MOLECULAR BIOLOGY OF PLANT-PATHOGEN INTERACTIONS

Organizers: Brian Staskawicz, Olen Yoder and Paul Ahlquist
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Keynote Address
E. Davidson see abstract **L001**

Mechanisms of Agrobacterium Transformation

Y 002 EARLY STEPS IN THE TRANSFORMATION OF HIGHER PLANT CELLS BY AGROBACTERIUM, Eugene Nester, Peter Christie, John Ward, Stephen Winans, Nicolas Toro, Calvin Young, and Milton Gordon*, Departments of Microbiology and *Biochemistry, University of Washington, Seattle, WA 98125.

The efficient transfer of DNA into plant cells by Agrobacterium tumefaciens requires the activity of both cis and trans acting factors coded by genes on the Ti-plasmid and on the chromosome. The chromosomal genes seem to be associated with attachment of the bacteria to the plant cells. The Ti-plasmid genes, termed vir, act in trans to process the T-DNA and transfer it into plant cells. These genes are not expressed in Agrobacterium growing in the absence of wounded plant cells, but are activated by phenolic plant signals through the virA and virG gene products.

We have been emphasizing the role of certain vir transcriptional units in our studies. These include virD, part of which codes for a site specific endonuclease. This protein becomes covalently attached to the 5' end of the T strand. virE codes for a single stranded DNA binding protein which may serve to protect the T-DNA in its transfer from bacteria to plant cells. The largest of the transcriptional units is the virB operon, which codes for 11 different proteins, many of which are associated with the bacterial membranes. The overdrive sequence which is located very close to the right border functions in cis and enhances the level of plant cell transformation by promoting the site specific cleavage at the right border by the virD encoded endonuclease. This cleavage is also enhanced by other vir gene products. Our data are most consistent with the transfer of a single stranded DNA molecule from Agrobacterium into plant cells. With increasing information on the function of the various vir genes, plasmid and chromosomal, it is now possible to propose detailed models for the transfer process.

Y 003 THE OVERDRIVE ENHANCER SEQUENCE STIMULATES PRODUCTION OF T-STRANDS FROM THE AGROBACTERIUM TUMEFACIENS TUMOR-INDUCING PLASMID, Joon M. Ji[1], K. Veluthambi[2], Stanton B. Gelvin[2], and Walt Ream[1], [1]Department of Biology, Indiana University, Bloomington, IN 47405 and [2]Department of Biology, Purdue University, West Lafayette IN 47907.
During crown gall tumorigenesis a specific segment of the A. tumefaciens tumor-inducing (Ti) plasmid, the T-DNA, integrates into plant nuclear DNA. Similar 23-base-pair (bp) direct repeats at each end of the T-region signal T-DNA borders, and efficient T-DNA transmission (transfer and integration) requires two discrete sequences: the right-hand border repeat and the 24 bp T-DNA transmission enhancer sequence called overdrive. T-DNA transmission apparently initiates when the virD endonuclease produces a site-specific nick in the bottom strand of the right-hand border repeat. Subsequent events generate T-strands, linear single-stranded DNA molecules containing the entire bottom strand of the T-region. Since cocultivation of Agrobacterium with wounded plant cells induces synthesis of the virD endonuclease and accumulation of T-strands in the bacteria, T-strands likely participate in T-DNA transmission. A synthetic right border repeat in its wild-type orientation promotes T-DNA transmission (virulence) at a low frequency; a conserved Ti plasmid sequence (overdrive) which normally flanks the right border repeat greatly stimulates virulence. Our previous work showed: overdrive functions fully in either orientation whereas the border repeat promotes T-DNA transmission best in one direction, and the exact spacing and sequence between a border repeat and overdrive can vary slightly without reducing border function. In the present study we investigated the influence of location on the ability of overdrive to stimulate right border function. The overdrive sequence normally lies either 15 bp or 16 bp to the right of two known right border repeats, but overdrive retained its activity when relocated, in either orientation, 75 bp to the left of the border repeat. The T-DNA transmission enhancer stimulated virulence significantly when moved 687 bp to the right of the border repeat, but distances greater than 3,000 bp diminished its activity. Upon cocultivation with tobacco protoplasts, strains carrying a functional overdrive accumulated substantially more T-strands than strains lacking the enhancer sequence. Introduction of a multicopy cosmid carrying the virG, virC, virD, and virE operons suppressed the phenotype of an overdrive deletion mutant, restoring wild-type levels of virulence and T-strand production. Apparently, extra copies of specific vir genes generated abnormally abundant amounts of vir proteins and rendered the overdrive sequence unnecessary. Thus, overdrive apparently stimulates early events in T-DNA transmission by enhancing the interaction between specific vir proteins and the right border repeat when the concentrations of these proteins limit the amount of T-strand production and T-DNA transmission.

Y 004 T-DNA-PROTEIN COMPLEXES WHICH POTENTIATE THE AGROBACTERIUM PLANT CELL TRANSFORMATION PROCESS. Patricia Zambryski, Elizabeth Howard*, Guido DeVos, and Vitaly Citovsky. Division of Molecular Plant Biology, Hilgard Hall, University of California, Berkeley CA 94720. *USDA-PGEC, 800 Buchanan St., Albany, CA 94710.

During the genetic transformation of plant cells by the soil bacterium Agrobacterium tumefaciens, a specific DNA segment, the T-DNA, is mobilized from its large Ti plasmid, transferred across the cell walls of the bacterium and the plant, and integrated as an unaltered fragment into the plant nuclear genome. This process is highly regulated and is triggered only in the presence of susceptible plant cells. In brief, 1) wounded plant cells produce low molecular weight phenolic compounds which, 2) are recognized by Agrobacterium as signal molecules, to 3) specicially induce the expression of virulence (vir) genes, essential for 4) interacting with the T-DNA element to mediate its transfer (reviewed in 1). Our recent work has focused on the characterization of the molecular reactions associated with the bacterial T-DNA element just prior to its transfer to the plant cell. We detect a free linear single stranded copy of the T-DNA, designated the T-strand (2,3). The T-strand is generated at about one copy per cell, and corresponds to the bottom strand of the T-DNA region on the Ti plasmid such that its 5' and 3' ends map to the right and left copies of the 25 bp T-DNA border sequences. The polarity of the T-strand fits exactly with the observed functional polarity of the T-DNA border sequences i.e., transfer occurs in the direction right border to left border. These properties make the T-strand the best candidate for the intermediate molecule that Agrobacterium transfers to the plant cell; other potential candidates, such as double stranded linear or circular T-DNA molecules are present at extremely low levels in vir-induced Agrobacteria. To study how the T-strand is transferred to the plant cell requires that we also characterize other bacterial components with which it is associated during its transit to the plant cell. We are specifically interested in DNA binding proteins which may facilitate T-strand transfer, and are currently assaying for two types of DNA binding proteins, with either specific or nonspecific DNA binding properties. A specific DNA binding protein is one which would bind to one (or both) ends of the T-strand to potentially "pilot" the T-strand through the bacterial and plant cells. A nonspecific DNA binding protein, such as single strand binding protein, would coat and protect the T-strand during its travels. Evidence for these types of potential T-DNA transfer facilitating proteins will be presented.

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Molecular Genetics of Bacterial Pathogens

Y 005 MOLECULAR BIOLOGY OF PECTATE LYASE PRODUCTION AND VIRULENCE IN ERWINIA CHRYSANTHEMI, Alan Collmer, Greg L. Cleveland, Alan D. Brooks and Sheng Yang He, Department of Botany and Agricultural Biotechnology Center, University of Maryland, College Park, MD 20742.
The extracellular pectic enzyme complex of the phytopathogen Erwinia chrysanthemi EC16 comprises exo-poly-α-D-galacturonosidase (exoPG), pectin lyase (PNL), and four isozymes of pectate lyase (PL). PL is considered to be the most important of these enzymes in causing plant tissue maceration because it is the endo-cleaving pectic enzyme produced in highest levels. To evaluate the role of the enzyme in soft-rot virulence, the PL-encoding pel genes were deleted from the E. chrysanthemi chromosome. The pelB and pelC genes were deleted by marker exchange-eviction mutagenesis. An nptI-sacB-sacR cartridge encoding kanamycin resistance and sucrose sensitivity was inserted in a cloned E. chrysanthemi DNA fragment in place of the pelB and pelC genes. The marked deletion was introduced into the chromosome by exchange recombination and then evicted by a second recombinational exchange with an unmarked deletion derivative. The pelA and pelE genes were subsequently deleted by similar methods, resulting in mutant UM1005 which was deficient in PLa, PLb, PLc, and PLe. E. chrysanthemi UM1005 produced less than 0.1% of the extracellular PL activity of the wild type but utilized polygalacturonic acid as a sole carbon source, presumably because exoPG could still digest the polymer extracellularly. The mutant was also able to cause maceration in potato tuber, pepper, and carrot tissues, although its virulence was substantially reduced. To identify the residual macerating factor produced by UM1005, a genomic library was constructed in pUC19. Two thousand Escherichia coli transformants were screened for their ability to sink into pectate semisolid agar. Seven clones were pectolytic and produced PNL (but not PL) as determined by spectrophotometric assays employing differential substrates. Although the level of PNL activity in the E. coli clones was higher than in E. chrysanthemi UM1005, the clones were unable to macerate plant tissues. Mutants deficient in PNL and exoPG are now being constructed to permit evaluation of the role of all of the components of the extracellular pectic enzyme complex of E. chrysanthemi in plant cell wall degradation, maceration, multiplication, and bacterial survival.

Y 006 DIVERSITY OF PATHOGENICITY GENES OF XANTHOMONAS, Michael J Daniels, Christine E Barber, Anne E Osbourn and J Maxwell Dow, Sainsbury Laboratory, John Innes Institute, Colney Lane, NORWICH NR4 7UH, UK.
Complementary strategies have been used to isolate genes encoding potential pathogenicity genes from cosmid genomic libraries of Xanthomonas campestris pathovar campestris, the agent of crucifer black rot. Genes encoding known products are recognised by plate tests to detect production of the factor directed by cloned genes. X.c. campestris protease genes are expressed in E. coli, but genes for cellulase and polygalacturonate lyase are not. However, X.c. translucens does not produce either enzyme and this pathovar can be used as an "expression host" for X.c. campestris genes. Genes involved in biosynthesis of the extracellular polysaccharide xanthan have been cloned by "complementation" of non-mucoid mutants of X.c. campestris. "Complementation" of mutations giving a non-pathogenic phenotype can be used to isolate genes of unknown function by plant testing of transconjugant strains arising from transfer of libraries into mutants. Using a broad host range promoter-probe plasmid with transcriptional fusions of DNA fragments to chloramphenicol acetyltransferase, in conjunction with chloramphenicol-treated seedlings, it is possible to identify promoter-containing fragments which show differential expression when bacteria enter plants(1). The promoter fragments can then be used as probes to isolate surrounding genomic sequences from cosmid libraries for analysis of possible roles in pathogenicity. Application of these several strategies yields a set of cosmid clones, the insert DNA of which may contain pathogenicity genes. Mutagenesis of cloned DNA with Tn5, mapping of the mutations and subsequent transfer into the genome of wild-type X.c. campestris by recombinational marker exchange yields a set of Tn5 mutants in otherwise identical genetic backgrounds which can be used to test the effect of mutations on pathogenicity. Using this approach we have found that (a) mutations which abolish protease(2) and cellulase have little effect on pathogenicity, (b) xanthan deficient mutants require higher inocula to give symptoms(3), (c) extracellular enzymes are subject to "global" regulation of synthesis and are exported by a mechanism specified by a cluster of genes(4).

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Y 007 MOLECULAR BASIS OF VIRULENCE IN PSEUDOMONAS SYRINGAE subsp SAVASTANOI, T. Kosuge, O. da Costa, T. Gaffney, J. Kelly, R. Nordeen, F. Roberto, Dept. of Plant Pathology, University of California, Davis, CA 95616. Production of the plant growth regulators, indoleacetic acid (IAA) and cytokinins, confers virulence in Pseudomonas syringae subsp savastanoi for its hosts oleander and olive. IAA is produced from tryptophan by the action of tryptophan monooxygenase and indoleacetamide hydrolase; genes for the enzymes, iaaM and iaaH, respectively, occur in an operon. The operon is found on a plasmid pIAA in oleander strains and on the chromosome in olive strains. Transcriptional and translation fusions to lacZ, primer extension and S1 nuclease mapping have been used to determine the transcription start site for the IAA operon promoter; the promoter region contains -10 sequences, TATGTT, and -35, TTGAAA.

Oleander strains convert IAA to IAA-lysine; iaaL the gene which encodes IAA-lysine synthetase occurs upstream from the IAA operon. The deduced amino acid sequence of the open reading frame of iaaL shows a hydrophobic region at the N-terminal end that is reminscent of a membrane-bound protein. Conversion to IAA-lysine influences the amount of IAA secreted by the pathogen; olive strains which do not produce IAA-lysine accumulate 3 to 5-fold higher amounts of IAA than oleander strains. IAA lysine synthetase is not specific for lysine; other dibasic amino acids such as ornithine also are used as substrates for the conjugation reaction.

Cytokinins are products of 5'-AMP and isopentenyl pyrophosphate metabolism. Cytokinin production appears to be influenced by IAA or a component of the IAA operon; loss of IAA production by insertional inactivation of iaaM or loss of pIAA invariably results in reduced cytokinin production.

The role of the two types of phytohormones in tumorigenesis has been well established in Agrobacterium tumefaciens; however, other non-tumorigenic plant pathogenic bacteria also produce cytokinins and IAA. The function of phytohormone production in non-tumorigenic plant pathogens remains to be determined.

Molecular Genetics of Fungal Pathogens

Y 008 REGULATION OF FILAMENTOUS GROWTH AND PATHOGENICITY IN USTILAGO MAYDIS. Flora Banuett and Ira Herskowitz. Department of Biochemistry, University of California, San Francisco, CA 94143.

Ustilago maydis is a fungus which causes corn smut disease. Completion of its life cycle is strictly dependent on the plant; its growth results in tumors on different plant parts. Two distinct forms characterize its life cycle: a non-pathogenic unicellular haploid form and a pathogenic filamentous dikaryotic form. Production of these forms and completion of the life cycle are governed by two loci, a and b (the incompatibility or mating type loci). a has 2 naturally occurring alleles; b has at least 25. The filamentous dikaryon results from fusion of haploids which differ at both loci: a1b1 haploids mixed with a2b2 haploids yield the pathogenic filament, whereas a1b1 mixed with a2b1 does not. We would like to understand how these loci regulate cell fusion, filamentous growth, and pathogenicity of U. maydis.

We have developed a plate mating assay based on (1) which has facilitated genetic analysis of this fungus. On charcoal media, the filamentous form exhibits distinctive white fuzz (the Fuz^+ reaction). We have obtained mutants of an a1b1 haploid unable to produce the fuzz reaction when mated to an a2b2 strain. The mutations in these strains are expected to alter a, or b, or other genes necessary for cell fusion or filamentous growth (fuz genes).

Previous workers (see 2) demonstrated the importance of b in filamentous growth of this fungus. Our recent work with diploid strains demonstrates that the a locus is also required for filamentous growth. Starting with a diploid of genotype a1/a2 b1/b2 (a#b#), which is Fuz^+ and solopathogenic, we obtained Fuz^- derivatives after treatment with low doses of UV. We found that one class of Fuz^- strains had become homozygous at b (a#b=), confirming results described previously. Surprisingly, the other class represented strains which had become homozygous at a (a=b#). These results show that different alleles at a are necessary for continuous filamentous growth. Fuz^- diploids differ in tumor inducing ability.

The behavior of a#b= and a=b# diploids provides a straightforward way to clone a and b by a simple functional assay. To clone b1 we will introduce DNA fragments from an a2b2 strain into an a1/a2 b1/b1 strain. Transformants will be screened for the Fuz^+ phenotype. We expect this analysis to be the starting point for future work that will lead to understanding how U. maydis subverts its host to alter cell growth control and cause tumors.

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Y 009 THE NEW USTILAGO GENETICS, Leong, S. A., Wang, J., Holden, D. W., Kronstad, J., Budde, A., and S. Covert,USDA-ARS and Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.
Recent progress on the development of tools for molecular genetic analysis of the corn smut fungus U. maydis will be discussed. We have developed a high efficiency, integrative transformation system based on resistance to hygromycin B. The DNA is both mitotically and meiotically stable and integration occurs by homologous recombination in 18% of the transformants. Ustilago hordei and Ustilago nigra have also been transformed. A cosmid library has been constructed and a number of genes have been isolated by genetic complementation and probing methods. A cDNA library was prepared in a yeast expression vector and cDNAs encoding metabolic enzymes were identified by complementation of yeast auxotrophs. The cDNA encoding orotidine-5'-monophosphate decarboxylase (pyr3) has been extensively characterized. The cloned genomic pyr3 gene was physically interrupted by insertion of a hygromycin B selectable marker and used to disrupt the wild type homologue in U. maydis. Finally, gene mapping studies using OFAGE (orthogonal field alternation gel electrophoresis) analysis of chromosomes will be described. Application of these tools to the isolation and characterization of genes involved in high affinity iron transport and mating type control will be discussed.

Y 010 GENETIC ORGANIZATION OF DOUBLE-STRANDED RNAs ASSOCIATED WITH BIOLOGICAL CONTROL OF CHESTNUT BLIGHT, Donald Nuss, Brendon Rae, James Tartaglia and Bradley Hillman, Roche Institute of Molecular Biology, Nutley NJ 07110.
The phenomenon of transmissible hypovirulence in *Endothia parasitica*, the agent responsible for the demise of the American Chestnut, is a premier example of a natural form of biological control of a plant pathogenic fungus. Double-stranded (ds) RNAs are thought to be the transmissible cytoplasmic elements responsible for the hypovirulence phenotype. The presence of dsRNAs correlates not only with reduced virulence, but also with suppressed synthesis of several abundant fungal proteins, reduced sporulation and, in certain strains, suppressed pigment production. While a number of the structural properties of these dsRNAs are consistent with a viral origin, they differ significantly from those exhibited by the dsRNA genomes normally associated with mycoviruses, e.g., the hypovirulence-related dsRNAs are not found within discrete virus-like particles and they contain a 3'-terminal stretch of polyadenylic acid base-paired with a 5'-terminal stretch of polyuridylic acid. Structural properties identified by direct analysis of the dsRNAs have provided landmarks useful in the characterization of a series of corresponding cDNA clones. Sequence analysis of selected independent cDNA clones, coupled with *in vitro* expression studies, has resulted in the identification of several gene products specified by the dsRNAs associated with the European hypovirulent strain EP713. Protocols recently developed for DNA-mediated transformation of filamentous fungi are being used in an attempt to determine the phenotypic effect of these gene products when expressed independently in virulent strains of *E. parasitica* and other plant pathogenic fungi.

Y 011 DEVELOPMENT OF A PLANT PATHOGEN FROM A "SAPROPHYTE." William E. Timberlake and Ralph A. Dean, Departments of Genetics and Plant Pathology, University of Georgia, Athens, GA 30602.

Aspergillus nidulans, an ascomycetous fungus that has traditionally been considered to be saprophytic, can be cultured on living plant tissues in the laboratory where it ramifies the tissues by hyphal growth and produces necrotic lesions in which the organism sporulates. Other disease symptoms, such as "water soaking" are also apparent with some tissues. Wounding of the plant tissues is a prerequisite for infection. Thus A. nidulans, although normally a saprophyte, has a demonstrable pathogenic potential.

We have found that A. nidulans produces substantial quantities of pectate degrading enzymes under controlled conditions, and production of these enzymes may be essential for successful colonization of living plant tissues. Enzyme production occurs only in the presence of appropriate substrates and is repressed by addition of glucose. We are attempting to construct mutant strains of A. nidulans that either do not produce pectate degrading enzymes, overproduce the enzymes or produce the enzymes even in the presence of preferred carbon sources, such as sucrose or glucose. The pathogenic potential of these strains will then be examined. The implications of converting a saprophytic organism into a vigorous pathogen by adding pathogenicity and virulence determinants to the saprophyte's genome will be discussed.

Plant-Microbe Signals

Y 012 PLANT SIGNALS TRIGGER EXPRESSION OF FUNGAL GENES REQUIRED FOR PATHOGENESIS, P.E. Kolattukudy, Ohio State Biotechnology Center, The Ohio State University, Columbus, OH 43210.

