The Plant Cell, Vol. 11, 2063, November 1999, www.plantcell.org © 1999 American Society of Plant Physiologists

MEETING REPORT

Highlights from the Ninth International Congress on Molecular Plant–Microbe Interactions

The President of the International Society for Molecular Plant–Microbe Interactions (IS-MPMI), Barry Rolfe, wrote most insightfully in a recent online article about the importance of basic scholarship to the cultural integrity of modern civilized society (http://www.scisoc.org/ismpmi/pubs/99spr.htm). "By participating in high-quality fundamental research," he argued, "first-hand access to the [global] 'club' of knowledge generators" is an advantage that is conferred upon the given nation and, presumably, the individual. For one week this summer (July 25–30), at the Ninth International Congress of the IS-MPMI in Amsterdam, ongoing work amidst the "club of knowledge generators" within the field of plant–microbe interactions was the theme. It is our hope that this Meeting Report will provide early accessibility to some of the Congress highlights.

SIGNAL PERCEPTION AND SIGNAL TRANSDUCTION IN PLANT–MICROBE INTERACTIONS

One of the most significant novel results to be announced at the Congress came from Thomas Boller (Friedrich Miescher Institute, Switzerland). His group has been studying elicitation of defense mechanisms by general components of pathogens, indicating that plants have the capacity to perceive typical fungal structures, including glycopeptides, chitin and ergosterol. Recently, the group has noted that plants also perceive the bacterial motor protein flagellin. Significantly, a highly conserved sequence in the N terminus of most bacterial flagellins is lacking in bacteria that coexist intimately with plants, such as Rhizobium and Agrobacterium (Felix et al., 1999). Synthesis of this peptide sequence (Flg22) created a novel and highly active elicitor that could be used to study the role of Ca^{2+} in the defense response and to document the full array of proteins to be rapidly phosphorylated in response to elicitation in Arabidopsis suspension cultures. Elicitation with Flg22 arrested seedling growth of most Arabidopsis ecotypes, permitting a ready screen of natural and mutagen-induced genetic variation in the response (Gomez-Gomez et al., 1999). Loci designated as *FLS1*, *FLS2*, and *FLS3* were characterized, and the *FLS2* gene was identified by positional cloning. FLS2 is a putative transmembrane protein kinase containing leucine-rich repeats (LRRs), resembling both *Xa21*, the rice gene for resistance to *Xanthomonas* and *CLV1*, the Arabidopsis gene involved in meristem function.

In a conceptually similar study, Dierk Scheel (Leibniz Institute of Plant Biochemistry, Germany) reported on an elicitor of defense responses in parsley cell cultures (PEP13; see Nennstiel et al., 1998) that corresponds to an amino acid sequence shared by many fungal transglutaminases. Purely biochemical approaches to receptor function are still extremely difficult, although Naoto Shibuya and colleagues (National Institute of Agrobiological Resources, Japan) were able to isolate a plasma membrane protein corresponding to the receptor for a glycan elicitor. Scheel has similarly taken a direct approach to the important question of how reactive oxygen species (ROS) are produced during plant defense mechanisms. His group purified an NADPH-dependent superoxide generating enzyme, using a novel gel assay for ROS production, that proved to be a membrane protein with some similarity to ascorbate oxidase.

PLANT–VIRUS INTERACTIONS

Bucking a trend, Jim Carrington (Washington State University, WA) applied a genetic approach to study restriction of viruses involving neither the hypersensitive response (HR) nor systemic acquired resistance (SAR). Long-distance movement of viruses is poorly understood. The C24 ecotype of Arabidopsis supports long-distance transport of tobacco etch virus (TEV), whereas the Col-0 ecotype does not. Engineering TEV to express the BAR gene provided a selection for Col-0 mutants that became resistant to glufosinate herbicide upon systemic infection with the TEV-BAR viral variant (Whitham et al., 1999). In this way, three mutant loci (*RTM1*, *RTM2*, and *RTM3*) were identified. *RTM1* encodes a novel protein with repeats of a sequence found in jacalin (an atypical lectin). This sequence probably plays a role in protein–protein interactions. RTM2 is another unusual protein that shows some similarity to a class of proteins with chaperone activities. These enigmatic gene products, while currently provoking more puzzlement than insight, will take us into new territory as studies on their cell biology develop.

