

# Plant Signaling 2000. Cross Talk Among Geneticists, Physiologists, and Ecologists<sup>1</sup>

Daniel J. Cosgrove\*, Simon Gilroy, Teh-hui Kao, Hong Ma, and Jack C. Schultz

Departments of Biology (D.J.C., S.G., H.M.), Biochemistry and Molecular Biology (T.K.), and Entomology (J.C.S.), Pennsylvania State University, University Park, Pennsylvania 16802

Plants respond in complex ways to their environment, to their internal physiological status, and to the activity of other plants, pathogens, herbivores, and organisms. Plant Signaling 2000, a symposium sponsored by the Penn State Intercollege Graduate Program in Plant Physiology (May 18–20, 2000), explored the machinery underlying these responses and their potential for cross talk. We recount here some of the major themes emerging from this interdisciplinary symposium, which ranged from genetic and biochemical analyses of signaling pathways in *Arabidopsis* and other model plants to field studies of plants responding to insect damage.

Signaling mechanisms underlying defense and development were the focus of this recent symposium. Interacting signal transduction processes are involved in many plant defense mechanisms against pathogenic bacteria and fungi, including the hypersensitive response, production of reactive oxygen species (ROS), systemic acquired resistance (SAR), and induction of pathogen-related (PR) genes. Lipid signaling via the octadecanoic pathway is of widespread importance for defenses against insect herbivory. Selective gene silencing has emerged as a key defense against viruses. In development, signals within and between cells regulate growth and differentiation in highly coordinated ways, in some cases using cellular and molecular signaling components very similar to those used in defense (e.g. transmembrane receptor kinases, mitogen-activated protein [MAP] kinases, and  $\text{Ca}^{2+}$ ). Participants in Plant Signaling 2000 were treated to a broad spectrum of lectures pertaining to recent advances in our understanding of these complex and interrelated processes.

## DETECTION OF PATHOGENS

In many plant species, ecotype differences in resistance to pathogens suggest a large natural variation in the recognition systems that detect pathogens. In gene-for-gene resistance systems, a specific host *R* gene is needed to detect a specific pathogen avirulence factor. Many *R* genes have been identified and their encoded proteins fall into classes such as protein kinases or transmembrane proteins with Leu-rich repeats (LRRs). One major class of *R* genes encodes for proteins that contain LRRs and a

nucleotide-binding site. *Arabidopsis* contains approximately 300 genes in this class including *RPM1*, an *R* gene that results in resistance to *Pseudomonas syringae*.

Pablo Tornero and coworkers at the University of North Carolina (Raleigh) used a novel screen to identify additional genetic components of the *P. syringae* resistance system in *Arabidopsis*. Mutants such as *Ira4* showed resistance but failed to show a normal hypersensitive cell death response, whereas *Ira5* showed a normal hypersensitive response, but had no resistance to *P. syringae* or to other normally avirulent pathogens. It is conceivable that *Ira5* might be a master regulator of a suite of resistance characteristics in *Arabidopsis*. Tornero's research indicates that gene-for-gene resistance is a complex process in which resistance genes not only determine specificity, but also function in cross talk with other resistance specificity systems.

In rice, bacterial blight is caused by *Xanthomonas oryzae* pv *Oryzae* (*Xoo*). Pamela Ronald (University of California, Davis) and coworkers identified a gene (*Xa21*) from a wild rice species that confers race-specific resistance to *Xoo*. *Xa21* is a receptor kinase with an extracellular LRR domain and a cytoplasmic domain with Ser/Thr kinase activity. The LRR domain likely interacts with a pathogen-produced ligand, possibly a protein encoded by the *avrXa21* locus, and this binding leads to subsequent activation of an intracellular kinase cascade, resulting in plant defense responses such as oxidative bursts, hypersensitive cell death, and defense gene activation.