Chemical signals from plants are known to play an important role in host-pathogen recognition and interaction that determine the events of disease development. The plant surface layers consisting of the epicuticular waxes, the hydroxyfatty acid polymer (cutin or suberin) comprising the cuticle, and the pectinaceous subcuticular layers are the major barriers which fungal pathogens must penetrate to infect plants (1). We have studied chemical signals from these surface layers which induce specific responses involved in pathogenicity by certain plant pathogenic fungi. The hydroxyfatty acid monomers of cutin have been shown to specifically induce transcription of the gene for cutinase, an extracellular enzyme implicated in the infection process of several fungal pathogens (2). Recently, we have discovered that cutinase induction requires interaction of cutin monomer from the plant with a specific "protein factor" from the fungus. Similarly, components of the subcuticular layer (pectin) induce Fusarium solani pisi to produce certain pectinolytic enzymes; addition of antibodies for these enzymes to spore suspensions severely inhibits their pathogenicity on intact host tissue (3). Recent observations indicate that certain components of the epicuticular wax of fruits induce growth and differentiation of fungal spores, with apparent host-pathogen specificity. Thus, fungal pathogens may be seen to have developed specific responses to breach the surface layers of the plant, based on recognition of signals generated from each layer.

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Y 013 REGULATION OF RHIZOBIUM SYMBIOTIC GENES BY PLANT SIGNALS, Melanie Yelton, Robert Fisher, Thomas Egelhoff, John Mulligan and Sharon Long, Dept of Biological Sciences, Stanford University, Stanford CA 94305-5020.

The nodulation (nod) genes of Rhizobium meliloti are required for establishing symbiotic root nodules on its host plant, alfalfa (Medicago sativa). These genes include nodABC, nodFE, and nodH. Transcript analyses and assays of nod-lac gene fusions show these genes are not expressed in free-living bacteria, but are induced by plant exudates. Induction of these nod genes requires the protein product of regulatory gene nodD. The most active inducing compound in the alfalfa-Rhizobium meliloti symbiosis is the flavone, luteolin. Studies in other systems have shown that the most active compound varies according to the plant-bacterial combination; the bacterial source of the nodD gene correlates with this, and may influence host range in some circumstances. The ability of a bacterium to respond to plant signals is dependent not only on nodD, but on the chromosomal background: R. meliloti nod genes can be induced by luteolin if they are carried along with nodD in Agrobacterium tumefaciens, but not in an E. coli or Pseudomonas background. We have further investigated the mechanism of nod gene regulation by studying the role of the nodD protein, NodD, and the role of the upstream DNA in inducible promoters. Using the gel retardation assay, we have found that extracts of R. meliloti expressing nodD are capable of interacting specifically with nod promoter fragments. Antibody directed against NodD removes from these extracts the ability to bind to nod promoters. NodD was substantially purified by means of an immunoaffinity column, and the purified preparation retained the ability to bind to nodD promoter fragments. Common to all nod gene upstream DNA is a highly conserved 50-bp long segment, the nod box, located at -26 to -28 bp from each nod transcript start site. A synthetic duplex oligonucleotide identical to half of the nodA nod box was found to compete with all intact nod promoters for interaction with NodD. Deletions in the nod promoter regions also indicated the role of nod box sequences in NodD interaction. Purified RNA polymerase and a new in vitro transcription-translation system for R. meliloti have been established as a means to reconstituting the molecular components of inducible symbiotic gene expression.

Y 014 *QUINONE REDOX POTENTIALS: A PARASITES BRAILLE,* David G. Lynn, Christopher E. Smith, and Gwendolyn D. Fate, Department of Chemistry, 6735 Ellis Avenue, The University of Chicago, Chicago, IL 60637

The discussion will center on the biological recognition within the parasitic angiosperms. These parasitic plants are clearly not microbes and so are strictly not pathogens, and yet the biological recognition appears to function, conceptually at least, in a manner related to that seen for microbe-plant interactions. A detailed discussion of the signal substances and how they function within the biological framework will be given. Since the recognition event results in specific developmental processes occurring within a eucaryotic parasite, similarities between the functioning of these signals and the general control of plant development will be highlighted.

Y 015 THE IMPORTANCE OF PHYTOALEXIN DETOXIFICATION FOR PATHOGENICITY, Hans VanEtten, Plant Pathology Department, Cornell University, Ithaca, NY 14853.
The synthesis of phytoalexins by plants in response to challenge by microorganisms is believed to be part of an active mechanism of disease resistance. Our research objective has been to elucidate the molecular basis by which a pathogen may overcome this resistance mechanism. The fungus Nectria haematococca causes a disease on pea even though the infected tissue synthesizes large amounts of the phytoalexin pisatin. Our studies indicate that virulent isolates of N. haematococca can grow in such tissue because they are able to detoxify pisatin. The enzyme pisatin demethylase, responsible for detoxifying pisatin, is a substrate-inducible microsomal cytochrome P-450. Conventional genetic studies have identified six different genes (PDA genes) coding for different levels of pisatin demethylating activity. The highest levels of virulence on pea are associated with those PDA genes coding for high enzyme activity. Our current hypothesis is that the PDA genes are a family of cytochrome P-450 genes, and that for parasitism on pea N. haematococca has evolved a specific cytochrome P-450 for the detoxificaton of pisatin. Recently, a N. haematococca PDA gene that encodes high enzyme activity was transformed into a Pda$^-$, non-virulent N. haematococcca isolate. Pda$^+$ transformants were virulent on pea, which verifies that detoxification of pisatin is required for pathogenicity by this fungus. Preliminary analysis of a number of other pea pathogens indicates that many of these fungi can demethylate pisatin. Detoxification of phytoalexins may be a common means to circumvent a plant resistance mechanism based on phytoalexin production.

Molecular Biology of RNA Viruses

Y 016 **CONSTRUCTION AND TESTING OF DESIGNED HYBRIDS BETWEEN BROME MOSAIC AND COWPEA CHLOROTIC MOTTLE VIRUSES**, Richard F. Allison, Michael Janda and Paul Ahlquist, Institute for Molecular Virology and Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.
The closest known relative of brome mosaic virus (BMV), type member of the bromoviruses, is cowpea chlorotic mottle virus (CCMV). The genomes of BMV and CCMV are each divided among messenger-sense RNAs 1, 2 and 3 (3.2, 2.9 and 2.1 kb). Despite their common genetic organization and the high degree of sequence similarity between their genes, BMV systemically infects monocotyledonous hosts while CCMV infects legumes. To facilitate study of the molecular basis for such host specificity and many other virus functions, we have cloned the complete genomes of both BMV and CCMV as cDNA copies whose in vitro transcripts are highly infectious to their respective systemic hosts. Each set of transcripts also induces viral RNA replication and packaging in barley protoplasts and produces infection lesions on Chenopodium hybridum, a local lesion host common to BMV and CCMV.
Using such infectious transcripts, all possible single component exchanges between the tripartite BMV and CCMV genomes were made and tested. Viral RNA replication was not observed in protoplasts with any heterologous combinations of RNAs 1 and 2, which encode trans-acting factors involved in RNA replication. However, substitution of the heterologous RNA3 into either genome produced viable hybrid viruses which replicated in barley protoplasts and initiated local lesions on C. hybridum leaves. In all cases BMV RNAs were preferentially replicated over CCMV RNAs even when CCMV RNAs provided the trans-acting replication components. In protoplasts CCMV and BMV coat proteins packaged RNAs 1 and 2 from the heterologous virus.
BMV-CCMV hybrids have also been constructed by exchanging selected segments within individual components of the tripartite BMV and CCMV genomes. E.g., BMV and CCMV RNA3 each encode two genes, a 5'-proximal 3a gene which may facilitate spread of infection between cells, and a 3'-proximal coat gene. When flanking noncoding sequences are properly adjusted, hybrids which reassort the 3a and coat genes within RNA3 are replicated in protoplasts and support local lesion infections of C. hybridum when co-inoculated with wild type BMV or CCMV RNAs 1 and 2. Tests of such hybrids in systemic hosts of BMV and CCMV suggest that 3a gene adaptation is involved in host specialization, but is not the sole determinant of host range.

Y 017 THE INTERACTION OF MODIFIED VIRUSES WITH THEIR HOSTS, W. O. Dawson and P. Bubrick, Department of Plant Pathology, University of California, Riverside, CA 92521.
A series of mutants of tobacco mosaic virus (TMV) with insertions and/or deletions in the coat protein gene was made by modification of the cDNA of the genome (pTMV204) followed by *in vitro* transcription. All mutants constructed multiplied as free-RNA viruses and moved efficiently in inoculated leaves of Xanthi tobacco. This included mutants that had the entire coat protein gene removed or deletions that extended 28 nucleotides into the 3'-non-translated region, as well as the bacterial chloramphenicol acetyltransferase gene substituted for the coat protein gene. The mutants moved systemically only to a limited extent. Most of the mutants produced coat protein-related polypeptides *in vivo*. The size and amount of protein found in inoculated leaves varied considerably among different mutants. In general, polypeptides that contained the normal coat protein carboxy terminus tended to accumulate in greater amounts. Three types of symptoms were observed in mutant-infected Xanthi tobacco: latency, yellowing, and necrosis. Most mutants that produced no or low amounts of coat protein induced no symptoms. The mutants that retained the carboxy terminus of the coat protein induced yellowing symptoms. Two mutants induced necrotic local lesions in Xanthi tobacco as well as almost all host plants tested. These results demonstrate the multifunctional role of the coat protein gene of TMV.

Y 018 MOLECULAR GENETIC AND FUNCTIONAL ANALYSES OF A PLANT VIRUS PROTEINASE AND ITS ROLE IN POLYPROTEIN PROCESSING. William G. Dougherty, James C. Carrington, T.Dawn Parks and Susan Cary, Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

Tobacco etch virus (TEV) is a member of the potato virus Y group. The RNA genome of TEV contains a single open reading frame coding for a 346,000 (346 kd) polyprotein. The processing of this polyprotein has been characterized using molecular genetic, immunological and biochemical approaches. The small nuclear inclusion protein of TEV is a viral-encoded proteinase. Virus-specific proteolytic activity is associated with this 49 kd TEV protein whether isolated in the form of nuclear inclusion bodies from TEV-infected plant tissue, or synthesized from cDNA sequences utilizing an SP6 transcription system and translation in a rabbit reticulocyte lysate. Monospecific antiserum reactive with the 49 kd protein specifically inhibits proteolytic activity. Nucleotide sequence studies, deletion analyses and site-directed mutagenesis of the 49 kd protein gene of TEV reveal the following. The 49 kd protein is 430 amino acids in length, containing, near the carboxy terminus, the conserved Cys-15aa-His motif present in all viral-encoded cysteine-like proteinases. A polypeptide in which the N-terminal 202 amino acids of this 49 kd proteinase have been deleted has the same proteolytic activity as the 49 kd protein. C-terminal deletion of 30 amino acids eliminates all activity. Replacement of the Cys with Ala in the putative active site abolishes proteolytic activity. The proteinase is active as a free entity or as part of a larger polyprotein.

During replication, this proteinase autocatalytically releases from the polyprotein (cis activity) and is active at three other sites (trans activity). Cleavage is between a Gln-Ser or Gln-Gly at the conserved sequence -Glu-Xaa-Xaa-Tyr-Xaa-Gln-Ser or Gly. This seven amino acid conserved sequence appears essential in defining a cleavage site, as shown by insertion and cleavage of synthetic sites at new positions in the polyprotein and by site-directed mutagenesis of an authentic site. Understanding mechanisms of viral proteinase recognition and specificity may be useful in engineering virus resistance. Alternatively, the specificity of the viral proteinase may be exploited to activate a resistance mechanism.

Y 019 GENES AND SEQUENCES INVOLVED IN THE REPLICATION OF THE COWPEA MOSAIC VIRUS RNAs, Rik Eggen, Ab van Kammen, Joan Wellink and Rob Goldbach, Departments of Molecular Biology and Virology, Agricultural University, Wageningen, The Netherlands.
The genetic information of cowpea mosaic virus (CPMV), type member of the comoviruses, is divided among two positive-sense RNA molecules referred to as B-RNA (5889 bases long) and M-RNA (3481 bases). Each RNA has a small protein, VPg, covalently linked to the 5'-end and a poly(A)-tail at the 3'-end. Whereas both RNAs are required for successful infection of plants B-RNA can accomplish its own replication in cowpea protoplasts. Together with biochemical evidence and interviral sequence comparisons this finding indicates that the genes involved in viral RNA replication are all located in this genome part. Based on the following observations it is proposed that the viral proteins involved in replication are active during only one single round of replication: (i) all attempts to isolate a template-dependent, re-initiating RNA replication complex have failed sofar; (ii) the amount of viral protein isolated as constituents of active, replication complexes is very low compared to the total amount of viral proteins in the infected cell; (iii) since each progeny RNA molecule requires a newly synthesized VPg the need for viral polymerase only keeps in step with the VPg requirement if it would not reinitiate.
To study the sequence requirements for replication a set of full-length cDNA clones of both B- and M-RNA has been constructed, from which infectious transcripts can be obtained by using the T7 in vitro transcription system. While the first clones obtained from B-RNA expressed only low levels of infectivity alterations of the terminal (both viral and non-viral) sequences improved their infectivity. The highest levels of infectivity (50% of protoplasts infected) were obtained with transcripts containing only 1 extra G-residue at the 5'-end and a poly(A)-tail of considerable length (140 residues). The cDNA clones have also been applied to study the proteolytic processing of the viral polyproteins. A model for the (regulating) role of the viral protease in both the replication and translational expression of the CPMV genome will be presented.

Viroids, Satellites and Defective Interfering Particles

Y 020 DEFECTIVE INTERFERING RNAs OF A SMALL RNA PLANT VIRUS. T. Jack Morris and Bradley I. Hillman. Department of Plant Pathology, University of California, Berkeley, CA 94720.
We have recently described a unique class of symptom modulating RNAs which are associated with infection of plants by a strain of tomato bushy stunt virus (1). The TBSV helper virus genome consists of a single linear ssRNA molecule of 4.8 Kb which, when inoculated alone, induces a lethal systemic necrosis in tobacco plants. The presence of linear ssRNAs of less than 500 nucleotides in the inoculum results in symptom attenuation, plant survival and concommitant reduction of viral synthesis. Cloning and nucleotide sequence analysis of several of the small RNAs has revealed that they consist of sequences derived from 5', 3', and internal segments of the helper TBSV genomic RNA. These co-linear deletion mutants have many properties in common with the defective interfering RNAs (DIs) commonly associated with animal virus infections. These TBSV-associated DIs meet all of the requirements for DI status in that: i) they interfer with the replication of the parent virus; ii) they are deletion mutants of the helper virus genome; iii) they do not contain an ORF of sufficient length to encode any translation products; and iv) they have retained the necessary sequences for both replication and encapsidation. This discovery marks the first definitive report of a defective interfering RNA (DI RNA) in association with a small RNA plant virus. The origin of these DI RNAs and their utility as valuable models for investigating cis-acting, viral regulatory sequences involved in both the replication and encapsidation of viral genomes and for studying symptom modulation and virus persistence will be discussed.

Reference: Hillman, B. I., Carrington, J.C. and Morris, T.J. A defective interfering RNA that contains a mosaic of a plant virus genome. Cell 51: in press.

Y 021 **TURNIP CRINKLE VIRUS SATELLITE DOMAINS INVOLVED IN VIRULENCE AND PROCESSING**, Anne E. Simon*, Henk Engel, and Stephen H. Howell, Department of Biology, University of California San Diego, La Jolla, CA 92093.
Turnip crinkle virus (TCV) supports a small family of satellite RNAs, one of which (RNA C) intensifies the symptoms of TCV when co-inoculated on turnip, while two other satellites (RNAs D and F) are avirulent. RNA C (355 b) differs from RNA D (194 b) and RNA F (230 b) mainly by the presence of a 3′-domain which is homologous to the 3′-end of the TCV genome (1). To study the functions of RNA C, we have constructed a chimeric satellite composed of the virulent and avirulent satellites, and we have inserted and deleted sequences at various sites in the virulent satellite (RNA C) cDNA template. Mutated satellite RNA was synthesized *in vitro*, and assayed for infectivity and symptom production in turnip. Small insertions (up to 60 bases) in the 5′ two thirds of the molecule had no detectable effect on infectivity or symptom production. However, small insertions and deletions in the 3′ one third of RNA C destroyed the infectivity of the satellite. The chimeric satellite constructed from the 5′ 155 bases of the avirulent satellite RNA F ligated to 200 bases of the 3′-domain of RNA C produced severe symptoms, which demonstrates the 3′-domain of RNA C determines virulence. We have also identified a region of RNA C involved in satellite processing. Deletion of nucleotides 79-84 resulted in the accumulation of large amounts of the dimeric form of the satellite. Only trace amounts of RNA the size of the inoculated (monomeric) transcripts were found. We have not observed self-processing by *in vitro* synthesized RNA C dimers under a variety of conditions, implying that RNA C is processed by a mechanism which differs from that used by circular satellites.

1. Simon, A.E. and Howell, S. H. (1986) EMBO J. **5**, 3423-3428.

*Current Address: Department of Plant Pathology, University of Massachusetts, Amherst, MA 01003

Transformation of Plants for Effective Virus Resistance

Y 022 CONTROL OF VIRAL INFECTION IN TRANSGENIC PLANTS BY EXPRESSION OF SATELLITE RNA OF CUCUMBER MOSAIC VIRUS, David Baulcombe, Martine Devic, Martine Jaegle and Bryan Harrison+, Molecular Genetics Department, Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2LQ; +Department of Virology, Scottish Crop Research Institute, Dundee, Scotland DD2 5DA.
Satellite RNA of cucumber mosaic virus (CMV) may be considered as either benign or virulent. The benign satellite RNAs do not produce symptoms and can attenuate the symptoms produced by CMV. Virulent satellites may still attenuate the effects of CMV, but superimposed on this they have the capability to induce symptom formation. Infections of CMV containing a benign satellite can cross protect against the effects of virulent satellite RNAs. Thus, the benign satellites can reduce viral disease in either of two ways: by interference with the virus or by cross protection. Consequently, when transgenic plants were engineered to express CMV satellite RNA they were effectively resistant to the effects of CMV, caused either by the virus or by the virus in contamination with a virulent satellite RNA.

The mechanism of interference with CMV is not clear. In some situations the attenuation also involves a reduction in the amount of virus and viral RNA suggesting a competition between satellite RNA and viral RNA. However, there are other examples where the symptom attenuation is not associated with a reduction in the amount of virus. This suggests therefore that the satellite RNA inhibits either the viral components which trigger symptom formation or the plant processes which mediate that response.

In order to identify sequences within the satellite RNA responsible for symptom attenuation and also the virulence capabilities, cDNA clones of different strains of satellite RNA have been introduced into the plasmid PM1 from which it is possible to produce infectious satellite RNA by in vitro transcription. This has allowed the analysis of mutant forms of the satellite or of chimaeric satellites incorporating elements from the different strains. In addition, a cDNA with a modified 5' end has been expressed in transgenic plants. Analysis of these satellite RNAs indicates that the symptom attenuating capability involves the 5' terminal sequence. The virulence capability is located in different regions of the molecule, depending on the strain of satellite RNA. Extension of these studies will allow disabled satellite RNA molecules to be engineered which are not capable of mutation to virulence.

Supported by the Agricultural Genetics Company.

Y 023 PLANT VIRUS RESISTANCE BASED ON THE SATELLITE RNA OF TOBACCO RINGSPOT VIRUS, W.L. Gerlach, D. Llewellyn and J.P. Haseloff, CSIRO Division of Plant Industry, GPO Box 1600, Canberra, Australia. 2601.

The satellite RNA of tobacco ringspot virus (STobRV) belongs to a class of small (300-400 bases) single strand RNAs which can be considered parasites of their helper viruses. STobRV replicates to high levels and is encapsidated by virus coat protein in TobRV infected plants. STobRV is not required for virus propagation and it has no detectable sequence similarity with the genome of its helper virus. Its presence during virus infection provides symptom amelioration to plants.

Transgenic tobacco plants expressing STobRV RNA transcripts or its complement show phenotypic resistance when infected with TobRV. This is correlated with amplification of STobRV to high levels in lesions and reduced systemic spread of the virus in infected plants. The level of protection is not markedly affected by the level of gene expression, presumably due to the amplifiable nature of the protecting STobRV sequences.

