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IS-MPMI Meeting Report

The 8th International Congress on Molecular Plant-Microbe Interactions was held in Knoxville, Tennessee, July 14 to 19, 1996. The meeting was a huge success, attracting almost 900 scientists. Below is a summary of some of the highlights of the meeting. Contributions to this meeting review were made by Madan Bhattacharyya, Ulla Bonas, Stanton Gelvin, Maria Harrison, Elisabeth Huguet, Konstantin Kanyuka, Jan Kijne, Jordi Mas, Charles Opperman, and Jonathan Walton.

Bacterial Pathogenesis.

Exciting advances were reported by laboratories working on *hrp*/*hrc* genes. These genes were first cloned in *Pseudomonas syringae* pv. *phaseolicola*, where *hrp* mutants were found to be necessary for the induction of the hypersensitive reaction (HR) on a resistant host, and nonhost plants, and for pathogenicity on susceptible plants. Since then *hrp* clusters have been identified in gram-negative bacteria such as *Xanthomonas*, *Ralstonia solanacearum*, *Pseudomonas*, and *Erwinia*, but not in *Agrobacterium*. Furthermore, 10 Hrp proteins, conserved among the phytopathogens, are homologous to components of the so-called type III protein secretion pathway that was first described for the animal pathogen *Yersinia*. Groups are now focusing on fine-tuning their analyses by creating nonpolar mutations, by sequencing to the right and left borders of the *hrp* clusters, and by trying to understand the role of individual Hrp proteins. In Christian Boucher's laboratory (CNRS-INRA) systematic nonpolar mutagenesis of the *Ralstonia solanacearum hrp* cluster, using the apha3 cassette, revealed that four genes are not *hrp* genes. Putative protein products encoded by these genes are likely to be secreted, as a mutation in a component of the Hrp secretion apparatus is more likely to have a true *hrp* phenotype. Sequencing out of the *hrp* clusters is in fashion, and in most cases revealed the presence of genes that could be involved in pathogenicity. In *Ralstonia solanacearum* analysis of the region to the left revealed the presence of six ORFs, four of which are HrpB-regulated, but no new *hrp* genes are present. Interestingly, there is a Cf-2 homolog next to *hrcC*. One ORF that is not regulated by HrpB is homologous to *fptA*, that encodes a receptor for siderophores. The expression of this ORF is, however, not subject to regulation by iron concentration. Posters from Steven Beer's laboratory (Cornell University) presented data on the borders of the *hrp* cluster. In *Erwinia amylovora*, the region to the left contains a homolog of the *Xanthomonas avr* gene *avrRxv*. In addition, there is a gene encoding a harpin-like protein (acidic, rich in glycine and serine, heat-stable) that induces HR on tobacco. Interestingly, the predicted product of *avrRxv* is homologous to the *Yersinia* YopJ protein, a secreted virulence factor (poster by Maureen Whalen, San Francisco State University). In *Erwinia chrysanthemi*, the left-hand region contains two genes that are homologous to HecB and HecA hemolysin activator proteins, while a phospholipase C gene, *plcA*, was identified on the right. In the animal system both hemolysin activator proteins and phospholipase C play a role in virulence. Alan Collmer's laboratory (Cornell University) reported on the biochemical dissection of the *Pseudomonas syringae* Hrp transport system. They studied the subcellular localization (i.e., cytoplasmic, periplasmic, or extracellular) of Harpin in different mutants affected in the conserved *hrc* genes. Their results indicate that products of the *hrcV, N, R, S, T,* and *U* genes are located in the inner membrane, while HrcC and HrcJ are in the outer membrane. Martin Romantschuk (University of Helsinki) and Sheng-Yang He (Michigan State University) reported some very interesting data on the production of extracellular filamentous structures by *Pseudomonas syringae* pv. *tomato* DC3000 grown on solid minimal medium. These structures were characterized as flagella (also present when bacteria were grown under noninducing conditions, and in liquid medium), and novel types of filaments that only appear under inducing conditions. These novel structures consist of the Hrp protein HrpA.

Fred Ausubel and Laurence Rahme (Massachusetts General Hospital) reported on isolation of virulence determinants from an isolate of *P. aeruginosa* that can infect humans, mice, nematodes, and *Arabidopsis*. They had already shown that some virulence factors are required for pathogenicity on both mice and plants (*Arabidopsis* and lettuce). This finding opens the possibility of using a screen based on plants to identify novel virulence factors in mammals. After Tn*phoA* mutagenesis of the *P. aeruginosa* isolate, 12 mutants with reduced pathogenicity on lettuce were identified, eight of which were also less pathogenic in *Arabidopsis* and mice.