Ronald also showed that the overexpression of the *Arabidopsis* NPR1 gene in rice increases resistance to *Xoo*. NPR1 is a key regulator of salicylic acid (SA)-mediated SAR, which is triggered by pathogens and chemicals and induces broad-spectrum immunity in plants. Using the yeast two-hybrid system, two classes of proteins that interact with NPR1 were isolated: four bZIP proteins and a Pro-rich NRP inter-

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\* Corresponding author; e-mail dcosgrove@psu.edu; fax 814-865-9131.

actor (PN1). Ronald proposed that the binding of the bacterial ligand to Xa21 triggers not only a cell death pathway, but also results in the production of ROS which, in turn, activates the SA-mediated signaling pathway involving PN1, NPR1, and BZIP proteins. These proteins then activate defense-related genes and SAR, thereby increasing resistance to *Xoo*.

## CELLULAR SIGNALING IN RESPONSE TO PATHOGENS

Plants respond to certain pathogens and specific elicitors with a burst in the production of ROS such as oxygen radicals, H<sub>2</sub>O<sub>2</sub>, and related substances. This oxidative burst, which occurs at the cell surface, is thought to function as a signal for downstream defense responses by the plant and to participate directly in chemical reactions that cross-link the cell wall and attack pathogen surfaces, thereby limiting the progress of invasion.

Philip Low (Purdue University, West Lafayette, IN) characterized the signaling pathway from initial perception of the elicitor oligo-GalUA (OGA) by soybean suspension cells to the subsequent activation of the membrane oxidase complex that produces ROS. Within 1 min of OGA application, two MAP kinases (44 and 47 kD) are transiently activated with a time course that closely parallels the oxidative burst. These proteins are Thr kinases that are activated by Tyr phosphorylation. The kinase inhibitors K252a and staurosporine specifically inhibited the 47-kD kinase *in vitro*, and the activation of both kinases *in vivo*. These findings suggest that the 47-kD kinase is upstream of the 44-kD kinase. The activation of the 44-kD kinase has a requirement for an increase in intracellular Ca<sup>2+</sup>, leading to the proposed pathway: Elicitor receptor → MAP kinase kinase → 47 kD MAP kinase → Release of intracellular Ca<sup>2+</sup> → 44 kD MAP kinase → Activation of the oxidase complex.

Application of OGA produced a transient rise in cytoplasmic Ca<sup>2+</sup>, which was measured in tobacco cells expressing aequorin, a Ca<sup>2+</sup>-dependent luminescent protein. Low found that the Ca<sup>2+</sup> source, whether it was from external or internal sources, made a significant difference in the cellular responses. Low also reported that oxalate inhibits the oxidative burst by a mechanism that does not involve chelation of calcium or inhibition of calcium signaling.

SA plays a critical role in plant disease resistance. Daniel Klessig (Waksman Institute, Rutgers University, Piscataway, NJ) demonstrated the multifaceted nature of SA's activity by describing its interactions with a growing number of proteins. In addition to the H<sub>2</sub>O<sub>2</sub>-scavenging enzymes ascorbate peroxidase and catalase, new SA-binding proteins are being discovered, some with much greater affinity for SA. An SA-induced MAP kinase (SIPK) activated by pathogens, elicitors, and wounding is among these pro-

teins. Work with the SA-insensitive mutant *npr1* indicates that NPR1 interacts with one or more bZIP transcription factors to induce PR-1 expression. Klessig placed SA in the disease-response pathway between the rapidly elicited changes in intracellular Ca<sup>2+</sup> and later gene induction events.

Klessig pointed out that pathogen-induced nitric oxide (NO) activates SIPK, but does not activate wound-induced protein kinases. SIPK activation depends on SA, as shown using SA-deficient (*nahG*) transgenic plants. This observation highlighted one of the central issues of the meeting and a major interest among attendees: the potential for cross talk versus exclusivity between disease- and wound (herbivore)-induced signaling pathways. In this light, Klessig pointed out that NO inhibits aconitase in plants, as it does in animals. Because the cytosolic aconitase in animals regulates free iron levels, the plant aconitase in the presence of NO is likely to influence free radical information via its impact on intracellular iron. The role of NO in regulating SA synthesis and ROS abundance, the fact that NO shares some activation targets with SA, and that SA evidently has the ability to moderate the effects of free radicals, make it clear that these two systems must be partially integrated in developing systemic resistance to disease.

