To facilitate the prediction of ABA and GA responsive genes in rice, Shen and his colleagues have developed methods to identify gene structure and to extract coding sequences as well as promoter and terminator regions throughout the *japonica* and *indica* rice genomes. Using a well-established cis-acting element module found in those elements mediating GA and ABA responses, Shen and his co-workers have identified 600 and 1000 rice genes that appear to be responsive to GA and ABA, respectively. Seventy seven of these

genes belong to the plant specific WRKY gene superfamily, which has been shown to be involved in both abiotic and biotic stress responses. The expression of some of the *WRKY* genes is altered by both hormones whereas others are affected by one and not the other.

Little is known about the molecular mechanisms of cell fate determination, cell patterning, and cell signaling in the anther. Using the transposon mutagenesis system, Dazhong Zhao (Ma Lab, Pennsylvania State University, University Park) screened Ds insertional lines for mutants defective in the anther development in Arabidopsis. One of the mutants identified is *ems1* (*excess microsporocytes1*): It produces excess microsporocytes but has no tapetal cells. Although male meiotic nuclear division normally occurs in the mutant, meiotic cytokinesis is arrested, resulting in the failure of microsporogenesis and male sterility. The cloning of the *EMS1* gene revealed that it encodes a putative, Leu-rich repeat receptor protein kinase. Further analysis has shown that EMS is a membrane protein that has autophosphorylation activity, and that *EMS1* expression is associated with the differentiation of the microsporocytes and tapetal cells. Zhao proposes that the EMS1 receptor kinase is involved in a novel signal transduction pathway that signals anther cell differentiation in Arabidopsis.

Sarah Assmann's (Pennsylvania State University) presentation focused on two abscisic acid (ABA) signaling pathways in guard cells. The first is mediated by an ABA-activated protein kinase (AAPK). In guard cells expressing a dominant negative version of AAPK, the stomata failed to close in response to ABA and there was a loss of the normal ABA sensitivity of guard cell anion channels. In the nucleus, AAPK phosphorylates a single-stranded RNA binding protein (AKIP1) changing its subnuclear localization and altering its affinity for dehydrin mRNA. Thus, in addition to its well-established role as a transcriptional regulator, ABA also appears to be involved in posttranscriptional gene regulation in this system. Assmann also spoke about the role of heterotrimeric G proteins in ABA signal transduction. Previously, her lab has used pharmacological tools to implicate G-proteins in guard cell K^+ channel regulation. Experiments on lines of Arabidopsis harboring T-DNA knockouts of the sole G protein α-subunit gene (*GPA1*) indicate that GPA1 plays a central role in ABA regulation of stomatal apertures via modulation of K^+ and anion channel activity. Evidence was also presented implicating the novel lipid metabolite, sphingosine-1-phosphate, as an intermediary in G protein-mediated ABA signaling.

June M. Kwak's (Schroeder Lab, University of California—San Diego) presentation also related to the mechanisms underlying ABA regulation of stomatal function. Previous pharmacological research implicated type 2A protein phosphatases (PP2As) as both negative and positive regulators of ABA signaling. The laboratory is attempting to elucidate the molecular mechanisms underlying these proposed counteracting PP2As. A T-DNA insertion in one of guard cell-expressed PP2A genes (*rcn1*) rendered the guard cells and their anion channels insensitive to ABA. Calcium imaging analyses also showed that ABA-induced cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) elevations showed reduced sensitivity in *rcnl*, whereas signal transduction steps downstream of the ABA-induced increases in $[Ca^{2+}]_{cyt}$ increases were unaffected. Kwak also presented data concerning NADPH oxidase disruption mutations that suggest a central role for NADPH oxidase in ABA activation of reactive oxygen species-activated Ca^{2+} channels and stomatal closure.

Jen Sheen (Harvard University, Boston) discussed the functional genomics of mitogen-activated protein kinase (MAPK) signaling cascades in plants. MAPK cascades are evolutionarily conserved signaling modules that connect diverse receptors/sensors to cellular and nuclear activities in diverse eukaryotes. Numerous studies have shown that plant MAPKs are activated by abiotic stresses, pathogens and pathogen-derived elicitors, and plant hormones. The Arabidopsis genome has 90 genes encoding putative MAPKs and their immediate upstream regulators, MAPKKs and MAPKKKs, the largest among all sequenced eukaryotes. However, little is known about the constitution of plant MAPK cascades and the specific roles that particular MAPK cascade genes play in particular plant signal transduction pathways. Sheen's group is attempting to determine the function of all Arabidopsis MAPK cascade genes using a comprehensive approach based on genomic information, transient expression assays, and transgenic and genetic analyses