Talks by Jan Leach (Kansas State University) on *Xanthomonas oryzae*, Ulla Bonas (CNRS, Gif-sur-Yvette) on *X. campestris* pv. *vesicatoria*, and Dean Gabriel (University of Florida) on *X. campestris* pv. *malvacearum* and *X. citri* covered members of the large *Xanthomonas avrBs3* gene family. This family includes genes with avirulence activity as well as pathogenicity factors (e.g., *pthA*). Jan Leach has identified a 27-kDa protein in the bacterium that binds to the AvrXa10 and AvrXa7 proteins under hrp inducing conditions. Ulla Bonas and Dean Gabriel presented data on the mutational analysis of nuclear localization signals that are present in the C-termini of the members of this gene family. Both groups found that the NLSs are required for gene function. A poster by Valerie Verdier-Michel (CIAT-OSTROM) on *X. manihotis* presented a pathogenicity gene that is a member of the *avrBs3* family. This gene is essential for the induction of the typical disease symptoms.

There was a major breakthrough reported with respect to the function of bacterial avirulence genes. A few laboratories have direct evidence that bacterial Avr proteins act inside the plant cell to induce cell death. Since the cloning of the first avirulence gene in 1984 by Brian Staskawicz and co-workers, approximately 30 different bacterial *avr* genes have been cloned. Their function, however, has remained enigmatic, with *avrD* being the only exception to date. At the meeting Noel

Keen reported that his group has identified a binding site for the elicitor syringolide in both susceptible and resistant soybean cultivars. Production of syringolide by *P. syringae* pv. *syringae* is *avrD*-specific, and recognition depends on the presence of the *Rpg4* resistance gene in soybean. Sheng-Yang He and Alan Collmer reported on studies using transgenic plants containing the *P. syringae* pv. *syringae avrB* gene in *Arabidopsis* that causes cell death, and co-bombardment of *avrB* and *gusA* genes. Lack of GUS activity after shooting RPM1 plants was used as an indication for HR induction. Alan Collmer also discussed data on the function of *hrmA*. It seems that *hrmA*, which is located to the left of the *hrp* cluster in *P. syringae* pv. *tomato* DC3000, functions as an *avr* gene on tobacco (in *P. syringae* pv. *tabaci*). Co-bombardment of tobacco suspension cells with plasmids containing the *gusA* and *hrmA* genes under the control of the 35S promoter revealed a lack of GUS activity in the presence of *hrmA*. The authors concluded that *hrmA* expression inside tobacco cells led to an HR and thus prevented expression of GUS.

Transient expression by particle bombardment of a susceptible *Arabidopsis* line with the bacterial avirulence gene *avrRpt2*, and its corresponding resistance gene *RPS2* with *gusA*, by Fred Ausubel and co-workers revealed that the *avr* gene product probably interacts with the R-gene product inside the plant cell. If RPS2 were overexpressed, expression of the bacterial avirulence gene is not required to elicit the HR (i.e., absence of GUS activity).

Brian Staskawicz and co-workers (U.C. Berkeley) found that *Agrobacterium*-mediated transient expression of a 35S-*avrPto* gene leads to a HR specifically in Pto-transgenic tobacco. Another example pointing to a direct interaction between the product of an avirulence gene and its "receptor" was given by Ulla Bonas and co-workers: Transient expression of *avrBs3* inside the plant cell under the control of the 35S promoter using *Agrobacterium* resulted in specific HR induction in the pepper line expressing the corresponding resistance gene, *Bs3*. Greg Martin et al. (Purdue University) reported on the direct interaction of the tomato resistance gene product Pto and the *avrPto* gene product using the yeast-two-hybrid-system. The B domain of Pto was required to interact with avrPto. (Interestingly, when the recessive allele of Pto was used, no interaction was seen, while when the recessive allele of Fen was used, interaction was observed.)

Challenges for the future will include understanding how the Avr proteins are translocated into the plant cells, and whether the Hrp proteins form a pilus that target these Avr proteins.

A number of exciting developments in understanding crown gall tumorigenesis were reported at the meeting. Gene Nester (University of Washington) reported that the *Agrobacterium tumefaciens* VirB proteins constitute a pilus that presumably serves to transfer T-DNA to the plant cells. To identify these pili, it was necessary to induce the bacteria at 19°C, a temperature much lower than that commonly used. He also presented genetic data implicating the VirA protein as the direct mediator of the phenolic *vir* gene inducing signal. Stanton Gelvin (Purdue University) presented data that implicated the ω region of the VirD2 protein in T-DNA integration into the plant genome. He also showed how *Arabidopsis* ecotype variants and insertional mutants could be used to understand the plant contribution to crown gall tumorigenesis. He specifically discussed an *Arabidopsis* ecotype into which the T-DNA could efficiently be transferred and transported to the plant nucleus. This ecotype, however, was recalcitrant to transformation because of a deficiency in T-DNA integration. Barbara Hohn (Friedrich Miescher-Institut) presented data to show that *Agrobacterium* could enter plant leaves through the stomata in the absence of wounding. She also discussed how VirE2 protein could protect the 3′ end of the T-DNA from degradation during transfer and integration. Peter Christie (University of Texas Medical School) discussed the interactions of the VirB proteins in making up the T-DNA export structure. He specifically presented data on the covalent interaction of VirB7 and VirB9 proteins through disulfide bonds.