Chris Lamb (John Innes Centre, Norwich, UK) highlighted the historical focus on H<sub>2</sub>O<sub>2</sub> and SA in the oxidative burst and resistance development, but then pointed out that the temporal and quantitative fit between the signals and the result is not very good. In Lamb's view, NO is in the right place, at the right time, in the right amounts to be a critical regulatory element in the development of the oxidative burst and pathogen resistance. For example, a spreading oxidative burst up-regulates phenylpropanoid synthesis, but too slowly to be a major signaling factor during SAR development, whereas NO acts faster and is a more likely signal. In fact, inhibiting NO synthesis (with exogenous inhibitors or transgenes) blocks defense responses despite an enhanced oxidative burst. This finding suggests that some balanced interaction among putative signals (peroxides, NO, and superoxides) is necessary for effective programmed cell death and SAR, or that additional critical signals are controlling elements.

Lamb also reported on a constitutive disease resistance mutant (*cdr1-D*) from *Arabidopsis* in which expression of PR proteins and other defense genes is elevated, a pattern suppressed in *nahG* crosses. The CDR1 sequence resembles an extracellular Asp proteinase. This resemblance, plus the fact that proteinase inhibitors can block resistance, suggests that an Asp proteinase-released peptide may be an important pathogen resistance signal. A constitutive disease sensitivity mutant (*cds1*) is defective in a gene that also appears to encode an Asp proteinase, strengthening this concept. Lamb put this observa-

tion in the context of a model in which a CDR1-produced peptide elicitor increases phenylpropanoid production, including SA. This produces pathogen resistance, but inhibits the octadecanoid pathway (which is central to wound responses), which in turn depresses insect resistance. These findings and other results indicate a mutually inhibitory cross talk between pathogen- and insect-resistance pathways and emphasize the complexity of the highly branched and interacting regulatory systems involved in these responses.

#### LIPID SIGNALING IN PATHOGEN AND HERBIVORE (WOUNDING) RESPONSE

Lipids serve as important signals in plant cell death and resistance to insects and pathogens. Richard Bostock (University of California, Davis) pointed out that sphingolipids, which regulate programmed cell death (PCD) in animals, comprise 70% of the lipids of some plant membranes. He showed that sphinganine-analog mycotoxins, which inhibit ceramide biosynthesis from sphinganine, promote PCD in tomato, and that exogenous ceramide can prevent mycotoxin-induced PCD. Moreover, in tomato lines resistant to mycotoxin-induced PCD, the level of ceramide levels remains constant, while levels decline in susceptible lines. Bostock also showed that arachidonic acid metabolism products are potent inducers of PCD. Integrating these results, Bostock suggested a signaling role for lipid metabolites, particularly ceramide-like sphingolipids, in plant PCD.

Bostock also addressed the specificity of plant responses to insect feeding, confirming the preliminary findings of others that octadecanoid products, including jasmonic acid (JA), inhibit portions of the SA-signaling pathway, and that the interaction between JA and SA is key to differential plant responsiveness to insects and pathogens.