In another approach to studies of plant viruses, David Baulcombe (Sainsbury Laboratory, UK) presented considerable progress on two forms of natural virus resistance. The *Rx* gene in potato mediates an unusually extreme degree of resistance to potato virus X (PVX) that is evident at the single-cell level in the

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absence of a typical HR (Bendahmane et al., 1999). Interestingly, expression of weak avirulence alleles of the coat protein (CP), or delayed expression of the CP, results in a classical HR, suggesting that an early and efficient recognition of PVX results in an effective antiviral response that is distinct from the cell death pathways. This system may be particularly valuable in dissecting the actual antiviral effector molecules controlled by classical *R* genes.

Baulcombe also presented exciting new insights into virus-induced silencing as an antiviral response and as a tool for functional genomics. Post-transcriptional gene silencing–like responses are now known to be activated by both RNA- and DNA-containing viruses, even in the absence of transgenes containing sequences homologous to the virus (Ratcliff et al., 1999). The homology dependence of virus-induced, as well as transgene-induced, silencing suggests that de novo production of nucleic acids with complementarity to the target RNAs must participate in the recognition and/or RNA degradation that occurs during the silencing response. New data suggest that discrete-sized, small RNA molecules of sense and antisense polarity relative to the target RNA are involved (Hamilton and Baulcombe, 1999). Exactly how these small RNAs are synthesized (and possibly processed to a discrete size) is not yet known, although a role for the host RNA-dependent RNA polymerase in this process is a strong possibility.

VIRULENCE AND AVIRULENCE OF BACTERIA AND FUNGI

avr Genes and *Pseudomonas syringae* Virulence

The *avirulence (avr)* genes of *P. syringae* and other phytopathogenic bacteria may interfere with pathogenesis in some plants and promote it in others. The interference results from the triggering of *R* gene–dependent defenses, but the basis for promoting parasitism and virulence is unknown. Jeff Dangl (University of North Carolina, NC) reported an unusual mechanism by which *P. syringae* pv *maculicola* M6 can inactivate an *avr* gene that would otherwise betray the pathogen to the *R* gene surveillance system of Arabidopsis. Strain M6 carries *avrRpm1* in the chromosome and consequently is avirulent in Arabidopsis Col-0, which carries the cognate *Rpm1* resistance gene. However, 3 hr after inoculating Col-0, M6 variants can be recovered that are virulent in Col-0 because of inactivation of *avrRpm1* through excision of the *avrRpm1* chromosomal region and insertion of a native transposon into the *avr* gene. Remarkably, this event can be triggered by a protease- and heat-sensitive factor that is present in the apoplastic fluids of Arabidopsis leaves mounting an *avr–R* gene—dependent defense. Barbara Kunkel (Washington University, MO) reported that *P. syringae* pv *tomato* DC3000 overcomes the partial resistance of Arabidopsis No-0 if the strain heterologously expresses *avrRpt2.* The partial resistance correlated with rapid induction of several plant defenses, including the expression of *pathogenesis-related* (*PR*) genes, and these responses are diminished when *avrRpt2* is expressed in the bacterium. Roger Innes (Indiana University, IN) reported a similar phenomenon involving *P. syringae* pv *tomato* DC3000 and *avrPphB* in Arabidopsis mutants in which *avrPphB–RPS5*—dependent defense signaling is compromised (see Warren et al., 1999). John Mansfield (London University, UK; see Jackson et al., 1999) reported a plasmid-borne block of *avr/vir* genes that contribute to virulence in *P. syringae* pv *phaseolicola.* When the 154-kb plasmid is cured, the race 7 strain elicits, instead of disease, an HR dependent on Hrp (hypersensitive response and pathogenicity) in previously susceptible bean cultivars. One of the genes on the plasmid, designated *virPphA*, could partially restore virulence in bean, or confer an avirulence phenotype in soybean. Thus, some *avr*-like genes can mask the ability of others to elicit hypersensitive defenses, which again suggests a role in defense suppression.