Among the many interesting *Agrobacterium* posters were those discussing T-DNA transfer from *Agrobacterium* to yeast (Paul Hooykaas laboratory, Leiden University), the processing and extracellular transport of VirB1 protein (Patti Zambryski laboratory, U.C. Berkeley), the processing of the VirB2 pilin-like protein (Cal Kado laboratory, U.C. Davis), and new strategies for the identification of homoserine-lactone autoinducers (Steve Farrand laboratory, University of Illinois).

Plant-Bacterial Symbiosis.

The Symbiosis part of the meeting was dominated by *Rhizobium* research. A major development was a revival of *Rhizobium* microbiology, recognizing the rhizosphere as a determining factor for efficiency of symbiosis and the bacterium as a living soil organism rather than as a Nod factory (over 40 posters dealt with regulation of rhizobial metabolism in one or another way). Furthermore, the organizers included bacterial protein secretion in the program as a separate topic, thereby accentuating the exciting developments in this area of research.

Frans de Bruijn (Michigan State University) highlighted the rep-PCR technology which enables generation of strain-specific fingerprints of symbiotic and other plant-associated bacteria. This excellent method will be of much use in studying bacterial population dynamics inside or outside plants. The topic of plant-regulated root colonization by rhizobia was covered by Don Phillips (U.C. Davis). More than 30 genes in *Sinorhizobium meliloti* have now been found that are regulated by betaines, isoflavonoids, or water-soluble vitamins. A mutation in a stachydrine-inducible gene required for stachydrine uptake significantly reduced competitiveness for root colonization. Interestingly, a mutation in *nodC*, the gene required for synthesis of the backbone of lipo-chitin oligosaccharides (Nod factors), severely impaired competition for colonization sites at low inoculum density. This result suggests that Nod factors also function at the plant root surface.

Dale Noel (Marquette University) reported that seed exudate (anthocyanins) and root exudate of *Phaseolus vulgaris* induce modifications of rhizobial lipopolysaccharide. Mutants that fail to respond appeared to be defective in root infection. Plasmid-borne nod genes are not required for this response. From this and other studies it has become very clear now that regulation of the synthesis of rhizobial surface polysaccharides is an important factor in establishment of an effective symbiosis, and that nod genes encode only a part of the nodulation story. Several reports hinted at functions for such polysaccharides. Juergen Ebel (Muenchen University) showed that cyclic B7-glucans from *Bradyrhizobium japonicum* (not active as elicitors) compete with fungal B7-glucan elicitors for spe-

cific membrane binding sites, thereby inhibiting phytoalexin production. Suppression of defense responses was also suggested for rhizobial Nod factors, low molecular weight exopolysaccharide and lipopolysaccharide (Karsten Niehaus, Bielefeld University). Such results raise the question whether *Rhizobium* indeed produces elicitors, the effect of which must be suppressed. I. de Kozak (CNRS, Gif-sur-Yvette) found that the presence of K-polysaccharides (KPS) is required for induction of genes from the flavonoid biosynthetic pathway in alfalfa leaves. Such polysaccharides or fragments thereof can be active as virulence factors even at very low concentrations, as shown by Graham Walker (MIT) for fragments of EPSII and KPS produced by *S. meliloti*. A role for KPS in nodulation was also discussed by Bradley Reuhs (CCRC, Athens). For example, a mutation in the gene *nolW* of *S. fredii* affects expression of KPS and at the same time extends the host range of this symbiont. Interestingly, *nolW* is homologous to the *hrpA1* gene, which nicely links virulence of rhizobia with virulence of bacterial plant pathogens.

Of course, Nod factors enjoyed ample coverage, with plant responses getting more and more attention. Jacques Bono (UMR CNRS-UPS) found a high-affinity binding site for Nod factors from *S. meliloti* to be associated with a plasma membrane preparation from *Medicago* cell cultures. This site, NFBS2, appeared to be absent in carrot cells. A root lectin from *Dolichos biflorus*, DB46, binds Nod factors from the homologous symbiont *B. japonicum*, reported Marilynn Etzler (U.C. Davis). Study of legume cell reactions to addition of Nod factors or related compounds requires application of various cell biological techniques, and the present multidisciplinarity of the field was nicely illustrated by various rhizobiologists now presenting plant cell results obtained with use of microinjectors, voltage clamp equipment, microtargeting guns, and such. An early response of host roots to addition of Nod factors is induction of Enod40 genes. Enod40 encodes a small peptide which may be involved in changing the plant hormone regime in the host root as a prelude to nodule primordium formation (Ton Bisseling, Wageningen University). Interestingly, Enod40 mRNA is bioactive as well, and may function as a riboregulator (Adam Kondorosi, CNRS, Gif-sur-Yvette). The latter speaker also reported that addition of cytokinin can mimic addition of Nod factors, and that the carbon/nitrogen metabolic status of the plant plays a role in control of root nodule initiation.