Many end products of fatty acid oxidation have received attention as volatile signals among plants or between plants and insects. Willi Boland (Max Planck Institute for Chemical Ecology, Jena, Germany) presented detailed studies of synthesis and signaling functions for these molecules. He showed how the same plant produces different mixtures of volatile terpenes in response to added JA, JA precursors, or phytodecanoic acid. These mechanisms may explain how different insect species, which produce diverse fatty acid-amino conjugates in oral secretions, could elicit different volatiles from their host plants. For example, oral secretions from two species of *Spodoptera* differ chemically and yield different volatile emission patterns from plants. The resulting volatile mixtures in turn may attract other insects, including parasites specific for the inducing insect (as shown by Jim Tumlinson's and Marcel Dicke's groups). Boland showed that the fatty acid component, not the amino group, is critical for activity, suggesting that

the insect may merely be providing fatty acid precursors to the plant pathway.

In the same vein, Ted Farmer (Lausanne University, Switzerland) described quantitative "oxylipin signatures" (octadecanoid linoleic acid and linolenic acid derivatives). This description provided a global picture of signaling in response to mechanical wounding, insect feeding, or pathogen infection, and identified a novel, wound-inducible C16 oxylipin signal, dinor-oxophytodienoic acid. This finding raises the possibility of other C16-derived signaling compounds and reveals the presence of a hexadecanoid pathway for synthesizing JA family members.

Farmer also described a "Lausanne Defense Minichip" to examine Arabidopsis gene expression. This cDNA microarray contains 150 expressed sequence tags corresponding to genes involved in pathogenesis, general defense and oxidative stress, fatty acid signaling and metabolism, aromatic amino acid metabolism, and signal transduction. Farmer and his colleagues developed expression profiles over 15 min to 24 h following mechanical wounding and compared them with the oxylipin signatures over the same time course to associate gene expression with signaling steps. By comparing expression of wound-induced genes in wild-type and a JA-insensitive mutant (*coi1-1*) of Arabidopsis, Farmer was able to classify wound-induced genes as JA dependent or independent. Water stress was found to induce approximately one-third of the genes on the microarray, including both JA-dependent genes and JA-independent wound-inducible genes. Farmer's group found many expression differences between Arabidopsis leaves subjected to mechanical wounding or damaged by the cabbage butterfly (*Pieris rapae*), indicating unique attributes in the chewing methods of insects.

#### AN ECOLOGICAL PERSPECTIVE ON PLANT-INSECT SIGNALING

Much of the complexity revealed in other presentations was placed in an ecological context by Ian Baldwin (Max Planck Institute for Chemical Ecology), who reported on studies of the North American native tobacco *Nicotiana attenuata*. Greenhouse and laboratory studies indicate that the plant responds specifically to the oral secretions of caterpillars with JA-induced nicotine synthesis (and other defenses). He is using mRNA differential display to dissect the molecular events and pathways involved in this response. Fifty of the most abundant transcripts that change when plants were treated with caterpillar oral secretions, mechanical wounding, or JA treatment were characterized. Baldwin predicted that more than 500 transcripts might be altered in the induced plants when rare messages are included. Working with Boland, Baldwin found that the fatty-acyl and amino-acyl conjugates in the caterpillar's oral secre-

tions induce an ethylene burst that then suppresses nicotine production. Because the caterpillar is resistant to nicotine and utilizes it for its own defense, it is not clear whether the insect or the plant benefits more (i.e. by reducing its large nitrogen allocation to nicotine) from this suppression.

## SYSTEMIC GENE SILENCING AND VIRAL DEFENSES

Plants have evolved mechanisms to detect and selectively destroy double-stranded RNA that are produced by either virus-encoded, RNA-dependent RNA polymerase from viral RNA, or by plant-encoded, RNA-dependent RNA polymerase from aberrant RNA. Recent advances in our understanding of transgene- and virus-induced post-transcriptional gene silencing were summarized by David Baulcombe (Sainsbury Laboratory, Norwich, UK). Baulcombe's group has used as a model system transgenic *Nicotiana benthamiana* harboring a green fluorescent protein (GFP) construct driven by the strong 35S-cauliflower mosaic virus promoter to study gene silencing. Systemic silencing of GFP expression can be induced by infection with potato virus X carrying the GFP sequence. Virus-induced gene silencing is similar to the mechanism by which antisense RNA silences expression of endogenous genes in that both phenomena involve production of short antisense RNAs of approximately 25 nucleotides made from the genes being silenced. This silencing is also characteristic of RNA silencing processes ("RNA interference") in *Caenorhabditis elegans* and *Drosophila melanogaster*. RNA silencing appears to involve a change in the epigenetic state of the plant genome (e.g. methylation of the transgene) because silencing may continue even after the virus is removed.