Xavier V. Daniel (Tobin Lab, University of California—Los Angeles) discussed the role of phosphorylation of CCA1 (a Myb-related transcription factor) by the protein kinase CK2 and the role of this process in controlling the circadian clock of Arabidopsis. CCA1 interacts in vitro with both α-subunits and β-subunits of the protein kinase CK2, and the reconstituted CK2 enzyme can phosphorylate CCA1 protein in vitro. Furthermore, whole-cell extracts from Arabidopsis plants contain a CK2-like activity that can phosphorylate CCA. Finally, overexpression of a β-subunit of CK2 shortened periods of circadian rhythms. These results suggest that the effects of CK2 on circadian rhythms may involve phosphorylation of CCA1. Daniel has been using a site-specific mutational strategy to test whether phosphorylation of CCA1 by CK2 is a mechanism that is normally used as a regulatory element in the circadian clock of Arabidopsis. He has identified Ser5 and Ser6 of CCA1 as phosphorylation targets of CK2. Sequence analysis of the CCA1 protein revealed four additional Sers in conserved CK2 motifs that could be targets for CK2 phosphorylation. When all six of these Ser residues were mutated and changed to Ala residues, the mutated form of this protein was a poor

substrate for CK2 phosphorylation. Research is ongoing to determine if the phosphorylation of CCA1 is important in refulating circadian rhythms. If so, then the circadian of plants overexpressing the unphosphorylatable mutant form of CCA1 should be different from wild-type.

Joanne Chory (Salk Institute, La Jolla, CA) reviewed new insights into how plants regulate and coordinate the expression of the nuclear and plastid genes that encode components of the plastid. Plastid genomes encode 60 to 80 proteins in higher plants, and more than 3500 nuclear genes are predicted to encode chloroplast proteins in Arabidopsis. In some cases, nuclear gene products control the regulation of genes in the chloroplasts and plastid gene products affect the regulation of certain genes in the nucleus. At least three different signaling pathways have been identified that originate in the plastid and control nuclear gene expression, but the molecular mechanism underlying this control is only now being revealed. Chory presented evidence that Mg-protoporphyrin, an intermediate of chlorophyll synthesis, acts as a repressor of the expression of several nuclear photosynthetic genes. The repressing effects of Mg-protoporphyrin require the protein GUN4. GUN4 activates Mg-chetalase, an enzyme that produces Mg-protoporphyrin.

PROTEASOME

Several participants discussed the role of protein degradation in signal transduction processes. Xing Wang Deng (Yale University, New Haven, CT) discussed the role of protein degradation during phototomorphogenesis in Arabidopsis seedlings, a process that is characterized by a reduction of hypocotyl growth, enhanced cotyledon expansion and unfolding, and development of the photosynthetic machinery. COP1 (constitutive photomorphogenic 1) is a nucleo-cytoplasmic regulatory protein that represses photomorphogenesis in darkness. Loss-of-function *cop1* mutants result in dark-grown seedlings phenotypically similar to light-grown wild-type seedlings. Eleven pleiotropic *COP* loci have been identified. Among them, *COP1* is the master repressor of photomorphogenic development. It acts within the nucleus as an E3 ligase that targets transcription factors involved in photomorphogenesis for degradation by the 26S proteasome in darkness. Light inactivates *COP1* and causes a reduction in its nuclear abundance. Most of the remaining *COP* genes encode for subunits of a highly conserved multisubunit protein complex, the COP9 signalosome that promotes deconjugation of a ubiquitin-like protein(Nedd8) from certain E3 ligases.

Joe Ecker (Salk Institute) discussed new developments in our understanding of *ethylene-overproducing* (*eto*) mutants of Arabidopsis. The dominant ethylene-overproducing mutant *eto2* was found to be the result of an alteration of the carboxy terminus of the 1-aminocyclopropane-1-carboxylate synthase that is encoded by *ACS5* and that serves as the first enzyme in ethylene biosynthesis. Eckert focused his discussion on the *ethylene-overproducing1* (*eto*)-1 gene product that apparently also interacts with the C terminus of ACS5. Pharmacological evidence supports the idea that ETO1 may target ACS5 to the proteasome. A high degree of phosphorylation apparently decreases the affinity of ETO1 for ACS5.