Secretion of Phytopathogenic Bacteria Effector Proteins

The cellular localization of phytopathogenic bacteria Avr proteins is important in pathogenesis because these proteins appear to be the primary effectors of parasitism. There is strong but indirect evidence that many effector proteins are injected into plant cells by the Hrp type III protein secretion system, and there is direct evidence based on cell fractionation and immunoblot analyses that these proteins can be secreted in culture by the Hrp systems of *Erwinia* spp. The secretion of several Avr proteins by the native Hrp systems of *P. syringae* and *Xanthomonas campestris*, bacterial pathogens with well-studied Avr-dependent plant interactions, has recently been observed in the laboratories of James Alfano (University of Nevada, NV; see van Dijk et al., 1999), Ulla Bonas (Martin-Luther University of Halle, Germany), Alan Collmer (Cornell University, NY), Brian Staskawicz (University of California, Berkeley, CA; see Mudgett and Staskawicz, 1999), and Christian Boucher (INRA/CNRS, France). Boucher's group reported that in *Ralstonia solanacearum* transcription of *hrp* genes is induced upon contact of the bacterial cell with the plant cell wall. Type III protein secretion systems are widely important in bacterial pathogenesis, and they are essential for the virulence of *Yersinia* spp and many other animal pathogens. Ulla Bonas showed that *X. c. vesicatoria* can secrete the *Y. pseudotuberculosis* YopE effector protein in culture (Rossier et al., 1999). Alan Collmer

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(Cornell University, NY), in collaboration with Olaf Schneewind (University of California at Los Angeles, CA), reported that *Y. enterocolitica* secretes *P. syringae* pv *tomato* AvrPto through recognition of an mRNA targeting signal that appears to be universal among the type III–secreted effector proteins of plant and animal pathogens (Anderson et al., 1999).

Ashbya gossypii as an Experimentally Attractive Fungal Plant Pathogen

Fungi cause the majority of plant diseases, and well-developed fungal models for genetic study of pathogenic processes include *Ustilago maydis*, *Magnaporthe grisea*, and *Cochliobolus* spp. Tom Gaffney (Novartis, NC) reported progress in developing *A. gossypii* as a new research model with several advantages. The fungus is a pathogen of cotton, tomato, and citrus, with an 8.8-Mb genome that is smaller and less redundant than that of yeast. Sequence analysis of the smallest of the seven chromosomes in *A. gossypii* (680 kb in length) reveals a high degree of synteny with yeast, with most rearrangements occurring at tRNA loci. Importantly, the *A. gossypii* genome can be conveniently subjected to one-step gene knockouts involving homologous recombination with only 45 bp of fungal DNA on either side of the marker.

RESISTANCE GENES

One of the most significant reports of the meeting came from Barbara Valent (Du Pont Company, DE). She reported on the isolation of the rice *Pi-ta* gene that confers resistance to rice blast strains that carry the corresponding *AVR-Pita* gene. *AVR-Pita* encodes an apparent zinc metallopeptidase of 223 amino acids that is putatively processed into a mature 176-residue form (AVR-Pita$_{176}$). *Pi-ta* encodes a member of the familiar nucleotide binding site leucine-rich repeat (NBS-LRR) family of proteins. How can a cytoplasmic NBS-LRR protein confer resistance to a secreted product of a fungal *AVR* gene? Transient expression studies showed that simultaneous bombardment of 35S gene constructs encoding either β-glu–curonidase (GUS) or Avr-Pita$_{176}$ led to eclipse of GUS expression (due to the HR) specifically in *Pi-ta*–containing varieties. Remarkably, yeast two-hybrid analysis provided convincing evidence for a direct AVR-Pita$_{176}$ interaction with the LRR region of the *Pi-ta* gene product. Direct interaction also occurred between the *Pi-ta* gene product (either LRR polypeptide or full-length protein) and membrane-blotted AVR-Pita$_{176}$. In a powerful control, the LRR domain encoded by a *Pi-ta* allele product that did not recognize AVR-Pita$_{176}$ interacted neither in the two-hybrid assay nor during protein gel blot analysis. These data are extremely significant, suggesting that secreted fungal proteins can be recognized by cytoplasmic NBS-LRR–bearing *R* gene products (perhaps after reaching the cytoplasm via a specialized fungal secretion system?) and that such recognition can involve direct interaction between *R* and *AVR* gene products.