The mechanism of the protection and its relationship to molecular properties such as satellite replication events and encapsidation are not understood. To obtain information on this we are testing the properties of mutated forms of STobRV in vitro and in vivo.

The sequences required for autolytic cleavage of (+) and (-) strand multimers of STobRV have been precisely defined by mutant analysis. By comparison of these autolytic cleavage sites with those of mutants and related RNAs, design rules have been deduced for the construction of oligoribonucleotides which can act as specific ribonucleases ("ribozymes") against target RNA molecules. As one application of this technique, we are exploring it as a new strategy for production of virus resistant plants.

Molecular Biology of DNA Viruses

Y 024 POST-TRANSCRIPTIONAL CONTROL OF CAULIFLOWER MOSAIC VIRUS GENES, T. Hohn, J. M. Bonneville, J. Futterer, K. Gordon, B. Pisan, H. Sanfacon, M. Schultze and J. Jiricny, Friedrich-Miescher-Inst., CH-4002 Basel.

Reporter genes attached to Cauliflower Mosaic Virus (CaMV) control regions were introduced into plant protoplasts by electroporation and the activity of the reporters (CAT and GUS) assayed after an incubation period. Results show that:

1) The 600 nt leader of CaMV 35-S RNA contains some sequences that enhance and others that inhibit expression of downstream genes.

2) Transcriptional polyadenylation signals of CaMV are recognized differently by protoplasts from different plants and tissues.

3) Polycistronic translation of CaMV constructs is possible, but requires a novel type of transactivation acting on sequences around the gene borders.

4) In contrast to real retroviruses, the CaMV gag and pol genes are expressed individually.

Y 025 EXPRESSION OF DNA VIRUS GENOMES IN PLANTS, Stephen H. Howell, Carmen Fenoll, Gail A. Baughman, Michel Schneider and Diane M. Black, Biology Department C016, University of California San Diego, LaJolla CA 92093.

Maize streak virus (MSV), a geminivirus with a one-component genome, encodes a major coat protein RNA which accumulates in infected cells. Using a maize protoplast transient expression system, we have defined the promoter which drives rightward transcription of the RNA encoding the coat protein. We have identified a 122 bp upstream segment that enhances promoter activity and functions as an upstream activating sequence (UAS). The UAS lies in the starting intergenic region of the viral genome in a domain that includes a hairpin loop (with conserved sequences in the loop) found in other geminiviruses. However, fine dissection of the UAS reveals that the hairpin loop is largely dispensible, while the GC rich boxes immediately upstream from the hairpin loop are necessary for rightward transcription. The MSV promoter UAS is interchangeable with a similar element in the cauliflower mosaic virus (CaMV) 35S RNA promoter. Proteins in extracts from maize nuclei bind to the MSV UAS and protect the GC rich boxes. Although the CaMV 35S promoter DR fragment is functionally equivalent with the MSV UAS, it does not effectively compete for the proteins that bind to the MSV UAS.

Gene VI of the cauliflower mosaic virus (CaMV) genome encodes a protein (P_{66}) in virus infected plants which accumulates in cytoplasmic inclusion bodies. When a segment of the CaMV genome bearing gene VI is transferred to tobacco plants by the *Agrobacterium* Ti-plasmid, the resulting transgenic plants display viral-like symptoms. That gene VI is responsible for the symptomatic phenotype was demonstrated by showing that symptom production was blocked by deletions and by a frame-shifting linker mutation in gene VI. Furthermore, in primary transformants, there was a strict correlation between the appearance of symptoms and the presence of gene VI product, P_{66}, detected by immunoblots.

Y 026 **MAIZE STREAK AND SQUASH LEAF CURL VIRUSES,** **Sondra G. Lazarowitz and Allison J. Pinder, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210.**

We have been studying the geminiviruses squash leaf curl (SqLCV) and maize streak (MSV), focusing on the structural and functional characterization of the viral genomic components. Our analyses have identified two highly homologous bipartite SqLCVs which can be distinguished by their host ranges in members of the Cucurbitaceae as well as in P. vulgaris and N. benthemiana. The host range of the more restricted virus SqLCV-NR is a subset of that of the broader host range virus SqLCV-BR. Sequence analyses have shown these two viruses to be nearly identical in their potential gene coding regions and have identified a 13 base deletion in the Common Region of SqLCV-NR as compared to SqLCV-BR. The role of this 13 base deletion and other viral sequence elements in the host range phenotypes of these viruses is being defined through site-directed mutagenesis and complementation analyses with cloned viral genomic components.

We have characterized and sequenced an infectious genomic clone of a South African isolate of the single component geminivirus MSV. Using agroinoculation we are assessing the effects of specific single-base changes, deletions and gene substitutions in the viral genome on replication, and systemic spread and symptom formation. Preliminary results indicate that the capsid is required for systemic spread. Mutant DNA lacking the capsid gene can be found replicating in the inoculated leaves, but appears to be impaired in the ability to systemically spread through the plant. Other mutations are aimed at determining the functions of the other potential gene coding regions of the genome, as well as specific sequence elements in the two viral intergenic regions.

Y 027 MOLECULAR GENETICS OF TOMATO GOLDEN MOSAIC VIRUS, J. Scott Elmer, Leslie Brand, Garry Sunter[1], William E. Gardiner[1], Charles K. Browning[1], David M. Bisaro[1] and Stephen G. Rogers, Plant Molecular Biology, Monsanto Company, 700 Chesterfield Village Pkwy, St. Louis, MO 63198 and [1]Department of Molecular Genetics and Ohio State Biotechnology Center, Rightmire Hall, Ohio State University, Columbus, OH 43210

We have adapted the "agroinfection" procedure of Grimsley et al. (PNAS 83: 3282-3286,1986) to develop an efficient, reproducible infectivity assay for the split genome geminivirus, tomato golden mosaic virus (TGMV). Agroinoculation with Agrobacterium T-DNA vectors carrying tandem copies of the TGMV DNAs can deliver both components of TGMV when used in mixed inoculation of wild-type host plants. A greater increase in infection efficiency can be obtained by agroinoculation of the TGMV A component to "permissive" transgenic plants that contain multiple tandem copies of the B component integrated into the host genome. An inoculum containing as few as 2000 Agrobacterium cells can produce 100% infection under these conditions. We have created mutations in the TGMV DNAs in vitro and have used agroinoculation of permissive plants to investigate the role of the coat protein in TGMV infection and have found that its coding sequence may be interrupted or substantially deleted without loss of infectivity. Certain of the coat protein mutants showed reproducible delays in time of symptom appearance as well as reduced symptom development. The most attenuated symptoms were seen with a mutant in which the coat protein coding sequence was almost entirely deleted. Analysis of the TGMV nucleotide sequence indicates that the A component has, in addition to the coat protein coding sequence, four overlapping open reading frames (ORFs) with the potential to encode proteins of greater than 10 kD. Similar analysis reveals that the B component has two such ORFs. We have investigated the functions of these putative proteins in both symptom formation and DNA replication by creating mutations in each of the ORFs. Our results show that the AL4 ORF, which is encoded within the N-terminal region of ORF AL1, is not essential for normal virus infection. In contrast, we find that disruption of the AL3 ORF results in delay and attenuation of symptom formation. We have also found that the products of the AL1 and AL2 ORFs are absolutely required for symptom formation. Studies of DNA replication show that only the AL1 open reading frame is essential for viral DNA synthesis. Analysis of mutations in the two ORFs of the B DNA show that both appear to be essential for systemic spread and symptom formation. The significance of these results for the development of vectors from the geminiviruses will be discussed.

Specificity in Plant-Microbe Interactions

Y 028 GENES THAT CONTROL HOST CULTIVAR SPECIFICITY IN THE RICE BLAST FUNGUS, Forrest Chumley, Kenneth Parsons, and Barbara Valent, Central Research and Development, The DuPont Co., Wilmington, DE 19898. The Ascomycetous fungus Magnaporthe grisea causes the disease of rice called blast. One of our goals is to understand the molecular basis for cultivar specificity seen in the rice blast system. Crosses between two races of the blast fungus that differ in virulence toward a cultivar have shown that single genes (avirulence genes) in the pathogen control specificity toward particular cultivars of rice. Four unlinked avirulence genes have been identified in crosses between races of the fungus that differ in virulence toward one of three rice cultivars tested, CO39, M201, and Yashiro-mochi. Analysis with more cultivars of rice seems likely to identify many more avirulence genes. We will utilize the transformation and gene cloning system developed in our lab to clone an avirulence gene as the first step toward determining how these genes act to govern cultivar specificity. We have now proven the utility of this system by cloning an M. grisea LYS+ gene (lysine biosynthetic gene). Prototrophic colonies were obtained when protoplasts of a Lys- mutant were transformed with a cosmid library of a prototrophic M. grisea strain. Cosmid DNA was easily recovered from these transformants using an eviction technique that employs Lambda DNA packaging and E. coli transduction. Transductants recovered contained cosmid DNA that subsequently was shown to transform Lys- strains to Lys+ with high efficiency. To our knowledge, this is the first example of cloning any gene from a fungal plant pathogen by complementation of function using the plant pathogen as recipient. This work demonstrates the feasibility of cloning M. grisea avirulence genes and other genes that play a role in pathogenicity.

Y 029 THE Rp1 LOCUS IN MAIZE. Albert H. Ellingboe, Department of Plant Pathology and Genetics, University of Wisconsin, Madison, WI 53706.

The Rp1 locus in maize confers resistance to Puccinia sorghia, the causal agent of the rust disease. Intercrosses between the 13 "alleles" at the Rp1 locus have yielded recombinants that suggest the Rp1 locus is a tandemly duplicated region with different specificities in each cistron. Mutations of alleles Rp1f and Rp1k have been obtained in plants heterozygons for these genes and containing Mu-1. These mutations contain specificities not present in any other alleles. Furthermore, the data do not support the interpretation of single cistron inactivation by Mu-1 insertion. Models based on unequal crossing over between the tandem array would be expected to give linear arrangement of loss with an excess of loss of genes in the center of the cluster. No linear arrangement explains the mutants observed. The data suggest that Rp1 is composed of a duplicated segment that has strict regulation of expression of the cistrons contained therein.

Y 030 RACE/CULTIVAR SPECIFICITY OF *PSEUDOMONAS SYRINGAE* PV. *GLYCINEA* INFECTION OF SOYBEAN, Thanh Huynh*, Douglas Dahlbeck† and Brian Staskawicz†, *Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, and †Department of Plant Pathology, University of California, Berkeley, CA.

Pseudomonas syringae pv. *glycinea* (*Psg*) is the causal agent of bacterial blight of soybean. Races of *Psg* are distinguished according to which soybean cultivars are resistant or susceptible to each race. Resistance or susceptibility of a host cultivar to a particular race of *Psg* is determined by the genotypes of both the host and pathogen. Host resistance is accompanied by the rapid death of host cells (the "hypersensitive reaction", HR) in the vicinity of the bacteria. *Psg* races containing the gene *avrB* (*Psg* races 0 and 1) specifically elicit the HR in soybean cultivars containing the resistance gene *Rpg1*. As a step toward determining how *avrB* in the pathogen and *Rpg1* in the host interact to determine race/cultivar specificity, we are studying genes in *Psg* race 0 that determine the bacterium's ability to induce the HR in *Rpg1*-containing hosts. Two classes of genes are required, the *avrB* gene and *hrp* genes. The *avrB* gene has been cloned and sequenced and encodes a 36 kD protein. The 36 kD *avrB* protein is present in bacteria recovered from infected leaves of either resistant or susceptible soybean cultivars. In bacteria grown in synthetic media, high level transcription of *avrB* is subject to catabolite repression by TCA cycle intermediates or structurally related amino acids (preferred carbon sources). In addition to *avrB*, which determines race-specificity, the *hrp* genes are required. Individual *hrp* mutations in *Psg* abolish not only the ability to induce the HR but also render the bacteria nonpathogenic (unable to multiply or cause symptom development in any soybean cultivar). All but one of the *hrp* mutations characterized so far map within a single 22 kb genomic region. Mutations in one approximately 2 kb region of the 22 kb *hrp* gene cluster abolish high level transcription of *avrB* in bacteria grown either *in planta* or in synthetic media. This *hrp* region may encode a positive regulatory factor for *avrB* transcription. *Psg* race 0 grown in media that allow high level *avrB* expression are competent to induce the HR when inoculated into *Rpg1*-containing soybean cultivars without additional bacterial RNA or protein synthesis. We are working with cultures of bacteria competent to induce the HR to identify the bacterial components responsible for inducing the HR and to determine how the *avrB* and *hrp* genes are involved in their synthesis.

Y 031 CLONING, STRUCTURE AND FUNCTION OF BACTERIAL AVIRULENCE GENES. D. Kobayashi, S. Tamaki, S. Hao, D. Trollinger and N. T. Keen. Department of Plant Pathology, University of California, Riverside, 92521.

We have cloned several avirulence (avr) genes from Pseudomonas syringae pv. tomato (Pst), which, when mobilized into P. s. pv. glycinea (Psg), elicit the hypersensitive response (HR) in soybean. One of these genes was identical to the avrA gene previously cloned from Psg race 6. Another characterized gene from Pst, avrD, is unlike others thus far found in any Psg race, but has considerable homology to DNA of all tested Psg races. The homologous avrD gene was accordingly cloned from Psg race 4 and compared to the Pst gene. Sequence data revealed that the coding regions are greater than 90% identical, although the cloned avrD gene from Psg race 4 does not express the avirulence phenotype. This data suggests that Psg contains a recessive allele of avrD which does not function to elicit the soybean HR. Furthermore, unlike other avr genes that we have tested, E. coli cells carrying the overexpressed Pst avrD gene elicit HR reactions when infiltrated into soybean leaves of precisely the same cultivars which respond hypersensitively to race 4 Psg cells carrying the same gene. We have not yet established, however, whether the avrD protein product in E. coli cells is itself a race specific elicitor of the soybean HR. The results have shown that avr genes may be cosmopolitan among P. syringae pathovars, and that avr genes may function as determinants of pathovar host range. Finally, the results indicate that avirulence genes occurring in a certain pathovar are modified such that they do not elicit a HR in the normal host plant species.

Y 032 CLASSICAL AND MOLECULAR GENETICS OF LETTUCE DOWNY MILDEW, Richard W. Michelmore, Rick V. Kesseli, Howard J. Judelson, Scot H. Hulbert, Terrence W. Ilott, and Erik Legg, Department of Vegetable Crops, University of California, Davis, CA 95616

Simultaneous genetic studies have been conducted on Lactuca sativa and Bremia lactucae at the classical and molecular level with the long term goal of determining the biochemical and genetic bases of specificity in lettuce downy mildew. Classical genetics demonstrated that the gene-for-gene hypothesis provided a rigorous description of the interaction between L. sativa and B. lactucae although additional genes could modify the basic interaction. Thirteen genes for resistance to B. lactucae (Dm) have been identified and mapped to four genomic clusters. Fine structure analysis of these regions is now underway. Corresponding avirulence genes are not tightly linked. Several cloning strategies will be aided by a detailed genetic map. Such maps are being developed for both host and pathogen using random cDNA and genomic fragments to detect RFLPs. Genes determining specificity are being mapped relative to RFLPs. Probes are now being developed to detect RFLPs in regions of the genome specifically containing Dm genes. RFLP markers have been used to study variation in both L. sativa and B. lactucae. Somatic fusion and somatic variation was detected in B. lactucae.

Identification of Genes for Plant Disease Resistance

Y 034 SOMATIC AND GERMINAL MUTATIONAL ANALYSIS OF THE *Rp1* DISEASE RESISTANCE LOCUS OF MAIZE, Jeffrey L. Bennetzen, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

The *Rp1* locus of maize determines resistance to the leaf rust pathogen *Puccinia sorghi*. Hard x-ray treatment of heterozygous *Rp1 Oy/rp1 oy* maize embryos generated seedlings with oil yellow sectors (*-/oy*) due to chromosome breakage between *oy* and the centromere. Since *Rp1* is distal to *Oy* on the short arm of chromosome 10, oil yellow sectors will usually be deficient for *Rp1*. Yellow sectored seedlings inoculated with *P. sorghi* spores show rust pustule formation in the oil yellow (*Rp1*-lacking) sectors and hypersensitive necrotic resistance in the green tissue, thereby demonstrating that *Rp1* is cell autonomous in its action. In cases where the hypersensitive reaction was initiated in green (*Rp1*) tissue adjacent to an oil yellow sector, the necrotic response appeared to be propagated poorly, if at all, through *Rp1*-lacking cells.

In an attempt to isolate insertion mutations at *Rp1* with the *Mutator* transposable element system, we found that *Rp1* inactivation was common both in lines with *Mutator* activity and in controls lacking any known active transposable element system. Some alleles of *Rp1* are "endogenously" inactivated as frequently as one in five-hundred, while other alleles are over twenty fold more stable. In a standard background, no mutations of the $Rp1^F$ allele were detected in 7339 progeny screened. In a *Mutator* background, 27 $Rp1^F$ inactivations were isolated in 35,356 seedlings tested. Although many $Rp1^F$ mutant seedlings have lost resistance to most or all of the *P. sorghi* isolates tested, several retain resistance to a subset of rust biotypes. Some of the resistance specificity profiles obtained in these mutants are unlike any previously identified *Rp1* alleles. These data indicate that the *Rp1* locus is composed of multiple resistance factors with variable intrinsic stabilities.

Y 035 PLANT DEFENSE GENES INDUCED BY VIRUS INFECTION, John F. Bol, Jan A.L. van Kan, Huub J.M. Linthorst, Douwe Zuidema, Ralph L.J. Meuwissen and Ben J.C. Cornelissen*, Dept. of Biochemistry, Leiden University, and * MOGEN International, Leiden, The Netherlands.

The hypersensitive response of plants to infection with viruses, fungi or bacteria induces a resistance of the plants to further infection by these pathogens and results in the extracellular accumulation of a number of pathogenesis-related (PR) proteins (Bol, 1988). The 10 acidic PRs induced in Samsun NN tobacco by tobacco mosaic virus (TMV) infection fall into 5 different groups; basic proteins with a 65% homology to 3 of these groups have been identified (see Table). The PRs with β-1,3-glucanase and chitinase activity (see Kauffmann et al., 1987) are probably involved in resistance to fungi and bacteria. Protein S is homologous to the maize inhibitor of insect α-amylase and bovine trypsine and may be involved in a defense against insects (Richardson et al., 1987). The function of other PRs is not known.

Extra cellular proteins induced in Samsun NN tobacco by TMV infection

Acidic PR proteins			Basic homologues		
name	MW x 10^{-3}	estim.no of genes	MW x 10^{-3}	estim.no. of genes	function
1a,1b,1c	15	8	19	8	unknown
2, N, O	40	?	33	?	β-1,3-glucanases
P, Q	28/30	4	32/34	4	chitinases
R	13/15	?	-	-	unknown
S	23	4	-	-	α-amylase inhibitor

We have isolated cDNA clones and genomic clones corresponding to the proteins listed in the Table, except proteins 2, N, O and R. In addition, clones were obtained corresponding to a protein called "C" that is strongly induced by TMV and salicylic acid (SA) but does not correspond to known PR's. As SA inhibits virus multiplication by up to 99%, the SA inducible proteins PR-1 and/or C may be involved in the antiviral response. Three PR-1 genes and two C genes have been sequenced and other PR genes are being characterized. Plants transformed with the putative promoter regions, fused to a reporter gene, are being used to analyze the regulatory elements involved in the induction of PR genes by pathogens or SA.

Bol, J.F.: Plant Gene Research Vol. V, D.P.S. Verma and R. Goldberg, Eds., Springer Verlag, (1988) in press; Kauffmann, S., Legrand, M., Geoffroy, P., Fritig, B.: EMBO Journal (1987) in press; Richardson, M., Valdes-Rodriguez, S., Blanci-Labra, A.: Nature 327, 432-434 (1987).

Y 036 GENETICS AND TAGGING OF RUST RESISTANCE (*Rp1*) IN MAIZE, A. Pryor, Division of Plant Industry, CSIRO, Canberra, Australia, 2601.