Reports on symbioses other than *Rhizobium*-legume interactions were relatively scarce: *nod*, *exo*, and *chv* homologs in *Nostoc* (Brigitta Bergman, Stockholm University), nodulins in *Frankia*-induced root nodules of *Alnus*, *Casuarina*, and *Datisca* (Katharina Pawlowski, Wageningen University), cytokinin production by the sugarcane endophyte *Acetobacter diazotrophicus* (Barbara Taller, University of Memphis), and several reports on cross-talk between mycorrhizal fungi and host plants. Maria Harrison (Noble Foundation, Ardmore, OK) described cloning and activity of a phosphate transporter from the VAM symbiont *Glomus versiforme*, and her choice of *Medicago truncatula* as a host plant promises a key role for plant genetics in this field in the near future.

Virus-Plant Interactions.

Two talks at the Congress illustrated progress in the molecular analysis of host functions required for virus replication. Paul Ahlquist and his group (University of Wisconsin) have focused on the replication of brome mosaic virus which takes place in infected cells at specific sites in the endoplasmic reticulum. The replication complex includes host factors and the interacting 1a and 2a proteins encoded in the BMV genome. To characterize the host factors involved either directly or indirectly in the replication complex the Ahlquist group have exploited the capability of yeast isolates expressing the 1a and 2a proteins to support replication of modified BMV RNA. The RNA was modified so that expression of visual or selectable marker genes was dependent on the BMV replication system. Expression of the marker genes allowed identification of mutant yeast isolates with modified capability to support the replication of BMV RNA. Many of the mutations have been complemented by transformation with DNA of the wild-type yeast isolates and we can look forward to imminent molecular characterization of the yeast genes facilitating replication of BMV. Interestingly, some mutants altered in BMV replication were able to support a replication of the insect virus flock house virus (FHV), suggesting that mechanisms of replication of different viruses may require expression of different host proteins.

Linda Hanley-Bowdoin (North Carolina State University) reported progress towards understanding the replication mechanism of the tomato golden mosaic geminivirus (TGMV). Its bipartite genome comprises two single stranded circular DNAs, A and B. Component A encodes two proteins involved in viral replication, AL1 and AL3. The AL1 protein is essential for viral replication. In contrast, the AL3 protein can be dispensed with, although its presence enhances levels of accumulation of viral DNA. For successful replication of TGMV several host-encoded proteins are also required. Recent experiments demonstrated that TGMV can somehow switch on a DNA replication machinery in quiescent plant cells. So far, one of the host proteins induced by TGMV in terminally differentiated plant cells has been identified. It is the proliferating cell nuclear antigen (PCNA), a processivity factor for DNA polymerase δ. There is also evidence that a geminivirus-encoded protein may interact with a homolog of the animal retinoblastoma suppressor of DNA replication. Thus, the further analysis of geminiviral replication may have implications for understanding of the control of DNA replication in plants generally as well as for the virological perspective.

The processes whereby viruses move through the infected plant were addressed in two talks by Bill Lucas (U.C. Davis) and Roger Beachy (Scripps Institute, San Diego). The Lucas presentation summarized the underlying complexity in these processes: Distinct mechanisms are responsible for cell-to-cell movement through plasmodesmata and for long-distance movement involving ingress in and egress from the vascular system. The movement mechanisms of RNA and DNA viruses have been studied and the current picture is of a series of complex interactions of virus-encoded proteins, viral genomes, and host factors. Most of these interactions can be considered only as concepts at the moment. However, the Beachy lab has developed tools which will facilitate direct identification of the subcellular components involved in movement. They have constructed a chimeric open reading frame encoding the movement protein (MP) of tobacco mosaic virus (TMV) coupled to the jellyfish green fluorescent

protein (GFP). Surprisingly, this fusion protein still functions as a MP so that the intrinsic fluorescence of the GFP can identify the subcellular location of the MP. Using this approach, they have detected the MP in plasmodesmata, associated with the cytoskeleton, and in amorphous structures in the cytoplasm of infected cells. Beachy and his colleagues have also developed a dominant negative mutant approach to the analysis of the MP of TMV: Transgenic expression of the defective MP (dMP) interferes with the MP of an inoculated virus and, consequently, there is a delay in the spread of the virus from the site of inoculation. Interestingly, this MP-mediated resistance is effective against RNA viruses that are unrelated to TMV, indicating that many different viruses may interact with the same cellular components. The broad spectrum nature of this MP-mediated resistance may also have practical benefit.