Brian Staskawicz (University of California, CA) reported on the isolation of the pepper *Bs2* gene that confers resistance to *Xanthomonas* spp disease. This required a technical tour de force to establish map-based cloning tools in pepper as well as an extraordinary struggle with an intron larger than the capacity of most binary cosmid vectors. The rationale for making this effort lay in an earlier observation that the corresponding avirulence gene, *AvrBs2*, makes a crucial contribution to bacterial virulence. Thus, the *Bs2* gene should provide durable resistance, since loss of *AvrBs2* leads to reduced pathogenicity. Races of *Xanthomonas* spp have been reported that can overcome *Bs2*, but careful analysis in the Staskawicz lab showed that all these strains were indeed less pathogenic and that the bacterium was unable to overcome *Bs2* without losing pathogenicity. This is encouraging, because the *Bs2* gene confers complete resistance to *Xanthomonas* spp on transfer to tomato, which will provide an extremely useful additional method to control this significant tomato disease.

In another technical and intellectual tour de force, Paul Schulze-Lefert (Sainsbury Laboratory, UK) described a molecular analysis of *Mla* gene function in barley. First, map-based cloning led to the identification of three distinct NBS-LRR gene classes, each of which cosegregated with the *Mla* locus. *Mla* encodes an extensive allelic series, and conceivably, some of these "alleles" may be of different gene families. Transient expression analysis will permit the identification of which of these members actually confers resistance in different stocks. Additionally, previous work permitted the identification of the *Rar1* and *Rar2* genes that are required for *Mla*-dependent resistance to powdery mildew (Jorgensen, 1994). The Schulze-Lefert lab has isolated the *Rar1* gene and shown that it is a member of a novel gene family encoding a zinc-binding protein shared by humans and *Caenorhabditis elegans* but not yeast. Intriguingly, in *C. elegans* strains in which the *Rar1* homolog is silenced, some phenotypes suggest perturbation of cell death. However, the most striking effect of mutations in *Rar1* in barley is abolishment of the rapid, *Mla*-dependent burst of ROS in response to incompatible powdery mildew races. The eradication of ROS production is not understood.

R Gene Evolution

R genes belonging to the extracellular LRR or NBS-LRR classes are often clustered at complex loci, which have

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been proposed to serve as repositories for genetic diversity to generate new *R* gene specificities. Jonathan Jones (Sainsbury Laboratory, UK) analyzed *R* gene clusters at the *Cf-4/9* locus in tomato and the *RPP5* locus in Arabidopsis (see Noel et al., 1999). Richard Michelmore (University of California at Davis, CA) similarly reported on the *Dm3* locus in lettuce and the *Pto* locus in tomato (Michelmore and Meyers, 1998). It appears from these analyses that there are two major sources of *R* gene novelty. First, there is divergent selection on solvent-exposed amino acids on the parallel beta sheet surface of the LRR. Second, recombination between *R* gene homologs can create novelty by "shuffling" these recognition surfaces. The rate of *R* gene shuffling almost certainly depends on the specific haplotypes that are paired in *trans.* Interestingly, homogenization of *R* genes by frequent recombination between gene family members at a given locus does not occur, probably because of suppression of mispairing between homologs due to divergence in non-conserved sequences. At some loci it is always possible to detect orthologs when different haplotypes are compared (*Pto* and *Dm3*), but at other loci too much shuffling has occurred for such an assignment to be possible (*RPP5* and *Cf-4/9*; see Parniske et al., 1997). These data provide insight into how plants accomplish two important objectives in gene-for-gene interactions, namely, formation of new *R* genes and *R* gene alleles with novel specificities for new pathogens or races, and maintenance of *R* gene function in the absence of pathogen (i.e., when selective pressure is low).