The distal four-five map units of the short arm of chromosome 10 of maize contain at least four genetically separate loci conferring resistance against rust fungi. The best studied locus *Rp1*, which specifies resistance against *Puccinia sorghi*, has 14 known alleles (*Rp1a,....Rp1m*) (1). Substantial recombination occurs between some of these apparent alleles further indicating the complex nature of this chromosomal region.

Of 8 *Rp1* alleles tested all were inherently unstable 'mutating' from resistance to full susceptibility at allele specific frequencies ranging from 0.008 to 0.00015 (2). This high frequency of instability confounds the isolation of insertional mutants of rust resistance tagged by the insertion of known maize controlling elements. From a population of about 2 x 10^5 F1 seedlings involving the most stable *Rp1d* allele, 32 susceptible plants were recovered several of which appear to be themselves unstable due to the presence of known controlling elements. These mutants and the genetic basis of the inherent *Rp1* instability are being characterized.

(1) Saxena K.M.S. and Hooker A.L. 1968 Proc. Nat. Acad. Sci, USA 61, 1300-1305.
(2) Pryor A. 1987 Maize Genet. Newsl. 61, 37-38.

Molecular Genetics of Bacterial Pathogens

Y 100 PLASMID INVOLVEMENT IN THE PRODUCTION OF CORONATINE BY PSEUDOMONAS SYRINGAE PV. TOMATO. Carol L. Bender, Dean K. Malvick, and Robin E. Mitchell*, Oklahoma State University, Stillwater, OK 74078-0285 and the *Department of Scientific and Industrial Research, Auckland, New Zealand.

Pseudomonas syringae pv. tomato PT23.2 produces the chlorosis-inducing phytotoxin, coronatine. Thirty-eight chlorosis-defective mutants of PT23.2 were generated using the transposon Tn5. Five mutants contained Tn5 insertions in the indigenous plasmid pPT23A; the remaining 33 mutants were either missing pPT23A (29 mutants) or contained putative deletion derivatives of this plasmid (4 mutants). These results suggested that pPT23A was involved in coronatine production in strain PT23.2. This plasmid was introduced into P. syringae pv. syringae PS61, which does not produce the phytotoxin coronatine. A bioassay for coronatine suggested that PS61 transconjugants containing pPT23A were now able to make the phytotoxin. In a more critical analysis, organic acids were isolated from PT23.2, PS61 and the transconjugant PS61 (pPT23A); these were derivatized to their methyl esters and analyzed by gas chromatography. The organic acids extracted from PT23.2 and PS61 (pPT23A) contained a distinct coronatine peak; coronatine production was absent in the wild type PS61. Therefore, the acquisition of pPT23A by PS61 resulted in coronatine biosynthesis, thus proving that pPT23A is involved in coronatine production in P. syringae pv. tomato.

Y 101 STRUCTURE AND PROTECTIVE ROLE OF THE CAROTENOID SYNTHESIS GENE(S) OF *ERWINIA STEWARTII*, Jeffrey L. Bennetzen,[1] Phillip J. San Miguel,[1] Richard A. Larson,[2] and R. W. Tuveson,[3] Department of Biological Sciences,[1] Purdue University, West Lafayette, IN 47907 and Institute for Environmental Studies[2] and Department of Microbiology,[3] University of Illinois, Urbana, IL 61801.

Total DNA from strain DC7R of *Erwinia stewartii*, the causal organism in Stewart's wilt of maize, was cloned as a *Sau*3A partial digest into the cosmid vector pLAFR3. Of the 2033 independent colonies generated by transformation of this recombinant DNA into *E. coli*, six were yellow, as are DC7R and most other *E. stewartii* isolates. Restriction enzyme analysis of the cosmid DNA from three of these yellow colonies indicated partially overlapping maps which shared a common 15.3 kb *Eco*RI/*Kpn*I fragment. This fragment was subcloned into pUC19 to construct the plasmid pESC43Y. Accumulation of yellow pigments in *E. coli* cells containing pESC43Y occurs primarily in late log growth phase and in stationary cells and is inhibited by glucose. Transposon mutagenesis has defined an approximately 6 kb region of pESC43Y involved in carotenoid synthesis.

Chemical analysis of *E. coli* cells containing the yellow pigment gene(s) of *E. stewartii* indicated the presence of carotenoids. Many bacterial species synthesize carotenoids, but a role for these pigments in bacteria has not been unambiguously demonstrated. We have found that *Erwinia* carotenoids grant slightly increased near UV resistance to *E. coli*. We have also observed that the plant phototoxins alpha terthienyl and harmine (which generate singlet oxygen and superoxides) are much more toxic to standard *E. coli* than they are to *E. coli* that contain *E. stewartii* carotenoids.

Y 102 PATHOGENICITY AND BIOLOGICAL CONTROL OF ERWINIA CHRYSANTHEMI

M. Boccara, A. Kotoujansky, E. Schoonejans and C. Neema.

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Erwinia chrysanthemi produces soft rot disease on a wide range of plants in tropical and subtropical countries. Unlike other soft rotting erwinia, E.chrysanthemi shows a certain host specificity and gives rise to a systemic type disease. Secretion of pectinolytic enzymes is esssential for induction of maceration symptoms but several outer membranes components are of importance for the virulence of E.chrysanthemi.

To control soft rot disease elicited by E.chrysanthemi two possible strategies have been developed in the laboratory and will be presented:

1) competition: Bacterial mutants unable to secrete or produce the pectinases can be used to limit wild type infection.

2) cross-protection: Outer membrane mutants which are avirulent are able to confere systemic resistance to wild type infection.

Y 103 STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE AVIRULENCE GENE avrBs3 FROM XANTHOMONAS CAMPESTRIS PV. VESICATORIA. Ulla Bonas and Brian Staskawicz*, Institut fur Genbiologische Forschung Berlin, Berlin, FRG and *Dept. of Plant Pathology, University of California, Berkeley, USA.

Bacterial spot on pepper and tomato is caused by *Xanthomonas campestris* pv. *vesicatoria* (Xcv). Race 1 isolates of Xcv induce a hypersensitive response (HR) in the pepper cultivar ECW-30R containing a dominant resistance gene, *Bs*3. We could show genetically that in the bacteria the avirulence gene *avrBs3* is responsible for the race specificity of this interaction. *avrBs3*, located on a self-transmissable plasmid in Xcv race 1, is 4.1 kb in size. The structural analysis reveals a remarkable region of 1.7 kb in the middle of a long ORF consisting of direct repeats. Data on the structural and functional analysis of *avrBs3* will be presented.

Y 104 CHROMOSOMAL ATTACHMENT GENES OF *AGROBACTERIUM TUMEFACIENS* CODE FOR β-1,2-GLUCAN SYNTHESIS AND EXPORT. Gerard A. Cangelosi, Rodolfo A. Ugalde*, and Eugene W. Nester. Dept. of Microbiology SC-42, University of Washington, Seattle, WA 98195; and *Instituto de Investigaciones Bioquimicas "Fundacion Campomar", Antonio Machado 151, 1405 Buenos Aires, Argentina.

We have identified three chromosomal loci of *Agrobacterium tumefaciens* which are required for attachment of the bacteria to plant cells, and for crown gall tumor formation. All three are involved in synthesis of a cyclic β-1,2-glucan. In cultures of wild type bacteria, the glucan is found in the culture supernatent (extracellular glucan), and in the cell pellet (cellular glucan, which could include cytoplasmic, membrane bound, and periplasmic glucan). Strains with mutations in the *chvA* locus synthesize cellular but not extracellular glucan. Strains with mutations in the *chvB* or *exoC* loci synthesize neither cellular nor extracellular glucan. *chvB* codes for a membrane protein of 230-250 kD which binds radiolabled UDP-glucose, and is required for in vitro β-1,2-glucan synthesis. Transposon insertions in the 3' one third of the gene for this protein result in truncated termination products with apparent molecular weights ranging from 150 kD to 230 kD. These truncated proteins are competent for β-1,2-glucan formation, attachment, and virulence. Transposon insertions farther upstream inactivate all three functions. These data suggest that the role of this membrane protein in attachment is directly related to its role in β-1,2-glucan formation. The protein is synthesized by *chvA* and *exoC* mutants. *chvA* mutants synthesize β-1,2-glucan in vitro, whereas *exoC* mutants do not.

Y 105 EXPRESSION AND FUNCTION OF THE ntrC GENE IN Bradyrhizobium japonicum, Greg Martin, Kenneth Chapman and Barry Chelm, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

In enteric bacteria the ntrC gene product can act as either a positive or negative regulator of several genes associated with nitrogen fixation and assimilation. In B. japonicum the ntrC gene product has a molecular weight of 59 kilodaltons, and is expressed under nitrogen limiting conditions and during symbiosis. The B. japonicum ntrC gene was cloned by using the ntrC gene from B. parasponia as a heterologous probe. The ntrBC operon from B. japonicum is structurally similar to the ntrBC operons of other Rhizobiaciae. The ntrC gene product is required for growth on nitrate but is not required for the formation of fix^+ root nodules. An insertion mutation in the 3' end of the gene causes the production of a truncated protein which is expressed on nitrogen rich media.

Y 106 MOLECULAR BASIS OF PATHOGENICITY IN ERWINIA HERBICOLA PV. GYPSOPHILAE, Ellen Clark, Yakir Ophir and Yedidya Gafni, Inst. of Field and Garden Crops, Volcani Center, Bet Dagan 50250, Israel.

Erwinia herbicola pv. gypsophilae is a bacterial phytopathogen which generates galls on its host, and has had a serious impact on the propagation of the flowering ornamental Gypsophila paniculata in Israel. We are examining the role of bacterial production of indoleacetic acid (IAA) in generating disease symptoms. Pathogenic strains of E. herbicola pv. gypsophilae produce IAA when cultured in minimal media supplemented with 0.2g/l L-tryptophan, an IAA precursor; the nonpathogenic E. herbicola strain used as a control in these experiments did not produce IAA under the same conditions. In DNA hybridization experiments performed under low stringency conditions, a cloned DNA fragment from Pseudomonas savastanoi containing coding sequences for the two enzymes in the IAA biosynthetic pathway cross-hybridized to plasmid DNA of E. herbicola pv. gypsophilae. A DNA fragment containing the cross-hybridizing sequence was isolated from a λ library of E. herbicola pv. gypsophilae plasmid DNA; further characterization of this cloned fragment will indicate whether it harbors genes involved in IAA biosynthesis in this pathovar.

Y 107 IDENTIFICATION AND CHARACTERIZATION OF THE PROMOTER REGION OF THE IAA OPERON OF PSEUDOMONAS SYRINGAE SUBSP. SAVASTANOI. Oswaldo da Costa e Silva, Thomas Gaffney and Tsune Kosuge. Dept. of Plant Pathology, University of California, Davis, CA, 95616.

The plant pathogenic bacterium Pseudomonas syringae subsp. savastanoi causes tumors on olive and oleander. The symptoms are dependent on the production of indole acetic acid and cytokinins by the bacterium in the host tissue. Two loci organized in an operon, iaaM and iaaH, are required for the biosynthesis of IAA. The DNA sequence of a 133 bp upstream fragment, which had promoter activity in transcriptional fusions, revealed a region that shared homology with E. coli promoters. The transcription start site of the iaa operon message was determined by S1 nuclease protection experiments, the promoter of the iaa operon has been identified and bares homology to E. coli promoters.

The mapped transcription start site is located 400 bp upstream from the begining of the first structural gene of the operon. From sequence analysis a 77 aminoacid ORF encoding a 8,000 Da protein product is present in this intervening region. The function of this protein, if it is indeed made in vivo, is still unknown at present.

Y 108 ROLE OF VIRE2 GENE PRODUCT IN AGROBACTERIUM-MEDIATED PLANT CELL TRANSFORMATION, Anath Das, Department of Biochemistry, University of Minnesota, St. Paul, MN 55108.

The virE operon of Agrobacterium tumefaciens Ti-plasmid, pTiA6, encodes two polypeptide of 7,500, and 60,500 daltons. The larger polypeptide, virE2, was cloned downstream from an E.coli tryptophan (trp) operon promoter.Under induction conditions cells containing the trpE-virE fusion plasmid over-produced the virE2 protein. The polypeptide partitioned into the insoluble fraction upon cell lysis. VirE2 protein was solubilized by urea treatment of the cell pellet . In gel retardation studies protein preparation containing the virE2 polypeptide strongly bound to single stranded DNA. Binding to DNA was not sequence specific. No affinity either for double-stranded DNA or single stranded RNA was observed. In protein blotting experiments, where proteins were first separated on denaturing polyacrylamide gels, then transferred to nitrocellulose filters and probed with radiolabelled DNA, the virE2 polypeptide showed DNA binding activity. In similar experiment, Agrobacterium induced with plant cells was found to contain a single-stranded DNA binding protein that, in polyacrylamide gels, comigrated with the virE2 polypeptide overproduced in E. coli and showed immunological reactivity with antibody raised against virE2 produced in E. coli . (Supported by NIH)

Y 109 AGROBACTERIUM TUMEFACIENS SURFACE LECTINS, Francis DELMOTTE, Chritiane DEPIERREUX,*Marie-France MICHEL and Michel MONSIGNY, Centre de Biophysique Moléculaire, C.N.R.S. and Université d' Orléans, 1 rue Haute, F-45071 - Orléans cedex 2; *Station d' Amélioration des Arbres Forestiers, I.N.R.A. d' Orléans-Ardon , FRANCE.

An early step in the tumor formation induced by Agrobacterium tumefaciens in wounded plants, is the attachment of the bacteria to plant host cells. To test the hypothesis that a sugar-protein interaction could be involved in the A. tumefaciens adherence to plant cells, we were interested to look at the presence of bacterial lectin. The A. tumefaciens surface lectin(s) was evidenced by spectrofluorimetry using fluoreceinylated neoglycoproteins, by flow cytofluorimetry using two fluorescent markers excited at 488 nm: a kanamycin derivative emetting at 590 nm and allowing to detect single bacteria, and the fluoresceinylated neoglycoproteins emetting at 520 nm. By flow cytofluorimetry at pH 7.0, the outer membrane associated lectin(s) of A. tumefaciens strain 2516 bound preferentially neoglycoproteins containing di-N-acetyl chitobiosyl or α-L-rhamnosyl moities. At pH 5.0, we determined by spectrofluorimetry that the same strain bound α-L-fucosyl moities and to a less degree neoglycoproteins containing α-D-mannopyranosyl or β-D-glucopyranosyl moities; this binding is saturable and pH dependent.

Y 110 REGULATION OF PHENYLPROPANOID PATHWAY GENE TRANSCRIPTS BY TRANS-CINNAMIC ACID, David J. Millar, Mehrdad Mavandad and Richard A. Dixon, Dept. of Biochemistry, Royal Holloway & Bedford New College, Univ. of London, Egham Hill, Egham, Surrey, TW20 0EX, U.K.

L-Phenylalanine ammonia-lyase (PAL) catalyses the first reaction in the biosynthesis of a wide variety of plant phenylpropanoid compounds from L-phenylalanine. The enzyme is encoded by a small multigene family in Phaseolus vulgaris, and treatment of cultured bean cells (c.v Canadian Wonder) with elicitor released from the cell walls of the phytopathogenic fungus Colletotrichum lindemuthianum results in a rapid increase in the levels of mRNA encoding one of the three PAL genes (gPAL 1), with subsequent increase in extractable PAL activity preceeding accumulation of phenylpropanoid defense compounds. This response is prevented by trans-cinnamic acid, the product of the PAL reaction.

S1 nuclease protection and RNA blot hybridisation analyses suggest that cinnamic acid acts to prevent transcription of PAL, and genes encoding other phenylpropanoid pathway enzymes, rather than by affecting the post-transcriptional stability of these mRNAs. Cinnamic acid does not appear to exert blanket inhibitory effects on transcription, as evidenced by 2-D IEF:SDS PAGE analysis of mRNA in vitro translation products from cells treated with cinnamate alone, or elicitor plus cinnamate. Among cinnamic-acid-induced polypeptides are likely to be found a proteinaceous inactivator(s) of PAL, whose appearance in cinnamate-treated cells is prevented by RNA synthesis inhibitors. The operation of a regulatory loop controlling flux through the phenylpropanoid pathway with cinnamic acid as a 'sensor' is discussed in relation to the timing and co-ordination of defense response gene activation and gene-product turnover.

Y 111 THE ABILITY OF THE NOD D GENE PRODUCT TO INTERACT WITH PLANT-SECRETED FLAVONOIDS IS INFLUENCED BY THE AMINO ACID SEQUENCE IN SEVERAL KEY REGIONS. M.A. Djordjevic, J.J. Weinman, J. McIver and B.G. Rolfe. Plant Molecular Biology, Research School of Biological Sciences, Australian National University, Canberra, Australia, 2601.

The positive activation of the expression of nodulation (nod) genes in several rhizobia is controlled by the product of the nodD gene as well as the availability of plant-secreted flavonoids (phenolic compounds of low mol. wt). In at least two Rhizobium strains the nodD product is also able to autoregulate its own synthesis. In R. leguminosarum biovars trifolii and viciae and in R. meliloti, certain intermediates of the phenylpropanoid pathways (flavonoids), stimulate the expression of the nod genes while other intermediates (isoflavonoids and coumarins) antagonise nod gene induction by stimulators. We have mutagenised the nodD gene of Rhizobium strain ANU843 with nitrosoguanidine to investigate whether the altered nodD products are able (1), to remain interactive with plant signal compounds (2), to induce nod gene expression (3), to autoregulate nodD expression (4), to complement nodD mutations in various Rhizobium strains and (5), to extend the host range of R. leguminosarum biovar trifolii to plants not normally nodulated by this strain. DNA sequence analysis has demonstrated that, in most nodD mutants, a single base change has occurred. Several classes of nodD mutants were isolated and characterised and the results obtained will be presented.

Y 112 CHARACTERIZATION OF GENES INVOLVED IN PATHOGENICITY IN PSEUDOMONAS SYRINGAE PV. TABACI, Karen Engst and Paul D. Shaw. University of Illinois, Urbana, IL 61801. Pseudomonas syringae pv. tabaci strain BR2 causes wild fire disease on tobacco and green beans. Eight mutants nonpathogenic on tobacco had been generated by using Tn5 mutagenesis. The EcoRI fragments from seven of the mutants were cloned into cosmid vector pLAFR3 by selecting for kanamycin resistance. Two clones have been isolated from a genomic library of the parental strain. One (22kb) was obtained by using a probe from the purified 28 kb EcoRI fragment containing Tn5 from mutant PTBR7.000. The Tn5-containing fragments from three other mutants have been subcloned into pTZ18R. One of these from mutant PTBR6.000 has been used as a probe to select a fragment of about 12 kb from the genomic library. These cloned fragments will be tested for their ability to restore pathogenicity to the mutants.

Y 113 INDIGENOUS PLASMIDS IN PSEUDOMONAS SOLANACEARUM ---- ARE THE PLASMIDS RELATED TO PATHOGENICITY OF THE PATHOGEN?, Fan Yun Liu and Xie Daoxin, Lab of Molecular Biology, Biotechnology Center, Chinese Academy of Agricultural Sciences, Beijing, He Liyuan, Plant Protection Institute, Chinese Academy of Agricultural Sciences, Beijing.

Fourteen of fifty-one wild strains isolated from fourteen host plant (potato,tomato,peanut, tobacco,sweetpotato,mulberry,ginger,pepper,olive,eggplant,remie,sesame,horsetail beefwood, black nightshade) in different area of China and fourteen of twenty-four "mutant" strains of Pseudomonas solanacearum carred one or two plasmids with molecular weight from less than five to one hundred and twenty megadaltons.

Some avirulent strains of Pseudomonas solanacearum were different from their original virulent strains by appearing of large plasmid with molecular weight from forty to sixty megadaltons.

It had been suggested that in these strains loss of pathogenicity might be consequence of a chromosomal fragment,from which a new plasmid is formed.