To identify genes required for gene silencing, Baulcombe's group used transgenic Arabidopsis plants carrying silenced GFP genes to screen for "loss of silencing" mutants. A gene (*sde1*) was identified that encodes for an RNA-dependent RNA polymerase. It is interesting that mutations in this gene result in complete loss of transgene-induced gene silencing, but not virus-induced gene silencing, suggesting that these two gene-silencing pathways are not identical.

Baulcombe also described countermeasures employed by some viruses against a plant's silencing mechanisms. For instance, a plant's silencing mechanism can be activated by leaf infiltration with *Agrobacterium* carrying 35S::GFP; a stable, systemic silencing of GFP expression results. Subsequent infection of the plant with potato virus Y reverses GFP silencing in the whole plant, indicating that the virus possesses mechanisms to suppress the plant gene silencing process. In other viruses, such as the cucumber mosaic virus, the reversal of GFP silencing occurs only in new leaves while other viruses such as

potato virus X lack this countermeasure entirely. Results from grafting experiments indicate that viral movement protein is able to block the movement of a plant signal for systemic gene silencing. Thus the game of cat and mouse played by virus and plant continues to grow in depth, complexity, and interest.

As described by William Lucas (University of California, Davis), the phloem provides a rapid and pervasive route for long-distance trafficking of RNA and other macromolecules involved in defense (including gene silencing) as well as in developmental signaling. Sieve cells of angiosperms lack nuclei and in gymnosperms and other plants the sieve cell nucleus may be transcriptionally silent. Thus proteins and nucleic acids found in the phloem are likely to be imported, via plasmodesmata, from companion cells and other similar cells. Analysis of phloem sap shows it to contain more than 400 proteins ranging in size from 10 to 200 kD. Microinjection of fluorescently labeled *Cucurbita maxima* phloem proteins into mesophyll cells led to an increase in the size exclusion limit of the plasmodesmata. This finding indicates that plasmodesmata of various cell types (not just phloem) can recognize and respond to these mobility proteins.

## HORMONE SIGNAL PATHWAYS

Joseph Ecker (University of Pennsylvania, Philadelphia) described recent results from his laboratory on ethylene signaling and biosynthesis in Arabidopsis. Previous work identified a set of genes encoding functionally redundant ethylene receptors, which are transmembrane proteins with a cytoplasmic His protein kinase. To identify new genes involved in ethylene signaling, Ecker's group screened for mutants that responded to trans-cyclooctene, an ethylene antagonist, as if it were ethylene. One of the mutants, called *ran1* (response to antagonist), could still respond to ethylene, indicating it has a broadened specificity. The RAN1 protein shares homology with a Cu<sup>2+</sup> transporter in yeast. Copper deficiency in the *ran1* mutant may result in a relaxed specificity of the ethylene-binding domain of ETR1 and other receptors. This explanation is supported by the fact that higher CuSO<sub>4</sub> in the medium reduces the ethylene-like response of *ran1* to the antagonist. Reduced expression of RAN1 by cosuppression caused a constitutive ethylene response. This finding supports the idea that Cu<sup>2+</sup> deficiency renders all ethylene receptor-linked kinases non-functional, allowing ethylene responses to occur.