R Gene Function

At the core of how gene-for-gene type resistance works is the structure and function of *R* genes and *R* gene signaling components. One of the early hypotheses to explain gene-for-gene interactions stated that *R* gene products interact directly with *AVR* gene products. Such a hypothesis is attractive because it provides a direct explanation for the large body of host and pathogen genetic data. However, this hypothesis may be too simplistic because many *R* gene products do not interact with corresponding *AVR* gene products. As shown by Jonathan Jones and Pierre de Wit (Agricultural University of Wageningen, The Netherlands), for example, various preparations of tomato Cf-9 protein failed to show affinity for *Cladosporium fulvum* AVR9. Using suspension cultures of tobacco cells expressing a *myc*-tagged version of the tomato *Cf-9 R* gene, the Cf-9 protein was shown to be glycosylated and associated with the plasma membrane (Jones). A Cf-9–containing protein complex may function to interact with AVR9 on the cell surface. Based on work from the de Wit lab (Kooman-Gersmans et al., 1996), AVR9 might bind to a distinct receptor within the complex. As reported by Keiko Torii (University of Michigan, MI), such a model is reminiscent of *CLV1*- and *CLV2*-mediated signaling to promote differentiation of the shoot meristem in Arabidopsis. *CLV2* looks very similar to *Cf-9* inasmuch as both proteins contain extracellular LRRs and short intracellular domains (Jeong et al., 1999). CLV2 function requires CLV1, a LRR-containing receptor kinase, within a high molecular weight complex containing additional signaling components. Biochemical as well as genetic approaches may prove fruitful to identify key components of the Cf-9 complex and to understand how Cf-9 activates Avr9-dependent defense responses without directly binding AVR9.

Resistance without Death?

Considerable progress in identifying signaling components within the major resistance pathways was reported. However, the relative role of cell death in defense induction and restriction of pathogen growth remains a key question. Andrew Bent (University of Wisconsin-Madison, WI) has begun to genetically differentiate pathogen arrest (using *P. syringae* in Arabidopsis) and the HR that results from the cell death pathway. Arabidopsis mutants with defense-no death (*dnd1* and *dnd2*) phenotypes after inoculation with avirulent *P. syringae* were isolated. These mutants exhibited gene-for-gene specific restriction of bacterial growth using several active *R* gene/*AVR* gene combinations, but without the HR (Yu et al., 1998). The mutants also displayed constitutive SAR, due to elevated salicylic acid (SA) levels, but the *dnd* mutants differ from most other SA-enhanced or constitutive SAR mutants in their failure to trigger cell death. The Bent lab has recently isolated *DND1* by positional cloning and discovered that it encodes an apparent cyclic nucleotide-dependent gated ion channel. How this protein fits within the known signaling cascades, perhaps as a cell-death activator functioning independently from mechanisms triggering defensive compounds or pathways, remains to be determined.

Interaction of a Plant NBS-LRR Resistance Protein with Its Cognate Bacterial AVR Protein within the Organism

Several plant *R* genes that encode putative NBS-LRR proteins that are expected to interact with cognate bacterial Avr proteins have been cloned. But there has been no report of physical interaction between the proposed molecular partners, for example, in the yeast two-hybrid system. Fumiaki Katagiri (Novartis Agricultural Discovery Institute, CA) reported that Arabidopsis leaf mesophyll protoplasts respond in a gene-for-gene manner to *RPS2*/

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avrRpt2 and *RPM1*/*avrB R*/*avr* when these cognate *R*/*avr* gene pairs are transiently expressed. Furthermore, immunoprecipitation of ^{35}S-labeled proteins with an antibody against the epitope tag of Avr proteins revealed the coimmunoprecipitation of RPS2 with AvrRpt2. Unexpectedly, AvrB also coimmunoprecipitated RPS2, but this may reflect prior observations of interference between *avrB*- and *avrRpt2*-dependent phenotypes in plants inoculated with *P. syringae* strains expressing both genes.