Y 114 CLONING AND CHARACTERIZATION OF THE MANNITYL OPINE CATABOLIC REGION OF AN OCTOPINE-TYPE TI PLASMID. S.K. Farrand, J. Tempé and Y. Dessaux, University of Illinois, Urbana, IL 61801 and University of Paris-Sud, 91405 Orsay, France.
Production of opines by plant tumors induced by members of the genus *Agrobacterium* is thought to promote colonization of these neoplasias by the inciting, opine-catabolizing bacteria. At present, there are eight or nine known opine families, with a given *Agrobacterium* strain usually able to catabolize only two or three. A number of Ti and Ri plasmids encode the catabolism of the four members of the mannityl opine family, mannopinic acid (MOA), agropinic acid (AGA), mannopine (MOP) and agropine (AGR). Analysis of overlapping cosmid clones has allowed us to map the region of the Ti plasmid encoding these functions to a 40 kb segment lying between occ and ori/inc. The locus for MOA catabolism has been located by subcloning to an 18 kb region divisible into two trans-acting segments. The repressor for MOA catabolism has been mapped by natural IS insertions to a 1 kb region within the MOA locus. Physiological and genetic experiments suggest that there are two pathways for MOP catabolism. One pathway appears to be associated with AGR catabolism while the other may be independent of this route. One of the MOP pathways also appears able to direct catabolism of MOA. The regions of the Ti plasmid encoding these two pathways show substantial overlap. Interestingly, the gene encoding MOP cyclase, an enzymatic function involved in catabolism of MOP and AGR, shows nucleic acid homology with the region of T_R of pTi15955 responsible for the conversion of MOP to AGR by transformed plant tissues. This suggests that a gene duplication is responsible for functions involved in mannityl opine biosynthesis in the plant and catabolism in the bacterium.

Y 115 TRANSCRIPTIONAL AND TRANSLATIONAL FUSION ANALYSIS OF THE PSEUDOMONAS SAVASTANOI IAA OPERON. Thomas D. Gaffney, Oswaldo da Costa e Silva and Tsune Kosuge. University of California, Davis, CA 95616.

The secretion of indoleacetic acid and cytokinins by the plant pathogen Pseudomonas syringae subspecies savastanoi induces tumors on olive and oleander. The two genes whose products are required for the conversion of tryptophan to indoleacetic acid, iaaM and iaaH, are adjacent and cotranscribed. The use of broad host range vectors which allow either transcriptional or translational fusion of iaaM to lacZ identified the IAA operon promoter region. One 133 base pair subclone derived from this region retained full promoter activity. Comparisons of identical constructs in Pseudomonas savastanoi and Escherichia coli indicated that lacZ expression directed from the IAA operon promoter was greater than twenty-fold higher in Pseudomonas. The IAA operon was expressed constitutively under a range of environmental conditions.

Y 116 EARLY EVENTS IN THE EXCISION OF THE T-DNA FROM THE AGROBACTERIUM TI-PLASMID, Stanton B. Gelvin, K. Veluthambi, R. K. Jayaswal, Department of Biology, Purdue University, West Lafayette, IN 47907.

We have investigated the early molecular events involving the excision of the T-DNA from the Ti-plasmid in Agrobacterium tumefaciens. Following induction of the vir genes of the Ti-plasmid by cocultivating the bacteria with tobacco protoplasts, new restriction endonuclease fragments of the T-DNA are generated, consistent with the double-stranded cleavage of the T-DNA at the four border sequences. Non-denatured blotting and S1 nuclease analyses also reveal the presence of single-stranded T-DNA molecules (T-strands) generated using all possible combinations of the four T-DNA borders. The generation of both the double-stranded cuts and the T-strands is dependent upon the vir genes A, G, and D. Most of these T-strands are derived from the botton T-DNA strand. However, T-strands can be derived from the top T-DNA strand. Deletion or inversion of the right T-DNA border abolishes the generation of discrete-sized T-strands. In both Agrobacterium and in an E. coli model system, the relative amounts of double-stranded border cutting and the generation of T-strands is approximately equivalent. Following cocultivation of A. tumefaciens cells, the entire Ti-plasmid overreplicates relative to the Agrobacterium chromosomal DNA. DNA sequence analysis and deletion mutagenesis experiments of the virD locus in E. coli indicate that both of the first two open reading frames of the virD locus are necessary for T-DNA border cleavage and the generation of T-strands.

Y 117 THE ENIGMATIC RELATIONSHIP BETWEEN Agrobacterium AND MAIZE, Nigel Grimsley[1], Peter Rogowsky[2], Clarence Kado[2], and Barbara Hohn[1], [1]Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basle, Switzerland, [2]College of Agricultural and Environmental Sciences, Department of Plant Pathology, California 95616.

Agroinfection, Agrobacterium-mediated delivery of viral or viroidal sequences to plants, provides a sensitive assay for T-DNA transfer. Strains of Agrobacterium carrying tandemly arranged genomes of maize streak virus (MSV) in their T-DNAs are known to agroinfect maize, and inoculations of maize seedlings at different positions demonstrated that meristematic tissues are the most susceptible to agroinfection. The ability of mutant strains of Agrobacterium to transfer DNA to maize was tested, and the results suggest that transfer occurs by a mechanism similar to that found in dicotyledonous plants. This observation questions the definition of a host-parasite interaction.

Y 118 AGROBACTERIUM TUMEFACIENS MUTANTS DEFICIENT IN CHEMOTAXIS TO ROOT EXUDATES, Martha C. Hawes, Laura Y. Smith, Alan J. Howarth, University of Arizona, Tucson, AZ 85721

A. tumefaciens is chemotactically attracted to many simple compounds that attract E. coli, but not to Luria broth, which is highly attractive to E. coli. A. tumefaciens strains B6, 15955, A348, ACH5, and A. rhizogenes R1000, but not E. coli, exhibited chemotaxis to excised root tips and to isolated root cap cells of Pisum sativum. In contrast, A. tumefaciens is attracted to excised root tips but not to isolated root cap cells of cotton and bean. The absence of the Ti plasmid had no discernable effect on chemotaxis. Mass movement of bacteria to pea root cap cells placed at the edge of semisoft water agar required at least 300 plant cells (ca. 10% of the number that slough from a single root daily); relative movement increased with increasing numbers of root cap cells. Several Tn5 mutants that are motile and capable of chemotaxis to several purified chemicals but not to pea root cap cells and/or excised root tips have been selected. All but one of the mutants caused tumors on pea, when applied at high inoculum concentrations.

Y 119 GENETIC ORGANIZATION AND REGULATION OF THE acc LOCUS ON THE *AGROBACTERIUM TUMEFACIENS* PLASMID pTiC58, G.T. Hayman and S.K. Farrand, University of Illinois, Urbana, IL 61801.

The agrocinopine A-agrocin 84 (acc) locus on pTiC58, the Ti plasmid of nopaline/agrocinopine A *Agrobacterium tumefaciens* strain C58 has been cloned. Thought to be coregulated with Ti plasmid conjugal transfer, the locus encodes sensitivity to agrocin 84, an antibiotic produced by *A. radiobacter* strain K84, and catabolism of the agrocinopine opines. The cloned region confers agrocin 84 sensitivity and transport of agrocin 84 and agrocinopine A. The locus was mapped by mutagenesis with the lac fusion transposon, Tn3-HoHo1. Insertions lying within a 4.4 kb DNA segment abolish agrocin 84 sensitivity and also block uptake of agrocin 84 and agrocinopines A and B. Analysis of selected Tn3-HoHo1 lac fusions indicates that the locus is transcribed as a single unit in a clockwise fashion, and is transcriptionally activated by agrocinopines A and B. A 2.4 kb DNA fragment mapping to the left of the region, when present in trans to the acc locus, represses agrocin 84 sensitivity and transport, transcription of the region and conjugal transfer of the Ti plasmid. This fragment probably encodes the repressor thought to regulate both opine catabolism and Ti plasmid conjugal transfer. Hybridization studies with Ti or Ri plasmids from agrocin-sensitive agrobacteria show close relatedness between the acc loci present on agrocinopine A-type Ti plasmids but essentially no homology with agrocinopine C-type Ti or Ri plasmids. The nopaline catabolic plasmid of strain K84, which also encodes agrocinopine A utilization, shows no homology to acc.

Y 120 GENERATION OF NONPATHOGENIC MUTANTS AND COMPLEMENTATION IN XANTHOMONAS CAMPESTRIS PV. GLYCINES 8ra, Ingyu Hwang, S. M. Lim, and P. D. Shaw, University of Illinois, Urbana-Champaign, IL 61801

Xanthomonas campestris pv. glycines strain 8ra causes bacterial pustule disease on soybeans. No plasmid has been detected in strain 8ra, and it is resistant to 50 µg/ml of rifampicin. N-methyl-N-nitro-N'-nitroso-guanidine was used to generate mutants, and fifteen nonpathogenic and five reduced pathogenicity mutants were isolated from two thousand colonies examined. Auxotrophic and color mutants were also obtained. A soybean cotyledon bioassay was developed to screen the colonies for pathogenicity. A cosmid gene library of chromosomal DNA from strain 8ra was constructed in E. coli HB101 by using a broad host range cosmid pLAFR3. Chromosomal DNA was partially digested with Sau3AI, and the fragments were fractionated on sodium chloride gradients. Fragments in the 20-30 kb size range were ligated into the BamHI site of pLAFR3, and the recombinants were packaged in vitro into E. coli HB101. Ten recombinant clones were chosen at random, and recombinant plasmids were isolated and digested with BamHI. The ten recombinant plasmids had different insertions with 3 to 6 BamHI restriction sites. In order to determine the completeness of the library, attempts were made to complement an auxotrophic E. coli HB101 mutant(leu^-, pro^-). The leu^- phenotype was complemented at a frequency of $5x10^{-5}$, but no pro^+ prototrophs were obtained. Complementation of nonpathogenic mutants is in process, and pathogenicity genes will be cloned and characterized.

Y 121 STUDIES ON THE MECHANISM, AND IDENTIFICATION OF REGIONS OF pSa REQUIRED FOR SUPPRESSION OF A. TUMEFACIENS ONCOGENICITY, Sheila M. Johnson and C. I. Kado. Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.

pSa is a 39 kb self-transmissable R-factor of the incompatibility group W. Originally isolated from *Shigella*, it has not been found to occur naturally in *Agrobacterium*. When introduced into *A. tumefaciens*, pSa completely suppresses the ability of the bacterium to cause crown gall tumors on plants. The suppression does not affect the physical properties of the Ti plasmid. Tn5 insertions and exonuclease deletions into a previously identified 7.6 kb fragment of pSa indicate that two separate loci within this fragment are required for oncogenicity suppression (OS). The mechanism of suppression is unknown. However, we found that the *vir*C promoter is still induced by acetosyringone in the presence of pSa. This suggests that pSa does not block the function of the *vir*A or *vir*G gene products, which are required for *vir*C gene induction by acetosyringone. It has been suggested that pSa suppresses oncogenicity by blocking bacterial production of indole-3-acetic acid (IAA). We have found, however, that *A. tumefaciens* harboring pTiC58, pSa, and pTET40, which carries genes constitutive for IAA production and produces five to ten times more IAA when grown in rich or minimal media than strains not carrying pTET40, still fails to incite tumors on *Kalanchoe* plants. This indicates that OS activity is not due to suppression of bacterial production of IAA.

Y 122 MOLECULAR ANALYSIS OF SPONTANEOUS RACE CHANGE IN BACTERIAL SPOT DISEASE, Brian Kearney and Brian Staskawicz, University of California, Berkeley, CA 94720

Xanthomonas campestris pv. vesicatoria (Xcv) causes bacterial spot disease on tomato and pepper. Resistance to the disease has been observed in a number of plant introduction lines of pepper and three resistance genes have been crossed into commercial cultivars of Capsicum annuum. The resistance gene Bs_1 provides hypersensitive resistance against Xcv Race 2 isolates carrying $avrBs_1$. We have studied changes in Xcv Race 2 isolates as they mutate to overcome resistance encoded by Bs_1. Thirteen independent mutants were isolated using the plant itself as selection. In all thirteen, mutation to overcome Bs_1 resistance was accompanied by a genomic alteration at $avrBs_1$. Four mutants were chosen for in depth study. In all four spontaneous mutation was accompanied by an insertion of 1.3 kb into either the coding region or presumptive regulatory regions of $avrBs_1$. Sequencing of the insertion established that mutation was caused by an endogenous transposable element which has been named IS476.

Y 123 COMPLEMENTATION FOR PATHOGENICITY IN A SPONTANEOUS NONPATHOGENIC MUTANT OF Xanthomonas campestris pv. oryzae. Segenet Kelemu and Jan E. Leach, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A genomic library of a pathogenic Xanthomonas campestris pv. oryzae race 1 isolate (PXO61) was constructed in the cosmid pLAFR5. Two cosmid clones were identified which restored pathogenicity to a spontaneous nonpathogenic mutant of a race 5 X. c. pv. oryzae (PXO112) on rice cultivar IR8. Restriction enzyme analysis of cosmids extracted from the complemented race 5 mutant revealed that the cloned DNA was rearranged. Cosmid clones extracted from the complemented mutant were used to transform Escherichia coli HB101 and then mobilized to the nonpathogenic mutant. Pathogenicity of the mutants containing the rearranged clone was restored on IR8. The complementing DNA is being subcloned and the gene(s) responsible for restoring pathogenicity are being characterized.

Y 124 CONTROL OF CYTOKININ PRODUCTION IN PSEUDOMONAS SAVASTANOI, Jeffrey L. Kelly, Francisco Roberto, and Tsune Kosuge, Department of Plant Pathology, University of California, Davis, CA 95616.

Production of both indoleacetic acid (IAA) and cytokinins by Pseudomonas syringae subsp. savastanoi (P. savastanoi) is implicated in tumor formation induced by this pathogen on olive and oleander plants. Oleander isolates of P. savastanoi secrete the cytokinins zeatin, zeatin riboside, isopentenyladenosine, and isopentenyladenine, producing up to 10 mg/L total when grown in minimal media. Cytokinins are secreted in stationary phase, in contrast to IAA secretion that appears early in a time course. Also, mutant strains producing no, or low levels, of IAA, secrete lowered levels of cytokinins. We are investigating this effect of IAA, and determining the control points of cytokinin production.

The gene from P. savastanoi encoding isopentenyltransferase (ipt), the first enzyme of cytokinin biosynthesis from 5'-AMP, was cloned and sequenced by R. Morris' group. Purified P. savastanoi RNA polymerase generates 2 transcripts in vitro from this gene, apparently using sequences resembling E. coli promoters. We are analyzing the ipt transcripts made in vitro and in vivo, and mapping their 5' ends. To further study ipt gene expression, gene fusions of the ipt promoter region with lacZ are being constructed. These will also allow monitoring the effect of IAA pathway metabolites on ipt expression.

Y 125 EVIDENCE FOR THE PRESENCE OF A CALCIUM REGULATED PROTEIN KINASE IN THE BEAN RUST FUNGUS, Lucille B. Laccetti[1], R.C. Staples[1] and H.C. Hoch[2], [1]Boyce Thompson Institute, Ithaca, NY 14853 and [2]Geneva Experimental Station, Cornell University, Geneva, NY 14456.

Germlings of the bean rust fungus, Uromyces appendiculatus, are capable of sensing the topographical surface features of the leaves of the bean plant, Phaseolus vulgaris. Specifically, germlings recognize the lips of stomatal guard cells and also synthetic surfaces with ridges 0.5 µm high that effectively mimic the guard cell lips. The recognition of this signal induces the germling to differentiate, a process involving the formation of specialized infection structures and multiple rounds of mitosis. The initial response of the germling to the signal occurs rapidly (within minutes), and it is possible that transduction of the signal involves the activation of calcium regulated protein kinase(s). Here we present evidence that such a calcium regulated kinase exists in the bean rust germling.

Y 126 A REPEATED DNA SEQUENCE FROM THE Xanthomonas campestris pv. oryzae CHROMOSOME DIFFERENTIATES Xanthomonas campestris PATHOVARS, Jan E. Leach, Marsha L. Rhoads, and Frank F. White, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

An EcoRI-HindIII DNA restriction fragment (2.5 kb) contained a major repeated sequence from Xanthomonas campestris pv. oryzae (Xco). The cloned repeated sequence (pJEL101) hybridized with no fewer than 14 bands in Southern blots of EcoRI restricted, total genomic DNA from 26 Xco isolates representing the Philippines, India, Japan, and Columbia. Out of 10 randomly selected clones from a pSa747 Xco genomic library (insert size = 30-40 kb), nine clones contained fragments which hybridized with pJEL101. pJEL101 hybridized to total DNA from three of 15 X. campestris pathovars tested. Of these three, only X. c. pv. oryzicola DNA hybridized extensively with pJEL101 and the pattern was clearly distinguishable from Xco. Thus, pJEL101 contains sequences which distinguish Xco from other pathovars of X. campestris. Preliminary data indicates the pJEL101 probe also differentiates Xco races.

Y 127 PLASMID INTEGRATION AND EXCISION IN PSEUDOMONAS SYRINGAE, Dallice Mills, Marilyn Ehrenshaft, Janet Williams, and Pradip Mukhopadhyay, Oregon State University, Corvallis, OR 97331.
Pseudomonas syringae pv phaseolicola causes halo blight disease in common bean, Phaseolus vulgaris. The chromosome and plasmid sites where the indigenous plasmid, pMMC7105 (150 Kb), integrated into the chromosome of strain LR716, as well as sites for excision of derivative plasmids, pMMC7115 (87Kb) and pEXC8080 (35Kb), were cloned and characterized. Both integration of pMMC7105 and excision of pMMC7115 appear to occur by simple homologous recombination at different copies of an insertion sequence-like (IS) element. The element was determined to be 1180 base pairs (bp) in length with 18 bp terminal inverted repeats. It has an open reading frame (ORF) on one DNA strand with four putative ATG translation initiation codons in phase with a unique UGA chain termination codon. These ORFs could encode polypeptides of 367, 312, 225 and 198 amino acids. The sites where recombination occurred to produce pEXC8080 have also been cloned and are being sequenced. These sites have homology with a repeated sequence that has been cloned from pMMC7105 and sequenced. This repeated sequence does not resemble known IS elements. Plasmid pUCD800 (Gay et al, J. Bact. 164:918, 1985) is being successfully used to capture transposing IS elements in this bean pathogen, to study their role in plasmid evolution and in host-pathogen interactions.

Y 128 MOLECULAR ANALYSIS OF A VIRULENCE REGION IN PSEUDOMONAS SYRINGAE, Pradip Mukhopadhyay, Janet Williams and Dallice Mills, Oregon State University, Corvallis, OR 97331.
One of the chromosomal regions encoding virulence genes of Pseudomonas syringae pv. syringae strain PS9020 has been cloned from a genomic library by complementing a Tn5-induced mutant, PS9021, unable to cause brown spot disease in bean. Marker-exchange mutagenesis indicates that a 3.9 kb HindIII fragment of the clone encodes the virulence factor(s). DNA sequencing of this fragment revealed the existence of two open reading frames (ORFs) on one strand, coding for putative polypeptides of sizes 40 kd and 83 kd, while another ORF coding for a 81 kd polypeptide was found on the other strand. Polypeptides of these respective sizes had been expressed in E. coli maxi-cells in earlier studies. Computer analysis of hydropathy profiles and homology of these ORFs to known genes suggest that the 40 kd protein has the characteristics of DNA-binding proteins and the 83 kd protein has properties similar to those of membrane proteins. By considering the phenotypes resulting from the Tn5 insertions within the ORFs, the 83 kd protein and possibly the 40 kd protein appear to be involved in the virulence of strain PS9020. Fusion of the lacZ gene of E. coli to a part of the ORF encoding the 83kd protein resulted in a hybrid protein inducible in E. coli. This hybrid protein is currently being used for preparation of antibodies towards the 83 kd polypeptide.

Y 129 RESPONSE OF PARSLEY CELLS AND PROTOPLASTS TO FUNGAL ELICITOR
Jane E. Parker, Klaus Hahlbrock and Dierk Scheel, Max-Planck Institut für Züchtungsforschung, 5000 Köln 30, F.R.G.

A crude hyphal wall preparation of Phytophthora megasperma f.sp. glycinea (PMG) contains molecules which elicit phytoalexin synthesis in its host plant soybean (Glycine max) and in the non-host plant parsley (Petroselinum crispum). A prerequisite for induction is the perception of appropriate signals by the plant cell. We are using parsley cell cultures and protoplasts to study the nature of elicitor molecules and the mechanism of signal recognition and transduction. Freshly prepared parsley protoplasts are responsive, in the absence of detectable cell wall, to PMG elicitor (J. Dangl et al. (1987) EMBO J., 6, 2551 - 2556). The response is comparable to cultured cells. The results indicate that inductive events are independent of cell wall factors and strongly suggest an interaction site within the plasma membrane. The response is readily visualised under UV light due to excretion of the auto-fluorescing coumarin derivatives, and represents an excellent functional assay system for active elicitor molecules and potential binding sites. Active components of the PMG elicitor in soybean are β-1,3-glucans. We present evidence that structurally different molecules of the same elicitor preparation stimulate phytoalexin accumulation in parsley. Proteins play an essential role in elicitation in this system and we are attempting to purify and characterise them further.