Michael Wassenegger (Max-Planck-Institut, Martinsried) described a fascinating convergence of two lines of research from his laboratory that may have a bearing on transgenic virus resistance of a completely different type. One line of research with viroid sequences expressed in transgenic plants led to the discovery of RNA-directed methylation (RdM). RdM is a specific methylation of DNA with sequence homology to RNA sequences accumulating at a high level in the nucleus that was revealed first in studies with transgenic plants expressing viroid cDNA. The second line of research involves a host encoded RNA-dependent RNA polymerase (RdRp). This enzyme has been purified and characterized biochemically by the colleagues of Wassenegger but, until now, does not have a defined function. The link between these two lines of research is the mysterious topic of transgene silencing. It is now clear that gene silencing and some types of transgenic virus resistance are related, RNA-mediated processes. There are also indications that methylation of the silencing/resistance transgene is a part of the underlying mechanisms. Bill Dougherty and his colleagues (Oregon State University) first suggested that the host-encoded RdRp was involved in these processes as the catalyst of anti-sense RNA production. The anti-sense RNA would be specific for the silencing/resistance transgene and would be required for silencing and resistance. Following from this suggestion, Wassenegger proposed that the anti-sense RNA would also mediate the RdM and thereby imprint the silenced state on the transgene. This model will be testable in the near future when cDNA clones have been isolated of the host-encoded RdRp. Wassenegger described candidate clones although, surprisingly, they do not encode proteins with the characteristic sequence motifs found in all other known RdRp proteins.

Two presentations dealt with virus-related aspects of disease resistance. One of these was from James Culver (University of Maryland) who discussed the relationship between the structure of the TMV coat protein and its role as an elicitor of the hypersensitive response in *Nicotiana sylvestris* carrying the *N´* resistance gene. A specific "elicitor site" was identified within the right side of the coat protein's four-helix bundle. Structural comparisons between coat proteins of different tobamoviruses revealed a functional conservation of this "elicitor site." In the virions of the U1 strain of TMV that can overcome *N´*-mediated resistance, this site is masked by a feature in the quaternary structure of the coat protein. The second presentation related to virus resistance was an update by Barbara Baker (USDA Gene Expression Center, Albany, CA) of the recent research on the tobacco *N* gene that confers resistance against TMV. The *N* gene was isolated about a year ago and it is still the only viral resistance gene cloned. A mutational and deletion analysis of the *N* gene was carried out in order to elucidate the function of each of the encoded protein domains: A domain with homology to the *Drosophila* Toll protein, a nucleotide binding site, and a leucine-rich repeat domain. Even small (one to three amino acids) alterations in the protein structure were shown to destroy N protein's function.

Although these are diverse topics, there was a recurring theme in that the problems addressed concern the interaction of a virus and its host plant. It is hoped that approaches like those in described in these keynote talks will be widely adopted so that, at the next MPMI Congress, there will be many papers dealing with the molecular analysis of host components affecting virus-host plant interactions.

Fungal-Plant Interactions.

The application of new techniques for the molecular genetic manipulation of filamentous fungi is leading to major new insights into the mechanisms of pathogenicity in this important group of organisms. Several groups reported the identification of essential pathogenicity genes using restriction enzyme mediated integration (REMI), insertional mutagenesis, mapped-based cloning, and targeted gene disruption. In one of the first applications of REMI to plant pathogenic fungi, Olin Yoder, Gillian Turgeon, and co-workers (Cornell University) reported the identification of genes involved in the biosyntheses of T-toxin by *Cochliobolus heterostrophus*: One encodes a polyketide synthase and the other a decarboxylase. Barbara Valent (DuPont) reviewed known pathogenicity genes in *Magnaporthe grisea*, many of which are necessary for appressorium development and function. A nascent *M. grisea* genome project has sequenced over 2,000 ESTs; included among those that could be identified (approximately 50% of the total) are homologs of genes also found in other pathogenic fungi. Regine Kahmann (Munich) also used REMI to generate pathogenicity mutants in *Ustilago maydis* and reported that up to 2% of all the mutants obtained were non-pathogenic. Many of these genes appeared to be involved in the mating response; however, homologs of signal transduction genes, such as SNIF7 of yeast and a G-protein alpha subunit, were also found. Keisuke Kohmoto and co-workers (Tottori University, Japan) used REMI to generate host-selective toxin-deficient strains of *Alternaria mali*, and also used PCR to identify a cyclic peptide synthetase gene that is uniquely present in toxin-producing strains.

Pierre DeWit (Wageningen) discussed studies on the *avr9* and *avr4* elicitors of *Cladosporium fulvum.* Although both are small peptides, they differ in several critical respects. Whereas the *avr9* gene was earlier found to be completely lacking in fungal isolates that can infect tomato plants with the *Cf9* gene, fungi that are virulent on *Cf4* plants contain alleles of *avr4* that produce Avr4 peptides with amino acid substitutions. Under some expression conditions, these "virulent" alleles of *avr4* can, like the avirulent allele, cause necrosis (hypersensitive response). Therefore *avr4* is, unlike *avr9*, not an "all-or-none" signal.