Earlier work from Ecker's group concerning ethylene biosynthesis identified several mutations (*eto1-eto5*) that resulted in ethylene overproduction. Molecular studies have provided new insights into the function of some of these genes. The *ETO1* gene has homology to proteins with peptide-binding domains. The *ETO2* gene encodes an isoform of the ethylene biosynthetic

enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS5). ACS5 activity is normally highly regulated; the observation that the *eto2-1* mutation resulted in a C-terminal truncation of ACS5 suggests that the C terminus might play a negative regulatory role. Ecker proposed that the ETO1 protein binds to the ETO2 C terminus and inhibits its activity. This interaction between ETO1 and ETO2 was supported by yeast two-hybrid assays; furthermore, ETO1 does not interact with the *eto2-1* truncated version of ACS5. Two other proteins, EOL1 and EOL2, with high levels of sequence similarity to ETO1, also can bind ETO2 in yeast two-hybrid assays. Moreover, when *ETO2* is expressed in *Escherichia coli*, its activity is inhibited by a co-expressed *ETO1*. An *eto2-2* loss-of-function mutation is epistatic to the *eto1* mutation, leading to less ethylene production than found in the *eto1* single mutant.

Joanne Chory (Salk Institute, La Jolla, CA) discussed recent work from her laboratory on Arabidopsis mutants altered in their perception of or response to brassinolide (BR). Although at least eight Arabidopsis loci have been identified with roles in brassinolide biosynthesis, only a single locus (*BRI1*) has been characterized with a role in perception or response. Chory reported that additional mutants (*bin2-bin5*) with phenotypes suggestive of a defect in BR perception or response have been identified, but not yet characterized.

Although there is strong suspicion that *BRI1* encodes the brassinolide receptor, biochemical support for this idea has been difficult to obtain. *BRI1* encodes a LRR receptor Ser/Thr kinase, one member of a large family of at least 133 genes in Arabidopsis. Most of the *BRI1* mutations are located in a 70-amino acid "island" between two clusters of LRRs forming a predicted extracellular domain. This island is the focus of attention for putative brassinolide binding. Plants expressing *BRI1::GFP* fusion proteins show localization of the GFP fusion to the plasma membrane, consistent with *BRI1* being an extracellular receptor. These plants have longer petioles than controls, suggestive of an enhanced brassinolide growth response. They also have greater brassinolide-binding activity compared with controls, and the binding activity adheres to an anti-GFP antibody. These results indicate that the *BRI1::GFP* fusion is functional and, moreover, that *BRI1* is associated with brassinolide-binding activity. However, whether binding is accomplished by *BRI1* itself or via a complexed protein remains to be seen.

Among the putative LRR kinases in Arabidopsis, two stand out as being very similar to *BRI1* in amino acid sequences and have been named *BRI2* and *BRI3*. The kinase domain sequences of the three *BRI* proteins are about 60% identical; furthermore, the 70-aa island is also present in *BRI2* and *BRI3*, and their sequence identities to the *BRI1* island range from 32%

to 52%. A T-DNA insertional mutation in *BRI3* has been found and the mutant is less sensitive to brassinolide than normal plants; however, the *bri3* mutant exhibits normal development, unlike *bri1* mutants, indicating that the defect of *bri3* in brassinosteroid response is mild. A *bri2* T-DNA insertion at the C terminus has no obviously unusual phenotype, suggesting that the insertion might not affect the *BRI2* gene function. In any case, it is possible that *BRI2* and *BRI3* are functionally redundant, and additional experiments are needed to fully dissect their roles in brassinosteroid response.

Jerome Giraudat (Institute des Sciences Végétales, Gif-sur-Yvette, France) highlighted the molecular genetic analysis of abscisic acid (ABA) signaling in Arabidopsis. Giraudat used infrared imaging to screen for Arabidopsis mutants defective in stomatal closure. More than 30 mutants were isolated, including new alleles of the known ABA biosynthetic genes *ABA1*, *ABA2*, and *ABA3*. Eight of the mutants were altered in ABA response; two of these were recessive alleles of a new locus called *OST1* and one was a dominant mutation, *ost2*. Further analysis of these new mutations promises to contribute to a better understanding of ABA signaling.