Y 130 CHARACTERIZATION OF A PHASEOLOTOXIN ENCODING GENE FROM PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA. David E. Clements, Charles Romeo, Morton Mandel and Tom Humphreys, Department of Biochemistry and Suresh S. Patil, Department of Plant Pathology and the Biotechnology Program, University of Hawaii, Honolulu, Hawaii 96822.

A cosmid clone was isolated from a genomic bank of Pseudomonas syringae pv. phaseolicola strain 4612 which complements a UV induced toxin minus (Tox^-) mutant of strain G50 (Clements et al. 1985, Phytopathol. 75:1355). A subclone containing a 2.8 kb fragment restored toxin production to the same Tox^- mutant. Using Tn3 HOHO1 mutagenesis of the 2.8 kb fragment, we have isolated four independent insertion mutations which fail to complement the Tox^- mutant. These insertions have been mapped to within 100 bp of each other. The analysis of insertion data leads us to believe that the gene involved in complementation of the Tox^- mutant may be no larger than 400 bp. This fragment is currently being sequenced.

Y 131 NUCLEOTIDE SEQUENCE OF THE GENE ENCODING IAA-LYSINE SYNTHETASE FROM PSEUDOMONAS SAVASTANOI. Francisco F. Roberto, Russell Nordeen and Tsune Kosuge, University of California, Davis, CA 95616.

Production and secretion of large amounts of the plant growth regulatory substances indole-3-acetic acid (IAA) and zeatin are necessary for the elicitation of the disease response on the plant hosts of the bacterium, Pseudomonas syringae subsp. savastanoi (P. savastanoi). Indole-3-acetic acid is further metabolized by the bacterium to an amino acid conjugate, IAA-ε-L-lysine through the action of IAA-lysine synthetase. The gene encoding this activity, iaaL, was recently cloned in E. coli. We report here the determination of the nucleotide sequence of iaaL and the deduced amino acid sequence of IAA-lysine synthetase. Also presented here is corroboration of the protein size as estimated from SDS-polyacrylamide gel electrophoresis of the iaaL gene product expressed in an in vitro transcription/translation system.

Y 132 STRUCTURE AND EXPRESSION OF THE AVRBS1 LOCUS OF XANTHOMONAS CAMPESTRIS PV. VESICATORIA. Pamela Ronald and Brian J. Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA 94720

Xanthomonas campestris pv. *vesicatoria* (Xcv) causes bacterial spot disease on susceptible cultivars of pepper producing water soaked lesions. Inoculation of Xcv containing the avirulence locus *avrBs1* induces a hypersensitive response (HR) on plants containing the resistance gene *Bs1*. In an effort to understand the biochemical basis of this incompatible interaction, we have cloned and characterized the *avrBs1* locus. A 2.3 kb subclone is sufficient for full avirulence activity on resistant pepper cultivars. Sequence analysis reveals 2 ORFs of 11kD and 50 kD. Deletion into the first ORF alters the HR inducing activity producing an intermediate phenotype. Any deletion into the second ORF completely destroys avirulence activity. If a deletion in the first ORF is driven by an exogenous promoter, full avirulence activity is restored indicating that the second ORF alone can induce the HR. An antibody to the 50 kD protein was made that reacts specifically with a 50 kD protein in extracts of E. coli and Xcv containing *avrBs1* fused to a highly expressed promoter.

Y 133 GENETIC ANALYSIS OF AGROBACTERIUM RHIZOGENES TL-DNA GENES INVOLVED IN ROOT INDUCTION. Farida Shaheen and Frank F. White, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Agrobacterium rhizogenes induces root proliferation on a wide variety of dicotyledonous plants at the wound site. The TL and TR-DNA of pRiA4 contain separate sets of genes. Each set of genes independently are capable of inducing roots on tobacco and tomato. On kalanchoe leaves TL and TR-DNA are both required to produce roots. These observations suggest the existence of multiple factors that promote root induction. The TR-DNA contains genes for auxin biosynthesis. An increase in auxin synthesis by plant tissue as mediated by TR-DNA alone is sufficient for root induction in certain tissues (e.g. tobacco stems). However the functions of the TL-DNA are less clear. To understand the role of TL-DNA genes on rooty phenotype, we have used two approaches: (1) strains containing double and triple mutations in the T-DNA of pRiA4 were constructed and characterized for rooty phenotype on kalanchoe leaves; and (2) segments of TL-DNA containing rolA, rolB and rolC genes were cloned individually or in combination in a binary vector. These recombinant clones were introduced into various Agrobacterium mutants and bioassayed on kalanchoe leaves and tobacco. Our result suggests that on kalanchoe leaves in addition to TR-DNA, rolB gene alone is sufficient to cause roots but fewer roots are formed compared to rolB & C genes together.

Y 134 AGROBACTERIUM-MEDIATED GENE TRANSFER TO PLANTS

Robert B. Simpson Barbara Bailey and Gabriella Meeker
The Plant Cell Research Institute, Inc. 6560 Trinity Court Dublin, CA 94568.

Using molecular and genetic analysis of transformed plants we have been studying the gene-transfer mechanism by which *Agrobacterium tumefaciens* transforms plant cells. The DNA (T-DNA) transferred and stably integrated in the plant chromosome was determined to be present in one to six copies. Approximately 30% of the copies were incomplete or rearranged indicating that the DNA transfer and integration mechanism are not perfect as is the case with transposable elements. In some cases the multiple copies were joined as direct or inverted repeat structures. In other cases multiple copies were found at unlinked locations in the plant chromosome. Also we have shown that two bacteria can frequently transform a single plant cell. In our plants we observed the T-DNAs from at least four different bacteria. Thus, multiple bacteria are the likely source of many of the multiple T-DNA copies in a plant cell. We will discuss possibilities of plant gene isolation based on insertional inactivation by the T-DNA or based on complementation of function by a genomic library introduced by *Agrobacterium*.

Simpson, RB, A Spielmann, L Margossian, & TD McKnight (1986) Plant Mol Bio 6, 403.
Spielmann, A & RB Simpson (1986) Mol Gen Genet 205 34
McKnight, TD, M Lillis & RB Simpson (1987) Plant Mol Bio 8, 439.
Prosen, D & Simpson (1987) Bio/Technology 5, 966

Y 135 INDUCTION OF PATHOGENICITY IN A FLUORESCENT PSEUDOMONAD. Francisca Van Outryve[b], Bernice Slutsky[a], Henk Joos[b], Jean Swings[b], and Marc Van Montagu[a;b]. Rijksuniversiteit Gent[a] and Plant Genetics Systems[b], Gent, Belgium.

The fluorescent pseudomonad P. marginalis produces a red rot on the heads of witloof chicory. Isolates of P. marginalis were collected from healthy and diseased plants. Using SDS PAGE, these isolates were grouped according to their protein fingerprint types. The pathogenicity of the isolates from healthy plants was tested and latent pathogenic isolates were found. One fingerprint type could thus contain nonpathogenic, pathogenic, and latent pathogenic isolates. A further comparison was made using an ED50 value determined by the number of cells that produced red rot in 50% of the tested leaves. The ED50 of the latent pathogen was consistently higher than that of the pathogen. However, when the latent pathogen was incubated with the extract of wounded plant tissue, its ED50 was significantly lowered. This induction process was specific to the latent pathogen and was maximized when bacteria were included during the production of the plant extract. The molecular nature of this induction event is currently being investigated. The pathogen may be constitutive with respect to regulatory controls governing virulence levels whereas the latent pathogen may be subject to these controls. Alternatively, the virulence levels of the two phenotypes may be determined via different mechanisms. Transposon mutagenesis and complementation experiments are being used to identify those genetic elements necessary for induction and pathogenicity in P. marginalis.

Y 136 **Identification of a protein covalently bound to T-strands in *Agrobacterium tumefaciens*.** Eric R. Ward and Wayne M. Barnes, Department of Biological Chemistry, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110.

When induced with the plant phenolic acetosyringone (AS), *A. tumefaciens* produces single-stranded DNA molecules corresponding to the T-region of the Ti plasmid. These so-called T-strands are putative plant transformation intermediates. We have determined that the T-strands are covalently bound to protein. Our evidence is: 1) like adenovirus DNA, T-strands are soluble in phenol unless first treated with a proteolytic enzyme, and 2) native T-strands are detectable in an assay we have developed which is specific for covalent protein-DNA complexes. This assay involves solution hybridization of a partially purified T-strand fraction to a radiolabelled oligonucleotide, electrophoresis of the labelled T-strand-protein complex through SDS-agarose in high ionic strength buffer, and electrophoretic transfer of the gel to nitrocellulose in low ionic strength buffer. Since protein, but not naked single- and double-stranded DNAs, bind to nitrocellulose under the conditions employed, any DNA signal bound to the filter must be tightly associated with protein.

We have constructed *A. tumefaciens* strains which, by virtue of tandem arrays of many T-DNA border sequences on their Ti plasmids, produce high levels of single and double-stranded AS-induced DNAs. Applying our covalent complex-specific assay to the double-stranded AS-induced DNAs has allowed us to determine that a protein moiety no bigger than 65 kilodaltons is bound to the 5' ends of previously determined lower strand nicks in the T-DNA border sequences. A likely candidate for the covalently attached protein is the border-specific endonuclease, known to be encoded by ORF 2 of the *virD* locus. We have therefore constructed a fusion of *virD* to *lacZ*. *E. coli* cells expressing this fusion generate increased levels of the nicked form of plasmids containing a substrate (T-DNA border) sequence. These relaxed circles are immunoprecipitable with anti-β-galactosidase antibody, while borderless control plasmids do not react. We shall next express the fusion gene in *A. tumefaciens*, where we expect that the T-strands, as well as the double-stranded AS-induced structures, will bind to anti-β-galactosidase antibody in covalent complex-specific nitrocellulose transfer experiments.

Y 137 MOLECULAR DETERMINANTS OF HOST RANGE IN XANTHOMONAS CAMPESTRIS PATHOVAR VESICATORIA, Maureen C. Whalen and Brian J. Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA 94720

The bacterial pathogenic species, *Xanthomonas campestris*, causes disease on a wide range of host plants. Each *X. campestris* pathovar has a highly specific relationship with a particular host. We are studying the molecular basis of host range specificity in *X. c.* pathovar *vesicatoria* tomato race 1 (XcvT). The inability of XcvT to cause disease on plant species other than tomatoes can be controlled by single genes. We have isolated a clone, XV9019, from XcvT that upon mobilization into the bean pathogen, *X. c.* pathovar *phaseoli*, confers avirulence activity associated with the donor strain. A mutangenized copy, XV9019::Ω, was exchanged back into the genome of XcvT and its effect on bacterial growth in bean leaves was monitored. XV9019 also confers avirulence activity in several other *X. campestris* pathovars: *glycines* on soybean, *vignicola* on cowpea, *alfalfae* on alfalfa, *malvacearum* on cotton, and *hocicola* on corn.

Y 138 A ZINC INDUCIBLE ESTERASE SECRETED BY PATHOGENIC STREPTOMYCES SCABIES, Jan M.A. Willard and Janet L. Schottel, Univ. of Minn., Dept. of Biochem., St. Paul, MN 55108. Streptomyces scabies, a gram positive filamentous bacterium, is the causative organism for scab disease on several types of underground vegetables, including potato. We are interested in enzymes secreted by the pathogen which may be involved in breaking down suberin, a waxy polyester compound that covers the underground parts of plants. Such enzymes may be required for pathogenicity. A secreted esterase has been detected in the culture filtrates of some pathogenic strains. This enzyme has been purified and partially characterized. We are interested in determining the mechanism of secretion, the regulation of enzyme production, and the role of this enzyme in pathogenicity. Antibodies to this protein have been used to immunoprecipitate an intracellular protein 20 kilodaltons larger than the secreted form of the esterase, but which does not have esterase activity. To determine whether this larger intracellular protein is an inactive precursor form of the esterase, which is processed upon secretion, the esterase gene has been cloned and is being sequenced. The DNA sequence and predicted amino acid sequence of the esterase gene are being analyzed and compared to the known amino terminal amino acid sequence of the secreted esterase. Production of the extracellular esterase is dependent on the presence of zinc in the medium. Zinc does not appear to be required for catalytic activity of the enzyme. Zinc may be involved in processing and secretion of the esterase, or zinc may be required for transcription or translation of the esterase gene. Total RNA isolated from uninduced cells and from zinc induced cells is being probed with the cloned esterase gene in order to measure the relative amounts of esterase mRNA.

Y 139 MOLECULAR GENETICS OF TABTOXININE-ß-LACTAM PRODUCTION AND RESISTANCE BY *Pseudomonas syringae* pv.*tabaci*, D. Kyle Willis[1,2], Thomas G. Kinscherf[2], Terese M. Barta[2], and Rebecca H. Coleman[1,2], [1]USDA/ARS and [2]University of Wisconsin, Madison, WI 53706

Pseudomonas syringae strain BR2R, the causative agent of wildfire blight of bean (*Phaseolus vulgaris*), produces a chlorosis-inducing phytotoxin designated as taboxinine-ß-lactam (T-ß-L). We have isolated ten Tn*5* induced mutants of this isolate that are altered in T-ß-L production and/or resistance. These mutants fall into three general categories as determined by an *E. coli* overlay assay for production and mutant overlay of BR2R for resistance: 1) intermediate for toxin production and toxin resistant (two mutants); 2) no detectable T-ß-L production and toxin resistant (one mutant); and 3) no toxin produced and T-ß-L sensitive (seven mutants). Southern blot analysis of the ten mutants with several restriction endonucleases has not provided evidence for linkage between any of the Tn*5* insertions. *In planta* assays showed that the mutants producing intermediate levels of T-ß-L were not reduced in virulence when compared to BR2R. In contrast, all of the Tox$^-$ mutants showed no pathogenic response in the leaves of bean. Subcloning and further molecular analysis of selected Tn*5* mutants is in progress. In addition to the Tn*5* generated mutants above, we have detected phenotypic variants of BR2R (designated as Type II) that are greatly reduced in T-ß-L production and *in planta* virulence. The relationship of these Type II derivatives to *P.s.tabaci*(*angulata*) and spontaneous Tox$^-$ *P.s.tabaci* derivatives will be discussed.

Y 140 THE MOLECULAR BASIS FOR CYTOPLASMIC AMINO ACID ACCUMULATION IN RESPONSE TO HYPEROSMOTIC STRESS IN BACTERIA, Janet M. Wood and Jocelyn L. Milner, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Proline and glycine betaine accumulate cytoplasmically to protect a variety of Gram negative bacteria against the deleterious effects of hyperosmotic stress. A common molecular basis for that compatible solute accumulation is emerging for a variety of organisms including Escherichia coli, Salmonella typhimurium, and certain Rhizobia and Klebsiellae. In Escherichia coli, neither synthesis nor catabolism of proline is modulated by osmotic stress. Proline porters II and III, which mediate the intracellular accumulation of exogenously provided proline, are activated and/or induced in response to decreased cellular turgor pressure, however. Choline uptake and the synthesis of glycine betaine from choline are induced by osmotic stress. Accumulation of exogenously provided glycine betaine can also be mediated by the osmoresponsive proline porters. Proline porter II activity observed in cytoplasmic membrane vesicles prepared from Escherichia coli K-12 is dependent on both the provision of a respiratory energy supply and the imposition of hyperosmotic stress. That system will permit us to further analyze the molecular basis for salinity tolerance. We are also interested in understanding the influence of salinity tolerance mechanisms on the outcome of plant-microbial interactions.

Y 141 PURIFICATION AND SOME PROPERTIES OF BACTERIOCIN PRODUCED BY STRAIN M2 OF PSEUDOMONAS SOLANACEARUM, Xie Daoxin and Fan Yunliu, Lab of Molecular Biology, Biotechnology Center, Chinese Academy of Agricultural Sciences, Beijing. He Liyuan, Plant Protection Institute, Chinese Academy of Agricultural Sciences, Beijing.

The bacteriocin from M2 of Pseudomonas solanacearum was purified by ammonium sulphate precipitation and anion exchange chromatography. This bacteriocin was designated as solanacearicin M2, which appeared as one band on PAGE. The bacteriocin showed antagonistic activity when it was recovered from the band through electrophoresis. Solanacearicin M2 consisted of two subunits with molecular weight of about 68,000 and 70,000.

There were no relationship between the bacteriocin production and pathogenicity of Pseudomonas solanacearum.

Fifty-nine of seventy-three strains of Pseudomonas solanacearum were bacteriocinogenic for indicator strain Po3 (a virulent strain isolated from potato). It had been confirmed that the bacteriocins were not encoded by plasmids in thirty-seven of the fifty-nine strains.

Y 142 CHARACTERIZATION OF THE DIFFERENTIATION-SPECIFIC GENES EXPRESSED DURING INFECTION STRUCTURE DEVELOPMENT OF THE BEAN RUST FUNGUS. Xiaoling Xuei[1], Srirama Bhairi[2], Richard C. Staples[2], and Olen C. Yoder[1]. [1]Department of Plant Pathology, Cornell University, and [2]Boyce Thompson Institute, Tower Road, Ithaca, NY. 14853.
The bean rust fungus, Uromyces appendiculatus, differentiates to form a series of infection structures at the tip of the germ tube in response to the lip of the stomatal guard cell as well as to a 0.5 um ridge on a plastic surface. Twenty differentiation-specific clones were isolated by cascade-hybridization. These were divided into six classes based on levels of cross hybridization among the clones. Analysis of transcripts revealed that expression of some of the clones was highly specific for the differentiated state whereas expression of others was upshifted during differentiation. One of the clones was 13kb in size and encoded several transcripts, including a promenant one of 1.0kb. The gene specifying the 1.0kb transcript was subcloned on a 2.1kb fragment, sequenced and the direction of transcription was determined.

Y 143 MOLECULAR CHANGES IN PLANT CELLS INITIATED BY ELICITOR TREATMENTS, Anne J. Anderson, Craig S. Tepper and Kim R. Rogers, Utah State University, Logan, UT 84322-4500.
Necrosis in suspension cultured bean cells was initiated about 6 hours after treatment with purified and crude elicitors from the α race of Colletotrichum lindemuthianum, a bean pathogen. The onset of necrosis was accompanied by accumulation of phenolics and phytoalexins in the cells and in the cell medium. Free radicals, detected by their EPR signal, and a product of lipid peroxidation, lipofuscin, also began to accumulate at this time. These data suggest that a complex set of responses including radical formation are involved in elicitor induced plant cell necrosis.

The mRNAs specifying enzymes concerned with phytoalexin synthesis, phenylalanine ammonia-lyase and chalcone synthesis accumulated earlier in the elicitor treatments. Two peaks of synthesis of these mRNAs were observed at 3 and 6-7 hours in elicitor treated cotyledon tissues. The relative magnitude of the two peaks was dependent on the elicitor used.

Signals and Specific Interactions

Y 200 ELICITOR-INDUCED pH CHANGES IN CULTURED SOYBEAN CELLS, Izydor Apostol, Philip S. Low, Peter Heinstein, Department of Chemistry and Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907

We have presented evidence previously that addition of an elicitor isolated from Verticillium dahliae stimulates fluorescence changes in several membrane-bound and intracellular fluorescent probes when added to cotton or soybean cells. Based on these data, we proposed that one of the initial events associated with elicitation was the movement of protons across membrane boundaries in the cell. In order to test this hypothesis, we have conducted more detailed ^{31}P NMR and fluorescent probe experiments, designed to monitor the pH within the various cell compartments during elicitation. The ^{31}P NMR demonstrates that the vacuole and cytoplasm indeed become more acidic immediately following stimulation with the elicitor. These pH transitions are further confirmed by monitoring the subcellular locations of the fluorescence transitions of the intracellular pH-sensitive dye, carboxyfluorescein, using fluorescence microscopy. Since inhibitors which block these pH changes associated with elicitation also inhibit elicitation, we suggest the rapid pH transitions are important in the stimulation of the defense response in plant cells.