Continuing on the theme of signal transduction in fungi, Donald Nuss (University of Maryland) presented evidence

that hypovirus-mediated virulence attenuation in *Cryphonectria parasitica* is mediated by a specific G-protein signal transduction pathway. Using differential display, he and co-workers also found that more than 135 genes are altered in their expression following hypovirus infection, a veritable genetic "reprogramming."

Successful pathogens must cope with noxious plant compounds. Jos Wubben and co-workers (John Innes Centre) reported that at least three pathogens use structurally and functionally related enzymes to degrade different saponins, that are pre-formed anti-fungal compounds present in the roots and leaves of some plants. Gene disruption and gene transfer experiments indicate that the enzymes are required by the fungi to be pathogenic, and, furthermore, that plants with reduced levels of saponins are more susceptible to attack. Although earlier genetic studies had strongly implicated detoxification of the phytoalexins pisatin and maackiain as critical pathogenicity traits of *Nectria haematococca* infecting pea and chickpea, respectively, disruption of the genes for the detoxifying enzymes (*PDA1* and *Mak1*) indicated that, in fact, these traits make only a small contribution to virulence (Sarah Covert, University of Georgia, and Hans Van Etten, University of Arizona). It has now emerged, at least for *PDA1*, that this gene is on a chromosome that is not transmitted normally through crosses and that other important virulence genes are tightly linked to *PDA1*. At least two of these other genes, called *PEP*, have now been identified and progress made towards their cloning (Corby Kistler, University of Florida).

An emerging common theme of fungal virulence genes is that they are often absent in other isolates of the same species and/or are genetically unstable. Genes of this sort include *avr9* of *C. fulvum*, *AVR2-YAMO* of *M. grisea* (Barbara Valent, DuPont), and the *PDA1* and *PEP* genes of *N. haematococca*. In addition, the atypical patterns of inheritance of the genes necessary for the biosynthesis of the host-selective toxins HC-toxin (Jonathan Walton, Michigan State University) and T-toxin (Olin Yoder and Gillian Turgeon) in species of *Cochliobolus* are due to their location on translocated, dispensable, and/or unstable chromosomes. The known genes involved in biosynthesis of both toxins are completely absent in toxin non-producing isolates.

Although mycotoxins have long been recognized as a major human health problem, their role in plant disease has not been clear. A. Desjardins, T. Hohn, and co-workers (USDA, Peoria) reported the results of one of the first field trials of an engineered plant pathogenic fungus in which they found that trichothecene-deficient strains of *Gibberella zeae*, the cause of wheat head blight, were significantly reduced in virulence.

Nematode-Plant Interactions.

Plant nematology was represented by six poster presentations and one symposium talk at the Congress. Several of the presentations presented very preliminary data on nematode-plant interactions, including alfalfa responses to both root-knot and root lesion nematodes, and methods to identify plant genes up-regulated during root-knot nematode infection in *Arabidopsis*. Charles Opperman (North Carolina State University) discussed collaborative work with Mark Conkling (North Carolina State University) on the *TobRB7* gene from tobacco in the Emerging Areas Symposium. *TobRB7* is strongly induced in the developing feeding site of the root-knot nematode, *Meloidogyne* spp. Opperman and Conkling have been able to show that the nematode-responsive region of the *TobRB7* promoter is a separate entity and can confer specific expression patterns in the developing giant cells. They have used two different approaches to develop transgenic root-knot nematode resistant plants. In the most successful approach, Opperman and Conkling have completed two years of field trials and numerous greenhouse experiments using antisense constructs of the *TobRB7* gene. *TobRB7*- antisense plants exhibit 70 to 90% reductions in root damage compared to the control plants in both field and greenhouse trials. Opperman also described their plans to design a minimal root-knot nematode promoter. Finally, he described approaches to understanding the nature of the inductive signal using both transgenic plants and transgenic plant-parasitic nematodes, a newly developed technology. Ke Dong and Opperman presented a poster on the genetic analysis of the soybean-soybean cyst nematode, *Heterodera glycines*, interaction. They have shown that unlinked genes control the nematode's ability to parasitize specific host genotypes. The genes controlling ability to attack soybean lines Peking and PI 90763 are both recessive, whereas the gene controlling PI 88788 parasitism is dominant. Using controlled crosses of inbred parental lines and a modified bulk segregant analysis of progeny, they have identified two molecular markers tightly linked to the PI 88788 gene. Bonnie Ferrie (U.C. Riverside) described the identification of genes up-regulated in resistant tomato within 12 h of root-knot nematode infection. In addition to a series of previously known pathogen defense genes, they identified one gene with significant homology to miraculin and to several proteinase inhibitors. They are currently defining the role of the gene (*LeMir*) in the resistance response. Niels Sandal (University of Aarhus) described molecular mapping of beet cyst nematode (*H. schachtii*) resistance genes in wild beet species. They have isolated several markers that are closely linked to beet cyst nematode resistance genes, and have used these markers to identify a 500-kb YAC from one of the wild beet species.