Zhiyong Wang (Carnegie Institution) discussed the role of protein degradation in responses mediated by brassinosteroid (BR). BR signal transduction is mediated by the cell surface receptor kinase BRI1 and is negatively regulated by the GSK3/SHAGGY-like kinase BIN2. BZR1 is a downstream signaling component that mediates both BR-regulated growth response and feedback inhibition of BR biosynthesis. BR treatment induces accumulation and dephosphorylation of the BZR1 protein. The BIN2 kinase directly interacts with and phosphorylates BZR1 in vitro and negatively regulates BZR1 protein accumulation in vivo. Experiments using a proteasome inhibitor suggest that phosphorylated BZRI is degraded by the proteasome. These results indicate that BIN2 phosphorylates BZR1 and targets BZR1 for degradation by the proteasome, and that BR signaling causes BZR1 dephosphorylation and accumulation most likely by inhibiting the BIN2 activity. Several putative BZR1-interacting proteins, including a putative E3 ligase and a DNA binding protein have been identified.

Ray Deshaies (California Institute of Technology, Pasadena, CA) provided new insights into molecular regulation of plant proteasomes. The COP9 signalosome (CSN) is a highly conserved eight-subunit protein complex initially defined as a repressor of photomorphogenic development in Arabidopsis. CSN directly interacts and regulated SCF-type E3 ligases, implying a key role in ubiquitin-proteasome-mediated protein degradation. CSN cleaves Nedd8 from the Cul1 (cullin) subunit of SCF ubiquitin ligases. The JAMM motif underlies CSN's Nedd isopeptidase activity. Among the other proteins that contain a JAMM motif is the Rpn11 "lid" subunit of the 26S proteasome. Although ubiquitin is recycled from proteasome substrates, the molecular basis of deubiquitination at the proteasome and its relationship to substrate degradation remain unknown. Deshaies reported that mutation of the predicted active site histidines in the JAMM domain of Rpn11 to Alas (rpn11AXA) was lethal and stabilized ubiquitin pathway substrates in yeast. Rpn11AXA mutant proteasomes assembled normally, but failed to either deubiquitinate or degrade a ubiquitinated protein (Sic1) in vitro. These findings reveal an unexpected coupling between substrate deubiquitination and degradation, and suggest a unifying rationale for the presence of the lid in eukaryotic proteasomes.

CHEMICAL GENETICS

Steve Briggs (Torrey Mesa Research Institute, San Diego) discussed how more and more investigators are now turning to quantitative genetic analysis and chemical genetics to gain insights into traits. Chemical genetics essentially substitutes a chemical treatment for a true mutation (i.e. the chemical produces phenocopies of a mutant). A library of chemicals capable of causing such plant phenotypes is analogous to a population of mutagen-derived mutants. The chemical library is used to identify plants with a desired phenotype, similar to doing a mutant screen. The advantages of chemical genetics include the ability to apply the chemical at selected times, tissues, and doses, the possibility of affecting simultaneously all members of a type of protein or other target, and the ability to make derivatives with differing selectivity and potency. Briggs described how a bead-based assay of gene expression made it easy to find chemicals that selectively induce members of the plant defense pathway.

Tom Kirchhausen (Harvard Medical School, Boston) provided many in the audience with their first glimpse of the stunning images produced by time-resolved 3D image acquisition of living cells. Kirchausen also explained how he had used two high-throughput visual microscopy techniques to screen 18,000 chemicals for possible effects on cell trafficking in vivo. These techniques were able to screen hundreds, even thousands, of chemicals per hour. Several promising drugs were discussed that add greatly to the pharmacological tools available for studying cell membrane trafficking.

A chemical genetics approach was also employed by Yunde Zhao (University of California—San Diego) to identify chemicals that affect auxin biosynthesis. This approach led Zhao to rediscover sirtinol, a molecule originally described in the literature as an inhibitor of vascular development. Zhao found that sirtinol leads to the increased expression of auxin-inducible genes and induces various auxin-related phenotypes (e.g. the inhibition of root elongation and the stimulation of adventitious rooting). A sirtinol-resistant (*sir1*) mutant has been isolated that, in addition to its resistance to sertinol, also has fewer lateral roots. The cloning of *sir1* should lead to important new insights into the regulation of auxin-inducible genes.