Y 201 INHIBITION OF THE HR AND K+/H+ EXCHANGE IN TOBACCO BY PECTIC CELL WALL FRAGMENTS. C. Jacyn Baker, Merelee Atkinson, and Dale Keppler, USDA, ARS, Microbiology and Plant Pathology Lab. Beltsville, MD 20705. Recently we established a close association between induction of the hypersensitive response (HR) in tobacco to bacteria and stimulation of a net proton uptake/K+ efflux. Low levels of pectate lyase will prevent the HR in tobacco plants and stimulate a transient H+ uptake/K+ efflux in tobacco suspension cultured cells. Further studies have demonstrated that the enzyme releases a pectic fragment from the cell wall which can cause these responses. The fragment is heat stable and can be inactivated by further treatment with pectate lyase. An active pectic oligosaccharide can also be generated from sodium polypectate by the same enzyme. Less than 1 ug/ml (dry wt.) will stimulate H+ uptake in suspension cultures and less than 50 ug/ml will inhibit the HR in plants. The magnitude of the transient pH response is directly proportional to the pectic oligosaccharide concentration, however the length of the transient period seems to be independent. Once tobacco suspension-cultured cells recover from a transient H+ uptake response the cells do not respond well to further additions of the oligosaccharide for at least 5 to 6 hr. The possible relevance of this response to the inhibition of the HR is being studied.

Y 202 ISOLATION AND CHARACTERIZATION OF TWO NEW MUTANTS BLOCKED IN T-2 TOXIN BIOSYNTHESIS, Marian N. Beremand, Patricia J. Black and Ronald D. Plattner, USDA/ARS, Northern Regional Research Center, Peoria, IL 61604.
Trichothecenes are a class of chemically related toxic secondary metabolites produced by various species of fungi. T-2 toxin is the major trichothecene synthesized by Fusarium sporotrichioides NRRL 3299. To facilitate the study of the biosynthesis, genetics, molecular biology and bioactivity of the trichothecenes, mutants blocked or altered in the production of T-2 have been produced by UV mutagenesis of this strain. Two new mutants, MB2972 and MB5493, have been isolated that produce little or no T-2. In turn, they accumulate high levels of sesquiterpenes that have fewer oxygenations than T-2; these accumulated compounds are not detected in cultures of NRRL 3299. Both are highly stable mutants and neither one displays any other discernible morphological or biochemical alterations as compared to the wildtype parent. These two mutants are the result of different genetic lesions. MB2972 accumulates two calonectrin analogues (3,15-di-ol,12,13 epoxy-trichothec-9ene and 3,15-ol,12,13 epoxy,3-acetate-trichothec-9ene). MB5493 accumulates trichodiene, the unoxygenated parent hydrocarbon for the trichothecenes. MB2972 and MB5493 also complement each other; when grown together in liquid shake culture they produce T-2.

Y 203 GENE EXPRESSION IN ELICITOR-TREATED BEAN CELLS, Joanna Ellis, Richard Blyden, Allen Jennings and Richard Dixon, Dept. of Biochemistry, Royal Holloway & Bedford New College, Univ. of London, Egham Hill, Egham, Surrey, TW20 0EX, U.K.
Treatment of cell suspension cultures of French Bean (Phaseolus vulgaris c.v. Immuna) with elicitor macromolecules from cell walls of the phytopathogenic fungus Colletotrichum lindemuthianum results in the de novo synthesis and accumulation of antifungal isoflavonoid phytoalexins and hydroxyproline-rich glycoproteins (HRGPs). We have studied the differential expression of transcripts encoding three members of the chalcone synthase (CHS) multigene family [1] in relation to the timing of accumulation of the 5-hydroxy and 5-deoxy classes of isoflavonoid phytoalexin (typified by kievitone and phaseollin respectively).

At early times post elicitation, when kievitone but not phaseollin accumulates, CHS gene 4 transcripts predominate, whereas gene 1 transcripts are the predominant type at later times when phaseollin is being synthesised. These data, and intact CHS enzyme polymorphisms revealed by chromatofocussing, are discussed in relation to the catalytically distinct "deoxy CHS" activity involved in the biosynthesis of phaseollin [2].

We are also studying the elicitor-inducible enzymes which post-translationally modify the HRGPs [3]. We discuss strategies for the cloning of such enzymes, including the use of artificial polyproline - IgG congugates and affinity purification of mRNAs.
[1] Ryder, T.B., Hedrick, S.A., Bell, J.N., Liang, X., Clouse, S.D. and Lamb, C.J. (1987). Mol. Gen. Genet., in press. [2] Dewick, P.M., Steele, M.J., Dixon, R.A. and Whitehead, I.M. (1982). Z. Naturforsch. 37c, 363. [3] Bolwell, G.P., Robbins, M.P. and Dixon, R.A. (1985). Eur. J. Biochem. 148, 571.

Y 204 INHIBITION OF FUNGAL GROWTH BY THE CHITINASES AND β-1,3-GLUCANASES INDUCED BY PATHOGENS IN PEA TISSUE,

Thomas Boller, Felix C. Mauch, Brigitte Mauch-Mani and Alexander Ludwig, Abteilung Pflanzenphysiologie, Botanisches Institut der Universität Basel, CH-4056 Basel, Switzerland.

Chitinase and β-1,3-glucanase were strongly induced in pea pods in response to infection. Crude protein preparations from infected pea pods, which contained high activities of chitinase and β-1,3-glucanase, inhibited growth of 15 out of 18 fungal species tested. Crude protein preparations from uninfected pea pods, with very low activities of chitinase and β-1,3-glucanase, did not inhibit fungal growth. Two isoenzymes of chitinase and two isoenzymes of β-1,3-glucanase were purified from pea pod tissue. Purified chitinase and β-1,3-glucanase, tested individually, did not inhibit growth of most of the test fungi. However, combinations of purified chitinase and β-1,3-glucanase inhibited growth of all fungi tested as effectively as crude protein extracts containing the same enzyme activities. Inhibition of fungal growth was caused by the lysis of hyphal tips.

Supported by the Swiss National Science Foundation, Grant 3.345-0.86.

Y 205 ELICITATION OF LIGNIN BIOSYNTHESIS AND ISOPEROXIDASE ACTIVITY BY PECTIC FRAGMENTS IN SUSPENSION CULTURES OF CASTOR BEAN

Robert J. Bruce and Charles A. West, UCLA, Los Angeles, CA 90024

Suspension cultures of castor bean (Ricinus communis L.) which have been treated with pectic fragment elicitor (PFE) rapidly accumulate lignin as a mechanism of disease resistance. In 6 d (maximally responsive, late log-phase) cultures, increases in lignin are first evident 3 h after addition of PFE with maximal rates of lignin synthesis between 4 and 10 h. The abundance of lignin in cultures after 12 h of elicitor treatment is 10-20 fold higher than in untreated control cultures, using an elicitor preparation with an average degree of polymerization of 7 (optimal). Plant peroxidases catalyze the polymerization of lignin as well as the synthesis of hydrogen peroxide. Treatment of cultures with elicitor causes coordinate changes in the activity of four of the six readily detectable extracellular isoenzymes of peroxidase present in healthy suspension cultures and also results in the appearance of three new cationic peroxidase isoenzymes. The differential expression of peroxidase isoenzymes during elicitation suggests that individual isoenzymes of peroxidase may have distinct functional roles during disease resistance. Work is currently in progress to determine the functions of the individual isoenzymes and the mechanisms of regulation of their expression. Supported by USDA 86 CRCR 12145.

Y 206 ROLE OF PECTIC ENZYMES IN PEA-FUSARIUM INTERACTIONS. Robin Buell and Lee A. Hadwiger, Washington State University, Pullman, WA 99164-6430

The role of pectic enzymes in Fusarium-pea interactions was examined by measuring the activity of pectolytic enzymes produced by Fusarium solani f. sp. pisi and f. sp. phaseoli both in culture and in planta. Fusarium solani f. sp. pisi and f. sp. phaseoli can both produce pectic enzymes in minimal media containing pectin as the sole carbon source. Both F. solani f. sp. pisi and f. sp. phaseoli can produce polygalacturonase, pectinmethylgalacturonase, polygalacturonate trans-eliminase, and pectinmethyl trans-eliminase in culture although the production of these four pectic enzymes is induced faster in f. sp. pisi than in f. sp. phaseoli. When pea endocarp tissue is challenged with F. solani f. sp. phaseoli, an incompatible pathogen on pea, no significant levels of pectic enzymes are produced by 30 hours after fungal inoculation. When pea endocarp tissue is challenged with Fusarium solani f. sp. pisi, a compatible pathogen on pea, significant levels of pectinmethyl trans-eliminase and polygalacturonate trans-eliminase are produced by 20 hours and 30 hours, respectively, following fungal inoculation. The production of these two lytic enzymes by F. solani f. sp. pisi can be correlated with growth of the fungus, decrease in host cell viability, and host cell maceration. The production of the lytic enzymes can also be correlated with the suppression of disease resistance response gene (DRRG) mRNA levels. Pectolytic enzymes are a virulence factor in the f. sp. pisi-pea interaction. Their role as a determinant in DRRG suppression is currently being investigated.

Y 207 ROLE OF CYTOKININS IN PLANT TRANSFORMATION EFFICIENCY

Linda A. Castle and Roy O. Morris, Oregon State University, Corvallis, OR 97331

Agrobacterium tumefaciens strains differ in their transformation and crown gall producing capabilities. One strain, C58 has emerged as a superior gall producer with a wide host range. A screen of 28 *Brassica* and *Raphanus* species was carried out by inoculating young plants with the strains C58, Ach5, and C58(pTiBo542). It was clear that C58 produced galls more frequently and that they were larger. It is known that nopaline strains such as C58 carry a cytokinin biosynthetic gene ,*tzs*, adjacent to the *vir* region on the Ti plasmid. Activation of *tzs* results in zeatin secretion from cells when they are exposed to wounded plant extracts. We sought to determine whether the presence of cytokinins at the wound site affected transformation efficiency. A second replicon containing *tzs* was introduced into Ach5 and C58(pTiBo542). However, in the heterologous systems, zeatin production was much lower than in wild type C58. It was not clear that these altered strains were more efficient in gall production on *Brassica* species. Deletion of *tzs* from C58 allowed us to examine the role of this gene from the opposite perspective.

Gall production is a function of transformation and tissue proliferation. We focused our study to the role of cytokinins in transformation efficiency by using the GUS gene fusion system to look at single cell events. Transformation efficiency of the wild type, *tzs* addition, and *tzs* deletion strains was quantitatively compared by histochemical and fluorometric assays. We also examined the role of exogenously added plant hormones in the transformation process. (This work was supported by USDA grant #85-CRCR-1-1645.)

Y 208 A PLANT-DERIVED PROTEIN THAT CONVERTS FUNGAL ENDOPOLYGALACTURONASE INTO AN AVIRULENCE FACTOR MAY BE AN ARABINOGALACTAN PROTEIN, F. Cervone, M. G. Hahn, G. De Lorenzo, A. G. Darvill and P. Albersheim. Univ. of Georgia/U.S. Dept. of Energy Complex Carbohyd. Res. Cen., USDA Russell Center, P.O. Box 5677, Athens, GA 30613.

Fungal endo-polygalacturonases (endo-PGs) play an important role during the early stages of plant pathogenesis. These enzymes hydrolyze the pectic component of plant cell walls, assisting in the colonization of plant tissue by invading fungi. Endo-PGs release mono-, di- and trigalacturonides from plant cell walls, carbohydrates that have no ability to elicit phytoalexin accumulation. We have purified a polygalacturonase-inhibiting protein (PGIP) from the cell walls of plants that alters the activity of the fungal endo-PG such that the enzyme generates larger, elicitor-active oligogalacturonides from its substrate. In the presence of excess PGIP, the products of extensive treatment of polygalacturonic acid by *Aspergillus niger* endo-PG are oligogalacturonides with an approximate average degree of polymerization of 12, the size that possesses maximum phytoalexin elicitor activity. PGIP thereby appears to convert endo-PG from being a virulence factor into an avirulence factor. Murine monoclonal antibodies (McABs) were generated against the purified PGIP. Immunoblots of purified PGIP reacted positively with the anti-PGIP McABs and also with other McABs that are known to react with an arabinogalactan epitope. These and other data suggest that PGIP is an arabinogalactan protein.

(Supported by grant #DMB-8518488 from the National Science Foundation)

Y 209 INFECTION OF TRANSGENIC TOBACCO PLANT PROTOPLASTS EXPRESSING MUTANT TMV COAT PROTEINS: THE EFFECT ON VIRAL REPLICATION, W. Gregg Clark and Roger N. Beachy, Washington University, St. Louis, MO 63130.

To further elucidate the mechanism of cross protection observed in transgenic tobacco plants expressing the TMV coat protein (CP), point mutations in the CP gene were created that would hinder the protein's ability to assemble properly on the viral RNA. One possible mechanism for the protection observed could be the re-coating of incoming viral RNA. These mutant CPs were analyzed for their ability to assemble on viral RNA *in vitro* (to determine their phenotype). They then were introduced into tobacco tissue under transcriptional control of the CaMV 35S promoter. From the resulting transgenic plants, leaf protoplasts were isolated, they were infected with TMV and viral replication was monitored. The results of these experiments and their implications, with respect to the mechanism of cross protection, will be discussed. In addition, deletion mutants initiating from the COOH-terminus of the CP gene were constructed, the mutant CPs were tested for *in vitro* viral assembly and are being introduced into tobacco tissue under transcriptional control of the CaMV 35S promoter The stable deletion mutant lines will be used as a source of material for leaf protoplast-infection experiments. The results from these experiments should clarify the significance individual domains of the CP may play in cross protection.

Y 210 "**Pathogenesis-Related Proteins of *Nicotiana tabacum*:**" John Cutt[1], Dave Dixon[1], John Carr[1], Mark Harpster[2], Pamela Dunsmuir[2], Daniel Klessig[1] 1.Waksman Institute at Rutgers University, PO Box 759 Piscataway, New Jersey 08855 and 2.Advanced Genetic Sciences, 6701 San Pablo Ave. Oakland, California 94608

We are investigating the "pathogenesis-related" proteins of *Nicotiana tabacum var.Xanthi*. cDNA and genomic clones which correspond to these polypeptides have been isolated and characterized. Sequence analyses of these clones and genomic southern blots indicate that the PR1 proteins are encoded by a small gene family (and corroborates the results of other labs working with *N.t.var.Samsun*). Southern analyses also support the cytogenetic evidence on the origin of the *N.t.* species from an interspecies cross of *N.tomentosiformis* and *N.sylvestris*. As well as determining the structure of these genes we are investigating i) the regulation of their induction in plants infected with Tobacco Mosaic Virus, ii) their localization in infected plants, and iii) their possible role in disease resistance. We have demonstrated that induction of these genes precedes the formation of local lesions consistent with their possible involvement in the hypersensitive response. Transgenic plants have been constructed which contain one or more of the PR1 genes under control of the CaMV 35S promotor. The effect of the constitutive synthesis of these proteins on the physiology and resistance status of the transgenic plants is being investigated.

Y 211 MODIFICATION OF NUCLEIC ACIDS BY THE HOST-SELECTIVE TOXIN FROM COCHLIOBOLUS CARBONUM RACE 1, Stephen J. Danko and Herman W. Knoche, Department of Biochemistry, University of Nebraska, Lincoln, NE 68583-0718.

HC toxin, the host-selective toxin from Cochliobolus (=Helminthosporium) carbonum race 1 has been structurally characterized as cyclo (D-prolyl-L-alanyl-D-alanyl-L-2-amino-8-oxo-9,10-epoxydecanoyl). Both the 8-oxo and 9,10-epoxy functions are essential for toxicity. HC toxin is selectively toxic to maize with the hmhm genotype and exhibits an EC_{50} of 180-230 ng/ml. The toxin has no short-term physiological effects on sensitive maize, but stimulates ion leakage from tissues, inhibits chlorophyll accumulation in etiolated leaves, and inhibits root growth after 6-12 hr. Because of these delayed responses to toxin, and because of the essentiality of the 8-oxo and 9,10-epoxy functions, HC toxin may act by covalent modification of host nucleic acids. When HC toxin is incubated with maize DNA in vitro, fluorescent products form within a few hours at pH 7.5 and 30°C. When individual nucleosides are incubated with HC toxin, fluorescent products can be detected after only 30 min. Both purine nucleosides, adenosine and guanosine, are modified, but pyrimidines are not. Fluorescent guanosine-HC toxin adducts have been isolated by reverse-phase HPLC. When ^{14}C-guanosine is incubated with HC toxin, the radiolabel elutes with both unmodified guanosine and the fluorescent product. When small DNA fragments are incubated with HC toxin and then fractionated by gel electrophoresis, the DNA migrates more slowly than does unmodified DNA, indicative of a structural change. These results indicate that HC toxin covalently modifies nucleic acids in vitro, and suggest that the mode of action of HC toxin in vivo may involve covalent modification of nucleic acids.

Y 212 CHARACTERIZATION OF ELICITOR AND PATHOGEN INDUCED GENE EXPRESSION IN ARABIDOPSIS THALIANA, Keith R. Davis and Frederick M. Ausubel, Dept. Molecular Biology, Massachussets General Hospital, Boston, MA 02114.

Plants utilize an array of defense mechanisms to prevent microbial infection. These defense strategies include inducible responses such as phytoalexin accumulation. Significant progress has been made in identifying specific genes that are activated by pathogens and/or compounds derived from microorganisms or plant cell walls (elicitors), however, little is known about the mechanisms involved in the activation of these genes. We have initiated studies on the activation of defense-responses in Arabidopsis thaliana with the goal of applying molecular biological and genetic approaches to identify genes involved in the regulation of defense responses. Our initial studies have focused on the response of suspension-cultured cells to treatment with the microbial elictors, endopolygalacturoic acid lyase and fungal cell wall components. Cultured A. thaliana cells respond to these elicitors by exhibiting increased levels of phenylalanine ammonia-lyase (PAL) and 4-coumarate:CoA ligase (4CL). The increase in PAL activity is preceded by a rapid increase in the steady-state level of PAL mRNA. Other studies have demonstrated that increased levels of mRNAs homologous to tobacco β-1,3-glucanase and PR protein 1a are also induced in elicitor-treated cells. We are currently determining if other enzymes involved in phenylpropanoid biosynthesis are induced in elicitor-treated cultures and if these enzymes are also induced in seedlings infiltrated with elicitors or phytopathogenic bacteria. These studies and their application for genetic analyses of the regulation of defense genes will be discussed. This work is supported by Hoechst AG.

Y 213 DIFFERENTIAL TOXICITY OF PHYTOALEXIN ENANTIOMERS TO FUNGAL PLANT PATHOGENS, Leslie M. Delserone, David E. Matthews, and Hans D. VanEtten, Cornell University, Ithaca, NY 14853.

Research on the enzymology of phytoalexin synthesis in leguminous crops suggests that a plant species' biosynthetic pathway could be modified to produce the opposite optical isomer of the native phytoalexin, or to produce a phytoalexin structure not normally synthesized by that species. We have preliminary evidence that these modifications might improve the resistance of some plant species to fungal pathogens. In in vitro experiments, some species of pathogens of red clover and garden pea were inhibited differentially by the enantiomers of the native phytoalexin. In each case, the fungus was inhibited less by the isomer normally synthesized by its host. Fungi also were assayed for differential sensitivity to phytoalexins not normally synthesized by their hosts. The phytoalexins in these cases differed from the native forms in the substitution pattern on the central, four-ring structure shared by most phytoalexins of legumes. Some species of pathogens of alfalfa and red clover were inhibited by these non-native phytoalexins. Based on these preliminary results, the introduction of genes coding for enzymes involved in phytoalexin synthesis from one plant species into another, altering the species' phytoalexin arsenal, might be a feasible approach to engineering plants for improved disease resistance.

Y 214 ISOLATION AND EXPRESSION OF TOMATO GENES INDUCED DURING *PSEUDOMONAS SYRINGAE* PV. *TOMATO* INFECTION. Demmin, D. S., Holzer, F., and Walling, L. Department of Botany and Plant Sciences. University of California. Riverside, California 92521.