A Negative Regulator of Systemic Acquired Resistance

The induction of SAR by necrotizing pathogens or by SA is dependent on Npr1 (also known as Nim1), a novel protein containing four ankyrin repeats. *npr1* mutants fail to induce the *PR* genes or broad-spectrum resistance after treatment by SA or pathogen. Xinnian Dong (Duke University, NC) reported the identification and cloning of the Arabidopsis SNI1 (suppressor of npr1-1, inducible 1) gene (Li et al., 1999). The phenotype of this recessive loss-of-function mutation suggests that the wild-type *SNI1* gene is a negative regulator of SAR. SNI1 is a novel protein showing marginal homology only with the N domain of the tumor suppressor Rb protein. Interestingly, the *sni1 npr1-1* double mutant still requires SA for induction of *PR* genes and resistance. These data suggest a model in which SA acts at two levels: first, by inactivating SNI1 via Npr1 activity, and second by positive activation of an unidentified regulatory factor, possibly a transcription factor.

PROGRAMMED CELL DEATH

The role of cell death in plant disease resistance or sensitivity is still a matter of debate. Julie Stone (Massachusetts General Hospital, MA) reported on work to address this issue in the Ausubel lab, using the mycotoxin fumonisin B1 (FB1) that elicits cell death. Arabidopsis protoplasts exhibit cell death in response to FB1, and inhibitor studies showed that this is an active process. SA contributes to FB1-induced cell death, as there is a correlation between susceptibility to FB1 and endogenous SA levels. Protoplasts prepared from transgenic *nahG*-expressing plants and *pad4* mutants, both of which have low SA levels, are more resistant to FB1, whereas *cpr* mutants with elevated SA levels are more susceptible to FB1 than is wild type. In contrast, *npr1* mutants are indistinguishable from wild type, suggesting that an NPR1-independent, SA-dependent pathway is required for cell death. FB1 was also used to select FB1-resistant (*fbr*) mutants. Interestingly, two of these were unaltered in their capacity to mount an HR and confer gene-for-gene disease resistance, but permitted reduced growth of virulent *P. syringae.*

Consistent data came from David Gilchrist (University of California at Davis, CA) to suggest that cell death programs are activated by *P. syringae* during disease and thus contribute more to disease sensitivity than to resistance. Plants may undergo apparent programmed cell death (PCD) during interactions leading to disease or defense, depending on the pathogen. For example, the fungus causing Alternaria stem canker of tomato produces AAL toxin, which induces apoptotic morphologies in both plant and animal cells and promotes disease. In contrast, the hypersensitive PCD associated with gene-for-gene resistance against the bacterium *P. syringae* occurs during defense. Inhibition of the tomato PCD signaling pathway, either by using peptide inhibitors of animal caspase or by transgenic expression of baculovirus p35 protein, enhances resistance against Alternaria. However, blocking PCD did not render tomato susceptible to normally avirulent *P. syringae* strains, and even more unexpectedly, blocking PCD rendered tomato resistant to normally virulent strains of *P. syringae.* These observations challenge current concepts of the interactions of *P. syringae* with plants, and they suggest that PCD has a broad and complex role in plant–pathogen interactions.

SYMBIOTIC PLANT–MICROBE INTERACTIONS

The work presented on symbiotic plant–microbe interactions focused on the association of plant roots with mycorrhizal fungi and rhizobia. Although mycorrhizal fungi have a very broad host range, most of the current research involves legume host plants, the unique hosts of rhizobia. Despite a striking difference in host range, genetic studies have revealed commonalities in the symbiotic interactions of mycorrhizal fungi and rhizobia.