Plant Responses to Microbes.

The first plant disease resistance gene (R) that follows Flor's gene-for-gene hypothesis was cloned in 1993 by Martin et al. Since then, many other R genes have been cloned. Research in these laboratories now focuses on determining how the R proteins function, and on the identification of the other components of the signal transduction pathways leading to the downstream defense responses.

A molecular and biochemical analysis of *Pto* (a tomato gene conferring resistance to *avrPto*-bearing strains of *Pseudomonas syringae*) and *Fen* (a tomato gene conferring sensitivity to the insecticide fenthion) and their signal pathways in tomato was presented by Greg Martin (Purdue University). *Pto* and *Fen* encode kinases sharing 80% identity at the amino acid level. Domain swapping experiments indicate that the specificity resides in the B domains of these proteins, while site-directed mutagenesis experiments indicate that the myristoylation motif is not required for Pto function. To dissect the signal pathways leading from Pto to the downstream response genes, a yeast two-hybrid system was used to identify proteins capable of interacting with Pto. In addition to *Pti*, three other interacting proteins sharing significant identity

with transcription factors were obtained. These proteins bind specifically to an ethylene response element (GCC box) present in the promoters of a number of defense response genes. Other significant advances include the demonstration, via the two hybrid system, that Pto and avrPto can physically interact with each other (Greg Martin's laboratory). This finding was complemented by work from Brian Staskawicz's laboratory (U.C. Berkeley) where transgenic plant approaches were used to demonstrate that *avrPto* and *Pto* can act together inside the plant cell to evoke a race specific reaction. Staskawicz's group has cloned an additional R gene, *Prf*, that is essential for *Pto* function. *Prf* contains motifs typical of many of the resistance genes and lies within the *Pto* gene cluster.

Barbara Baker (U.C. Berkeley) reported a structure-function analysis of the N protein and concluded that all domains of the N protein are essential for N to confer resistance to tobacco mosaic virus. Likewise, Fred Ausubel (Massachusetts General Hospital) reported that all domains of the Rps2 protein, with the exception of the membrane-spanning domain, were critical for resistance to *Pseudomonas syringae*, suggesting that Rps2 may operate in the cytoplasm.

David Jones (Jonathan Jones laboratory, John Innes Center) reported progress towards understanding the molecular basis of tomato resistance to *Cladosporium fulvum*. Four resistance genes, *Cf-9*, *Cf-2*, *Cf-4*, and *Cf-5*, have been cloned and sequence analysis revealed that all four proteins have large extra-cytoplasmic domains containing variable numbers of leucine-rich repeats. The N-terminal repeats are more variable, whereas the C-terminal repeats are conserved, supporting the hypothesis that the N-terminus may be involved in specificity determination and the C-terminal portion in signaling. A poster from Martin Parniske (Jonathan Jones laboratory) described the structure, function, and evolution of the *Cf-9* gene cluster. This is the first resistance gene cluster to be sequenced. The *Cf-9* cluster contains four homologs of the *Cf-9* gene that share a high level of sequence identity with *Cf-9*. Sequence analysis of the intergenic regions identified recombination breakpoints that may have given rise to gene duplications. The positions of the breakpoints enabled the sequence of recombination events to be reconstructed, providing insight into how these resistance genes may have evolved.

In addition to the race-specific elicitors, there are a wide variety of molecules, both pathogen- and plant-derived, that can be recognized by plant cells. However, the identity of the plant receptors for these molecules has remained a mystery. Biochemical approaches beginning with the characterization of the signal and its binding site, followed by the purification and cloning of the binding proteins/receptor, are being used in a number of laboratories with exciting results. Two groups (Juergen Ebel, Muenchen University; and Naoyuki Umemoto, Kirin Brewery) reported the purification and cDNA cloning of a soybean binding protein that recognizes a β-glucan elicitor from *Phytophthora sojae* cell walls. The poster presented by Umemoto indicated that their cDNA clone encodes a protein with elicitor binding activity. Antibodies to the protein inhibit the interaction of elicitor with soybean membranes and the subsequent induction of phytoalexin synthesis. The protein resides on the plasma membrane and is a likely candidate for the elusive β-glucan elicitor receptor. Thomas Boller (Friedrich Miescher-Institut) previously demonstrated that plants have receptors for chitotetraose and ergosterol, components of both pathogenic and symbiotic fungi. He reported that plants also have the ability to recognize a conserved region of flagellin and may therefore recognize the presence of bacteria in this way.