VISUAL MICROSCOPY

To achieve an understanding of plant function that is comprehensive enough to allow for the predictive modeling of plants by systems biology-based approaches, it is necessary to obtain detailed information concerning the expression of genes and the activity of proteins in every cell of the plant. Because these measurements often involve reiterative measurements, there is a real need to develop high-throughput microscopy techniques that can measure and record fluorescence with sufficient spatio-temporal resolution. One of the highlights of the symposium was a tour of the CEPCEB's core facility that houses state-of-the-art microscopy instrumentation. The academic coordinator of this microscopy facility, David G. Carter (University of California—Riverside), also gave a lecture wherein he described specifically some of the new imaging tools available, discussed their benefits and limitations, and described protocols which can be used to evaluate and exploit their performance. Carter described a fully automated microscopy system that can visit every well in a microtiter plate, automatically find the best plane of focus, and then take series of multichannel images.

Venugopata R. Gonehal (Meyerowitz Lab, California Institute of Technology) presented a practical demonstration of how such microscopical techniques can be used to address a biological question. He described a new microscopical method employing intracellular tags that allows for the high-resolution visualization of cells in the developing shoot apical meristem of Arabidopsis.

SYSTEMS BIOLOGY APPROACHES

The primary goal of plant systems biology is to achieve a knowledge of plant function that is comprehensive enough that useful models predictive of plant responses can be generated. Modeling provides a means for understanding the integrated behavior of organizationally complex systems in relation to their underlying molecular determinants. Large amounts of complex data may be integrated to aid in experimental design and testing of hypotheses, pinpoint missing components and pathways, and elucidate underlying biological design principles.

The powerful predictive abilities of systems biology were underscored by Timothy Galitski's (Institute for Systems Biology, Seattle) talk on the signaling network controlling the developmental transition of the yeast (*Saccharomyces cerevisae*) to a filamentous form under conditions of low nitrogen. He discussed how interacting proteins and protein complexes are organized into networks of modules, and how the elements of the signaling network controlling the yeast developmental transition to a filamentous form were clustered. His model identified module organizer proteins and module connector proteins that appear to be important for the transition process to occur, as well as a previously unknown or underappreciated role for peroxisomes in this process.

Mary Wildermuth (University of California–Berkeley) discussed the application of modeling toward understanding plant pathogenesis. In particular, her work focused on the biosynthesis of plant defense molecules (e.g. salicylic acid, jasmonic acid, and ethylene) in Arabidopsis in response to powdery mil-

dew (*Erysiphe orontii*) and the regulatory and metabolic pathways and networks mediated by these molecules. She described how coupled experimental and computational approaches (including DNA microarray analysis, metabolic profiling, and the identification/discovery of cis-acting regulatory elements) are being employed to look for functional patterns that can then be integrated into a formal model. Eventually this formal model will be extended to examine what elements and strategies are shared in different pathosystems and how the regulatory and metabolic networks of hosts and pathogens are integrated.

One of the great challenges of postgenomic era is how to interpret the immense data sets that are currently being generated as a coherent model. Laurence Lejay (Coruzzi Lab, New York University) discussed systems based approach to designing and exploring experimental spaces in Arabidopsis that allows them to build models describing how multiple input signals intersect to regulate genes in pathways. To explore the interactions of light, C and N signaling, they employed a tool initially developed for software testing called Combinatorial Design that enabled them to explore a large experimental space of combinations of light, carbon and nitrogen, using a small number of treatments. Initially, they considered six input signals; light, carbon, inorganic N, Gln, Glu and starvation. If the value of each input is binary, examining all possible combinations would result in 64 treatments. This experimental space would grow exponentially if every input were represented by three different concentrations (e.g. 729 samples). Combinatorial design reduced the number of the experimental treatments to a small number that would systematically sample and effectively cover the same experimental space. In the case of the 64 combinations, combinatorial design reduced the experiments to 6, while it reduced the 729 treatments necessary in the second example to a mere 14. The Combinatorial Design results were modeled into regulatory circuits using Boolean logic. Because of its parsimony, combinatorial design should enable scientists to model genome-wide regulatory circuits efficiently.

The growth of shoots from their apical meristems is an incompletely understood and incompletely described aspect of plant development. In the past, the laboratory of Elliot M. Meyerowitz (California Institute of Technology) has identified regulatory genes necessary for a proper relationship between cell division rates and cell differentiation in the shoot apical meristem. The genes identified (among others, a receptor kinase and its ligand) indicate that an important process is cell-to-cell communication between adjacent meristematic regions. His lab is developing new optical methods to visualize in four dimensions the exact domains of gene expression and patterns of cell division in the meristem, and are starting to use this information to produce an in silico model of a shoot apical meristem.

Peter V. Minorsky
Department of Natural Sciences
Mercy College
Dobbs Ferry, NY 10522