Previous work by Giraudat and others demonstrated that the *ABI1* and *ABI2* genes encode members of the 2C class of protein Ser/Thr phosphatases. Dominant mutations in these genes (*abi1-1* and *abi2-1*) reduce the plant's sensitivity to ABA. To elucidate the functions of *ABI1* and *ABI2*, a suppressor screen was conducted. Intragenic revertant mutations were recessive and caused hypersensitivity to ABA, indicating that *ABI1* and *ABI2* are most likely negative regulators of ABA response. Other revertants were found to be new alleles of the ethylene-signaling gene *EIN2*, whereas *CTR1* alleles were identified as *abi1-1* enhancers. The previously isolated ethylene-insensitive *etr1-1* and *ein2-1* mutations were found to increase the sensitivity to ABA, whereas the constitutive ethylene response mutant *ctr1* showed reduced sensitivity to ABA. These results indicate that the ethylene-signaling pathway modulates the control of seed germination by ABA.

Simon Gilroy (Pennsylvania State University) presented evidence for a role for phospholipase D (PLD) in ABA signal transduction. PLD is transiently activated in aleurone cells and guard cells in response to ABA. Such activation is rapid (within 5 min of ABA treatment) and insensitive to protein synthesis inhibitors, suggesting activation of preexisting enzymes, rather than ABA-related induction of PLD gene transcription. The product of PLD action on membrane lipids, phosphatidic acid, induced an ABA-like response; moreover, blocking PLD activity with 1-butanol inhibited ABA action. Thus PLD appears to mediate ABA signaling in these cells. Gilroy also presented results indicating involvement of a G-protein upstream of PLD.

## SEX SIGNALS IN FERNS

Jody Banks (Purdue University) presented a genetic analysis of sex determination in the fern *Ceratopteris richardii*. Unlike seed plants, most ferns are homosporous, with a free-living gametophyte. In *C. richardii*, each spore can develop into either a male gametophyte bearing antheridia or a hermaphroditic gametophyte bearing both antheridia and archegonia. What determines which alternative pathway a spore will take? It turns out that the hermaphrodite releases a sex-determining hormone called antheridiogen ( $A_{CE}$ ). Spores that germinate in the presence of  $A_{CE}$  develop into male gametophytes, whereas those that germinate in the absence of  $A_{ce}$  become hermaphrodites. ABA blocks the response to  $A_{CE}$ . More than 140 mutants, classified into five types, have been identified. The *her* mutants (five loci) form only hermaphrodites, even in the presence of  $A_{CE}$ , whereas the *tra* mutants (two loci) form male gametophytes without  $A_{CE}$ . The *fem* mutants (two loci) form females, but not hermaphrodites or males; the *man* (many antheridia) mutants (one locus) produce an increased number of antheridia; and the *abr* (ABA resistant) mutants (four loci) are insensitive to ABA.

The single mutant phenotypes suggest that the *TRA* genes are either positive regulators of femaleness or negative regulators of maleness or both, and that the *HER* and *FEM* genes have opposite functions from the *TRA* genes. Double mutant analysis indicates that all mutations, except *fem1*, have clear epistatic relationships, allowing the placement of genes in a particular order. The *fem1 tra2* double mutants exhibit an "intersex" phenotype different from either single mutant, with shallow multiple meristems, no archegonia, and very few antheridia. Banks presented a model in which the *TRA*, *MAN1*, *FEM1*, and *FEM2* genes constitute a regulatory loop, with *HER* genes mediating the input from  $A_{CE}$ , and *TRA* and *FEM1* controlling the female and male outputs, respectively.