The recognition of specific signal molecules by plant cells is followed by the activation of signal transduction pathways that frequently result in an oxidative burst, and then to a variety of downstream events aimed at protecting the plant from pathogen attack. These events may induce systemic immunity to protect against subsequent attacks. Phil Low (Purdue University) proposed that a multiplicity of pathways lead to the oxidative burst, and used pharmacological methods to demonstrate that different external signals may be transduced by distinct pathways. For example, inhibitors of phospholipase A inhibited the oxidative burst induced by *Verticillium*, but had no effect on an oligogalacturonide or a mechanically-induced oxidative burst.

Hydrogen peroxide arising from the oxidative burst can induce systemic expression of defense genes and act as a local trigger for cell death. Maria Alvarez (Chris Lamb laboratory; Salk Institute, San Diego) reported further on the role of H_2O_2 in systemic acquired resistance (SAR) and demonstrated that an avirulent pathogen or H_2O_2 induced by a local injection of glucose/glucose oxidase would provide immunity to subsequent challenge by a virulent pathogen. This treatment also induces chromatin condensation and a low frequency of apoptotic cell death in the systemic leaves, suggesting that a low level systemic HR occurs in parallel to the initial local HR. Inhibition of the avirulent pathogen-induced burst by treatment with diphenylene iodonium (DPI) prevents systemic cell death and subsequent immunity, indicating the requirement for the oxidative burst in this process. Requirements for reactive oxygen intermediates in the process of HR lesion formation and cell death are also being defined by genetic approaches. Jeff Dangl's group (University of North Carolina) reported the identification and characterization of a new set of mutants (pheonix, or *phx*) that are extragenic suppressors of the previously reported *lsd* (lesion simulating disease resistance reaction) mutants. These mutants identify additional steps in the disease resistance pathways. Jean-Pierre Metraux (University of Fribourg) identified a salicylic acid-insensitive mutant that defines steps in the modulation of the SA response.

Investigations of plant interactions with symbiotic nitrogen fixing bacteria focused on the determination of the receptor for the initial signal molecules (Nod factors), and dissection of signal transduction pathways that lead to the formation of a functional nitrogen-fixing nodule. J. Bono (Universite Paul Sabatier, Toulouse) reported the identification of two binding sites with different affinities for Nod factors in the plasma membrane fraction of *Medicago* cells. The lower affinity binding site was also found in non-leguminous species and is probably not a specific Nod factor receptor. However, a higher affinity binding site appears a more promising candidate. Marilynn Etzler (U.C. Davis) reported the purification and cloning of an unusual lectin from *Dolichos* also capable of binding Nod factors. The lectin has nucleotide phosphatase activity and is present in young root hairs. Experiments demonstrating the biological relevance and specificity of the interaction between these proteins and specific Nod factors have still to be performed in both cases.

The early events following the perception of Nod factors include rapid calcium spiking in the root hair cells. This was demonstrated by Sharon Long (Stanford University) in a series of elegant experiments using microelectrodes and calcium sensitive dyes that enabled real-time imaging and visualization of the process. Experiments using plant mutants and non-specific Nod factors support the hypothesis that calcium spiking is an early and essential response to Nod factors for the subsequent development of the symbiosis. A poster by Jannine Sherrier (John Innes Centre) proposed a novel hypothesis that Nod factors might act by disrupting cellulose crystallization through direct action at the plasma membrane. This hypothesis was supported by their demonstrations that Calcofluor, an inhibitor of cellulose crystallization, could act as a functional analogue of *Rhizobium* lipochito-oligosaccharides, and could induce both the deformation and swelling of root hairs as well as the induction of an early nodulin, Enod12A.

Genetic approaches, that have been used for many years to analyze processes in the bacterial symbiont, are now being applied to analyze the plant partner in this symbiosis. Jens Stougaard (University of Aarhus) outlined progress towards the generation of nodulation mutants in *Lotus japonicus* using T-DNA tagging and transposon mutagenesis. A number of Nod- mutants have been created, but unfortunately most of these are not tagged. A poster by Varma Penmetsa (Texas A&M University) reported the identification of a number of nodulation mutants of *Medicago truncatula*, including a super-nodulating mutant *Sickle*. *Sickle* plants make over ten times the number of nodules compared to a wild-type plant and are insensitive to ethylene, indicating that ethylene is a key determinant in the regulation of nodulation.

Martha Hawes (University of Arizona) outlined progress on the analysis of root border cells. These are a somewhat ignored but important population of cells that are released from the root into the surrounding environment. She demonstrated that these cells are recognized by root pathogens and also release Nod gene inducers. The cells have unique protein profiles that suggests that they are distinct from the root cap cells, and it was proposed that their function is to regulate the ecology of the rhizosphere.