Tremendous progress has been made in understanding the molecular mechanisms controlling endo- as well as ectomycorrhizal association. Francis Martin (INRA-Nancy, France) provided an example in which an ectomycorrhizal gene has been cloned and shown to become activated during a specific stage of association (Martin et al., 1999), whereas Maria J. Harrison (Samuel Roberts Noble Foundation, OK) presented the example of a xyloglucan endo-transglycosylase related gene whose expression pattern suggests involvement in senescence of the arbuscule. Furthermore, studies with several mutant hosts are in progress and will reveal key events of symbiotic interactions. For example, Paola Bonfante (University of Torino, Italy) and Martin Parniske (Sainsbury Laboratory, UK) reported that the cell biological and genetic characterization of the *Lotus japonicus LjSym4* gene in the interaction

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with AM fungi revealed that the root epidermis plays a crucial role in the control of infection. This control is far more stringent in the epidermis than in the cortex, which is strikingly similar to the Rhizobium–legume interaction. For example, in pea, the *PsSym2* gene is known to control infection thread growth specifically in the root epidermis. Henk Franssen (Wageningen University, The Netherlands) reported on the progress on the cloning of this pea gene using synteny between pea and the model legume *Medicago truncatula.*

Legumes that have been effectively selected as model plants are *M. truncatula* and *L. japonicus.* Jens Stougaard (University of Aarhus, The Netherlands) reported the cloning of the first *sym* gene (*Nin*) of *Lotus* (see Schauser et al., 1999). *Nin* is essential for the formation of nodule primordium and the progression of infection threads. However, it is not involved in perception or transduction of the rhizobial Nod factors. Because *nin* mutants still respond to these signal molecules, NIN is most likely a transcriptional activator containing heptad leucine repeats and acidic activation domains.

Several groups reported on *Medicago* mutants disturbed in an early step of Nod factor perception/transduction. Christine Galera (CNRS/INRA, France) described mutants from three complementation groups that are unable to mount responses to Nod factors or establish symbiosis with AM fungi. Sharon Long (Stanford University, CA) reported that Nod factors do not induce Ca^{2+} spiking in root hairs of one of the groups.

In addition to the genetic analysis of rhizobial infection, cell biological and biochemical studies were reported. Anne Mie Emons (Wageningen University, The Netherlands) described that Nod factors cause an increase of the subapical fine bundles of actin filaments within 3 min after application of the signal molecule (Miller et al., 1999; Norbert et al., 1999). Nick Brewin (John Innes Centre, UK) reported the cloning of a gene encoding a component of the infection thread matrix (see Rae et al., 1992). The gene is very similar to previously described nodule-enhanced extensin genes of *Medicago* and *Vicia.* Brewin presented that this infection matrix extensin can be insolubilized by hydrogen peroxide. Preliminary data showed that both oxalate oxidase and diamine oxidase might be the source of hydrogen peroxide. Based on these observations, it was proposed that hardening of the infection thread matrix in a spatially controlled manner can contribute to proper infection thread growth.

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The Role of BP-80 and Homologs in Sorting Proteins to Vacuoles

A recent article (Miller et al., 1999) and accompanying editorial (Smith, 1999) raised issues that are of substantial importance to those of us who putter in the pipes of the secretory pathway. Miller et al. (1999) convincingly showed that tobacco homologs of the vacuolar sorting receptor, BP-80, physically interacted with a ~46-kD *Nicotiana alata* proteinase inhibitor precursor protein (Na-PI) in stigma cells. This is an important result and the authors deserve congratulations for their fine work. We, however, have serious reservations about conclusions from this experiment. As these conclusions have important ramifications with respect to mechanisms for sorting proteins into the two different Golgi-to-vacuole pathways, it would seem appropriate to bring these issues to the attention of the plant cell biology community.

As was carefully discussed in both the paper and editorial, two pathways that carry soluble proteins from Golgi to vacuoles have been defined. One is followed by the vacuolar sorting receptor BP-80 and involves clathrin coated vesicles (CCVs) for at least a portion of the pathway. All evidence available to date indicates that this pathway leads to a prevacuolar compartment serving the lytic vacuole, defined together as organelles containing an acidic pH and proteases that can process barley proaleurain to mature form (Jiang and Rogers, 1998). The BP-80 transmembrane domain and cytoplasmic tail direct chimeric reporter proteins to this destination. Indeed, the transmembrane domain alone is sufficient in this regard, and the cytoplasmic tail appears