## PHOTORECEPTOR FOR PHOTOTROPISM

*Arabidopsis* has more than 10 photoreceptors, including the phytochrome family, the cryptochrome family, and the recently identified receptor for phototropism, dubbed "phototropin." Winslow Briggs (Carnegie Institution of Washington, Stanford, CA) described some of the biochemical and photochemical properties of this photoreceptor. The first phototropin gene (*NPH1*) was identified by Briggs and colleagues via an *Arabidopsis* mutant with defects in phototropism and in light-modulated phosphorylation of a membrane protein. *NPH1* is a Ser/Thr protein kinase with two LOV domains, named after their homology to domains responsive to Light, Oxidation state, and Voltage in other proteins.

Briggs reported that expression of *NPH1* in insect cells leads to a functional, blue-light regulated kinase with an excitation spectrum characteristic of a flavin chromophore. When the LOV domains alone are expressed in *E. coli*, their absorption spectrum resembles the action spectrum for phototropism, indicating that the LOV domains by themselves fold correctly and bind chromophore. This allowed isolation of the bound flavin, which turned out to be the cofactor FMN, bound non-covalently in a 1:1 ratio of LOV domain:FMN. Thus the *NPH1* protein with its two LOV domains is a two-chromophore photoreceptor.

Spectral analysis (S. Crosson and K. Moffat, University of Chicago) indicated that light induced the formation of a flavin thioadduct as well as a change in protein conformation. Recent x-ray crystallography data of the LOV domain from a fern phototropin shows that a highly conserved Cys is located in the flavin-binding pocket of the LOV domain. Briggs proposed that signal transduction by this protein begins with light-induced formation of a flavin-Cys bridge and a consequent change in protein conformation, which activates the kinase activity.

## CALCIUM SIGNALING

Changes in cytoplasmic calcium are recognized as a common signaling element in plant and animal cell function. Jeffrey Harper (Scripps Research Institute, La Jolla, CA) described recent insights into how these changes are generated, how information is encoded in the spatial and temporal dynamics of the  $Ca^{2+}$  signal, and how this information is decoded by cellular response elements.

The phenotype of transgenic tobacco lines overexpressing *CAX1*, an *Arabidopsis*  $Ca^{2+}/H^{+}$  antiporter, resemble  $Ca^{2+}$ -starved plants, despite the fact that total  $Ca^{2+}$  in the plant was high. The physiological defects in these transgenic lines can be corrected by  $Ca^{2+}$  supplementation. These results indicate that the endogenous function of *CAX1* is in maintaining low cytoplasmic  $Ca^{2+}$  and reinforce the view that the correct, coordinated regulation of organelle  $Ca^{2+}$  transporter activities is essential in supporting plant cell function. Harper's investigations of the control of the *Arabidopsis* endoplasmic reticulum  $Ca^{2+}$  pump *ACA2* further suggested that such transporters show complex feedback regulation through  $Ca^{2+}$ -dependent regulators such as calmodulin and  $Ca^{2+}$ -dependent protein kinases.

Harper also described use of a GFP-based  $Ca^{2+}$  sensor, named "Cameleon," in plants. When  $Ca^{2+}$  binds to the calmodulin domain of the Cameleon protein it induces a conformational change which can be detected by altered fluorescence resonance energy transfer between its component cyan and yellow fluorescent protein domains. By monitoring the efficiency of fluorescence resonance energy transfer,

$\text{Ca}^{2+}$  levels can be measured. The Cameleon protein can be tailored in its  $\text{Ca}^{2+}$  sensitivity, targeted to specific cell types or organelles, and even fused to signal transduction or  $\text{Ca}^{2+}$ -regulatory elements (e.g. ACA2) to report  $\text{Ca}^{2+}$  levels in the microenvironment of a particular signaling system. This new technology presents exciting opportunities for following  $\text{Ca}^{2+}$  dynamics in plants.

The symposium was unusual in its attempt to bring molecular biologists and ecologists into dialogue around the theme of plant signaling mechanisms. Emerging from this dialogue was an appreciation that cross talk between signaling systems may have important consequences for the plant's subsequent responses to other biological and environmental assaults, and thus its survival and fitness.