A total leaf gt10 cDNA library representing mRNAs expressed during an incompatible infection of *P. tomato* was constructed. The library was screened with a cascade hybridization enriched probe. Greater than 200 clones expressed during during incompatible infection have been isolated and purified. Temporal expression of these genes after infection and wounding is being characterized. We have determined that proteinase inhibitor genes are induced during bacterial pathogen infection of tomato. Using cDNA, pTi24 and pTi48, we have determined that proteinase inhibitor genes are induced to high levels 24 hours after infection during the incompatible infection and induced by 36 hours during a compatible infection. Temporal expression of the genes that code for phenylalanine ammonium lyase and pathogenesis related proteins are also being examined.

Y 215 ISOLATION OF A HYPERSENSITIVITY RELATED cDNA CLONE FROM *NICOTIANA TABACUM* VAR. XANTHI NN, Daniel B. Golemboski, David D. Dunigan and Milton Zaitlin, Cornell University, Ithaca, NY 14853.

The hypersensitive resistance response exhibited by some species of *Nicotiana* is controlled by the N gene. A procedure was used that allowed for the selective isolation of differential complementary DNA sequences that are present in the RNA population of *N. tabacum* var. Xanthi NN, which contains the N gene, and a variety of *N. tabacum* (i.e. Turkish Samsun nn) which does not respond hypersensitively to infection by tobacco mosaic virus. This method has resulted in the subsequent isolation of a single cDNA clone that hybridizes to genomic DNA from Xanthi NN but not from Turkish Samsun. Northern analysis reiterates these results. An F2 population derived from a cross between Xanthi NN and *N. tabacum* var. Petit Havana (nn) segregates at the expected 3:1 ratio of hypersensitive to non-hypersensitive. Southern analysis has shown that the Xanthi NN specific clone also segregates with the hypersensitive response.

Y 216 MODEL FOR REGULATION OF SYSTEMS IN THE RESISTANCE AND SUSCEPTIBLE INTERACTIONS BETWEEN PATHOGENS AND PEAS. Hadwiger, Lee A., Robin Buell, David Christian, Catherine Daniels, Chin Chiang, Washington State University, Pullman, WA 99164-6430.

Resistance in peas is controllable in that chitosan, an antifungal wall component of Fusarium solani can singly induce immunity in pea endocarp tissue to F. solani f. sp. pisi and heat shock or protein synthesis inhibitors can block induction of immunity and render the pea tissue susceptible to f. sp. phaseoli. The following mechanisms are proposed to be involved in determining resistance or susceptibility. Plant β-glucanase (BG) and chitinase (CH) release fungal wall fragments. Chitosan fragment release is influenced by plant saponins possibly by enhancing chitin synthetase and assisting action of chitin deacetylase. Chitosan then complexes with plant DNA and induces disease resistance response genes (DRRG) and also increases phenylalanine ammonia lyase, BG, CH, pisatin and lignin. Since DRRG mRNA levels are detectable in healthy tissue and are induced to higher levels in all resistance responses tested, we propose cell maintenance functions for some of their gene products which aid the viability of cells adjacent to those challenged. Our model also implicates pectolytic enzymes in reduction of DRRG activity and loss of cell viability in susceptible tissue. Relationships between DRRG and single Mendelian traits (MT) affecting resistance and mechanisms of DRRG activation will be discussed. We propose some MT represent special chromatin sites (e.g., attachment to nuclear membrane, nuclear matrix, chromosome translocations) and that elicitor components can alter these chromatin structures allowing pleiotrophic gene responses in unattached portions of chromosomal loops.

Y 217 THE MOLECULAR BASIS OF PLANT RESISTANCE TO POTATO CYST NEMATODE, K.E. HAMMOND[+], D.J. BOWLES[+], and H.J. ATKINSON[*], Departments of Applied Zoology[*] and Biochemistry[+], University of Leeds, LEEDS LS2 9JT, U.K.

Cyst-nematode species G. rostochiensis and G. pallida are the major pest of the potato crop. Future control is likely to depend on improving the resistance status of otherwise desirable cultivars. An understanding at the molecular level of the basis of resistance is thus an essential prerequisite. Theoretically because cyst-nematode parasitism depends upon inducing the formation of a specialised plant organ - the syncytium - by modifying existing host cells, resistance could either be a positive attribute able to counter infection or arise from the absence of an appropriate plant gene(s) for susceptibility.

Utilizing the interaction between cv Maris Piper (H_1 genome) and two pathotypes of G. rostochiensis as a model system we have explored the timing, range and co-ordination of changes in gene expression during both a compatible and an incompatible interaction in synchronously infected roots of potato plants. Analysis of the in vitro translation products of various polysomal mRNA populations, isolated from 4 points early in the infection sequences, by 2D IEF-SDS PAGE/fluorography has revealed changes in host gene expression to occur within 6 hr of nematode invasion and qualitative, quantitative and stage specific differences in gene expression between the compatible and incompatible interactions. Several compatible and incompatible specific polypeptides, potentially of either host or nematode origin, have already been identified. Work is currently in progress to isolate the corresponding coding sequences and to explore the significance of the changes in gene expression in relation to the manifestation of plant resistance to nematode attack.

Y 218 EFFECTS OF THE AAL-TOXINS ON VARIOUS LEVELS OF TOMATO PLANT CELL DEVELOPMENT, Hanneke Witsenboer[1], Carla van Schaik[1], Raoul Bino[2], Jacques Hille[1], [1]Free University, Dept. of Genetics, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands' [2]Institute for Horticultural Breeding (IVT), P.O.B. 16, 6700 AA Wageningen, The Netherlands

The fungus Alternaria alternata fsp. lycopersici is the causal agent of Alternaria stem canker in tomato. The disease is characterized by the formation of dark brown cankers on stems and necrosis of leaftissue between the veins. The fungus produces host-specific toxins that play a major role in the pathogenesis. Resistance to the pathogen is inherited as a single gene expressing complete dominance. Resistance to the purified toxin is controlled by a dominant gene expressing incomplete dominance when heterozygous . Since the AAL-toxins play a very important role in tomato disease development and because of a postulated cellular site of the toxins the effects of the AAL-toxins were studied on the cellular level and on higher levels of tomato plant differentiation to get insight into mechanisms involved in tomato resistance to Alternaria alternata fsp. lycopersici . Differences between susceptibility and resistance were shown during root induction, in leaves and in pollen. However differences were not observed at cellular levels. Therefore, the expression of resistance seems related to some higher level of tomato plantcell development.

Y 219 TMV INDUCTION OF GENE EXPRESSION IN TOBACCO PLANTS, Jeannine Horowitz, Robert B. Goldberg,University of California, Los Angeles,CA 90024.
Our research has dealt with the characterization of mRNAs that are induced in Samsun NN tobacco as the result of an infection by TMV. In this tobacco genotype the virus is localized at the site of entry, in contrast to Samsun nn tobacco where the virus spreads systemically. We have determined that the induction of Samsun NN genes by TMV is regulated mostly at the transcriptional level and that these genes are also present in the Samsun nn genotype but are not induced at the same levels by TMV. The genes are induced in the different organs of the tobacco plant when these organs are infected by TMV but they are also induced in non-infected organs of the infected plant. As shown by in situ hybridization, TMV-induced genes do not follow a cell-type specific expression but are expressed at higher levels in cells adjacent to the initial site of infection. TMV-induced genes have been isolated and sequenced and are now being used in 5'deletion analysis and DNA binding experiments to locate cis-acting sequences and trans-acting factors involved in the pathogenic induction.

Y 220 A 32 KB REGION OF THE *RHIZOBIUM TRIFOLII* SYMBIOSIS PLASMID IS SUFFICIENT FOR NODULATION AND NITROGEN FIXATION ON CLOVER, Roger W. Innes, Melissa A. Hirose and Peter L. Kuempel, University of Colorado, Boulder, CO 80309-0347.
Bacteria in the genus *Rhizobium* are able to fix atmospheric nitrogen in symbiotic association with leguminous plants. Most of the genes identified in *Rhizobium* that are required for this symbiosis have been localized to single large plasmids (symbiosis plasmids, pSym), which range in size from 180 kb in *R. trifolii* to 1600 kb in *R. meliloti*. It is still unknown, however, for any species of *Rhizobium*, how many pSym encoded genes are actually required for successful development of a nitrogen fixing symbiosis. We have used a combination of *in vivo* and *in vitro* cloning methods to subclone large fragments of the *R. trifolii* strain ANU843 pSym. A single subclone (pRI4003) containing only 32 kb of pSym was obtained that restored nitrogen fixation ability (in association with clover) to a pSym cured derivative of strain ANU843. This clone contained the *nifHDKE, nifA, nifB,* and *fixABCX* genes previously identified in other *Rhizobium* strains, as well as the entire 14 kb nodulation region previously identified in ANU843. Using Tn*5* mutagenesis, we identified a new region downstream of *nifE* that is essential for nitrogen fixation.

Y 221 A VISUAL ASSAY FOR Ac TRANSPOSITION IN DICOTS. Jonathan Jones, D. Gilbert, L. Harper, P. Maliga and H. Dooner. AGS, 6701 San Pablo Ave, Oakland CA 94608
Streptomycin sensitivity leads to bleaching in the cotyledons of sensitive tobacco seedlings. We have engineered a nuclear streptomycin resistance (SPT) gene that allows transformed seedlings containing this construct to green on streptomycin.
We have engineered the corn transposon Ac into the nuclear SPT gene in such a way that Ac excision restores SPT expression. When seed of plants transformed with this construct are plated on streptomycin, many seedlings display a green/white variegation in their cotyledons. This phenotype is likely to be caused by somatic excision of Ac from the SPT gene during embryogenesis. Southern blot analysis of green germinal revertants supports this contention.
We have used this assay to (i) evaluate the influence of Ac dosage on transposition frequency and (ii) to obtain germinal excision frequencies for Ac in tobacco. These frequencies are high enough to suggest that it is feasible to transposon tag interesting genes in dicots using Ac as an engineered transposon.

Y 222 ELICITATION AND SUPPRESSION OF ISOFLAVONE AND PHYTOALEXIN ACCUMULATION IN COTYLEDONS OF CICER ARIETINUM AS CAUSED BY POLYMERIC COMPOUNDS FROM THE FUNGUS ASCOCHYTA RABIEI

Helmut Kessmann, Doris Meier and Wolfgang Barz, Westfälische Wilhelms-Universität, Lehrstuhl für Biochemie der Pflanzen, Hindenburgplatz 55, 4400 Münster, FRG

The phytopathogenic deuteromycete Ascochyta rabiei (Pass.) Lab. is the causal agent of "Asco - chyta blight" on chickpea (Cicer arietinum L.). After fungal infection the pterocarpan phyto - alexins medicarpin and maackiain accumulate in a resistant cultivar of chickpea to much higher levels than in a susceptible cultivar.

As part of a comprehensive program to elucidate the biochemical events during plant-pathogen interaction and to determine the molecular basis of fungal virulence and host resistance we have studied the influence of polymeric compounds obtained from the fungus on the accumulation of isoflavones and phytoalexins.

The elicitor, a crude polysaccharide fraction, was prepared from mycelium of the fungus. A crude, dialyzed homogenate of germinated spores suppresses the constitutive isoflavone and elicitor induced phytoalexin accumulation in cotyledons of chickpea. The suppressing material could be quantitatively liberated from germinated spores by washing with lithium chloride so - lution. Some elicitor material was also present in this fraction and could be separated by ultrafiltration (M_r > 75 kD). Ultrafiltration techniques have further revealed that the "suppressor" has a molecular weight between 10 and 25 kD. At least three compounds with significant suppressor activity were separated and purified by reversed-phase HPLC. Investigations on the structure of the most active suppressor compound is under way. Suppressor activity binds to Concanavalin A, is stable to heat (20 min, 121°C) and to proteolytic enzymes but sensitive to periodate and trifluoracetic acid treatment. Data on elicitor-suppressor counteraction will be presented.

Financial support by Deutsche Forschungsgemeinschaft.

Y 223 NUCLEAR PROTEIN BINDING TO A *CIS*-ACTING SILENCER IN A DEFENSE GENE PROMOTER, M.A. Lawton, K. Kragh, S.M. Jenkins, M. Dron, S.D. Clouse, R.A. Dixon and C.J. Lamb, Plant Biology Laboratory, Salk Institute, P.O.Box 85800, San Diego, CA 92138

Plant defense gene activation is an early step in the induction of defenses in response to elicitors, wounding or infection. Functional assay in electroporated protoplasts showed that the promoter of a gene encoding the phytoalexin biosynthetic enzyme chalcone synthase (CHS) contains an upstream silencer between -173 and -326, as well as an elicitor-regulated activator between the TATA box and -173. Binding of nuclear proteins to the silencer *in vitro* was detected by gel retardation and DNase I protection footprints. Footprinting identified binding to three discrete sites in the element. Binding was specifically competed by CHS promoter sequences and by the promoter of a co-ordinately induced defense gene, but not by unrelated sequences eg. CHS cDNA. Electroporation of the silencer element enhances in *trans* the activity of the CHS promoter, presumably by competition for binding of the *trans*-acting repressor(s). This suggests an approach to engineer increased expression of a set of co-ordinately regulated biosynthetic genes involved in a multigenic defense response.

Y 224 **CHARACTERIZATION OF RAPESEED MYROSINASE,** M Lenman, J Rödin, L-G Josefsson, and L Rask, Dep. of Cell Research Swedish Univ. of Agricultural Sciences, Uppsala, Sweden.

Many cruciferae contain glucosinolates, low molecular weight compounds consisting of a glucose residue derivatized in a thioglukoside bond to an amino acid. The glucosinolates seem to always be accompanied by a group of isoenzymes, thioglucoside glucohydrolase, also called myrosinase, which catalyzes the hydrolysis of the nontoxic glucosinolates to goitrogenic isothiocyanates, nitriles or thiocyanates. Partly purified rapeseed myrosinase was used as antigen to produce mouse monoclonal antibodies. Two sets of monoclonal antibodies precipitating myrosinase activity were obtained. One of them reacts with at least three rapeseed components of approximately 70 kD, all of them with myrosinase activity. The other set of monoclonal antibodies reacts with two components of 52 and 55kD, both lacking myrosinase activity. Gelchromatography experiments resolved two myrosinase-containing fractions of approximate m.w. 700.000 and 200.000. The high molecular fraction contains a complex consisting of two myrosinases and the 52 and 55kD proteins whereas the smaller fraction only contains 70kD chains.

Y 225 GENETIC CONTROL OF CULTIVAR SPECIFICITY ON RICE IN THE BLAST FUNGUS, Hei Leung, Emerlito S. Borromeo, Marichu A. Bernardo, International Rice Research Institute, P.O. Box 933, Manila, Philippines.

The blast fungus Magnaporthe grisea (ascomycete) is an important pathogen of rice and many grasses. Isolates from rice are usually female sterile, thus preventing genetic analysis of cultivar specificity on rice. Using a naturally-occurring hermaphroditic isolates Guy11 collected by J. L. Notteghem, we obtained viable progenies from crosses between rice isolates and determined the inheritance of cultivar specificity. In a cross between Guy11 and a laboratory tester, over 50% ascospores were viable. Random-spore and tetrad analysis showed that two loci, Pos1 (pathogenicity on Oryza sativa) and Pos2, were involved in conditioning virulence to rice line 51583 and Sha-tiao-tsao, respectively. A buf mutation was shown genetically to be epistatic to virulence. The segregation patterns of two isozyme loci, lactate dehydrogenase-1 and lactate dehydrogenase-3, were normal suggesting that lethality was random among ascospores. Fertility and ascospore viability were low in crosses between field rice isolates. In a cross between rice isolates, Guy11 and CH104-3, only 10% of the ascospores were viable, but all progeny were virulent to 51583 and Sha-tiao-tsao. From this cross, we identified three different loci - Pos3, Pos4, and Pos5 - controlling virulence on rice line K59, 28558 and Kinandang Patong, respectively. Joint segregation analysis showed an excess of parental types with respect to virulence and avirulence. Fertility and virulence were maintained in progeny recovered from backcross, F_2, and F_3 generations, an important criterion for routine genetic and molecular analysis of the fungus.

Y 226 REGULATION OF CASBENE SYNTHETASE DURING ELICITATION OF CASTOR BEAN SEEDLINGS WITH PECTIC FRAGMENTS, Augusto F. Lois and Charles A. West, University of California Los Angeles, Ca 90024.

Treatment of castor bean seedlings with an elicitor preparation of polygalacturonic acid results in the accumulation of the diterpenoid phytoalexin casbene. Casbene formation is preceded by a transient increase in the activity of casbene synthetase, the terminal enzyme in the synthesis of this phytoaelexin. RNA blot hybridization with cDNA complementary to casbene synthetase RNA was used to measure changes in the mRNA amounts at various times after elicitation. Total hybridizable casbene synthetase mRNA amounts increased after elicitation to a maximum at six hours, and then decreased steadily with almost the same kinetics as observed previously for the changes in the translatable mRNA activity. *In vitro* transcription studies with castor bean nuclei isolated at various times after elicitation showed that the increase in casbene synthetase mRNA is the result, at least partly, of an increase in its specific rate of transcription initiation. A.L. is a McKnight trainee; this research was supported in part by USDA Grant 86 CRCR-1-2145.

Y 227 SUBCELLULAR LOCALIZATION OF CHITINASE AND ß-1,3- GLUCANASE IN BEAN LEAVES. FUNCTIONAL IMPLICATIONS FOR THEIR INVOLVEMENT IN PLANT PATHOGEN INTERACTIONS. Felix C. Mauch and L.Andrew Staehelin, University of Colorado, Boulder CO 80309.

Plants respond to an attack by potentially pathogenic organisms and to the plant stress hormone ethylene by an increased synthesis of hydrolases such as chitinase and ß-1,3-glucanase. These two enzymes have no known function in the primary metabolism of higher plants but may act as defense hydrolases against fungal pathogens with chitin-ß-1,3-glucan cell walls. To explore the involvement of chitinase and ß-1,3-glucanase in defense responses we have localized these enzymes at the subcellular level in ethylene-treated bean leaves embedded in LR White using protein A-gold and polyclonal antibodies against these enzymes. Our micrographs indicate that in leaf cells the major accumulation of chitinase and ß-1,3-glucanase occurs in the vacuole. Within the vacuole label is found predominantly over electron dense protein deposits. A second site of accumulation of ß-1,3-glucanase is the cell wall, where label is seen nearly exclusively over the middle lamella regions surrounding intercellular airspaces. Thus, it appears that ß-1,3-glucanase is partly deposited into vacuoles and partly secreted into cell walls. In addition, we observed with both kinds of antibodies some labeling of Golgi cisternae and of transport vesicles, suggesting that chitinase and ß-1,3-glucanase could be processed in the Golgi apparatus on the way to their ultimate sites of deposition. Based on these observations we postulate that: a) the cell wall associated ß-1,3-glucanase could play a role in pathogen recognition processes by releasing glucan elicitors from the fungal cell walls that trigger other plant defense responses, and that b) the vacuolar chitinase and ß-1,3-glucanase function as last line of defense, becoming " functional" only when an attacking fungus kills a few host cells. At that time the stored vacuolar enzymes would be suddenly released in concentrations capable of destroying the pathogen. This work was supported by NSF-grant DCB-8615763.

Y 228 TOWARDS A MORE DETAILED STRUCTURE OF PECTINS OF PRIMARY CELL WALLS, Andrew J. Mort, Padmini Komalavilas, and Niels O. Maness, Department of Biochemistry, Oklahoma State University, Stillwater OK 74078-0454.

Many plant pathogens degrade cell walls during the course of infection. Enzymes that degrade cell walls appear to be virulence factors. However, various reports have shown that some of the fragments produced from the enzymatic digestion of the walls may actually induce resistance responses in the plant.

We are studying the substrate for many of these enzymes—the pectins of cell walls. Our current model system is suspension cultures of cotton (*Gossypium hirsutum* L.). By treating intact cell walls with liquid HF at -23°C, we can obtain fragments which represent the backbone of two regions of pectin, the homogalacturonan and the repeating disaccharide region of rhamnogalacturonan (RGI). (We have not looked at what has been called RGII.) Since treatment with HF does not remove esters, we can determine the positions of acetate and methyl esters in the fragments. We have shown that the acetate esters are all in the RGI region on O-3 of about 50% of the galacturonic acid residues. The methyl esters are all in the homogalacturonan regions. The homogalacturonan regions are at least 30 galacturonic acid residues in length. These alkali labile acetate and methyl ester substituents may have a great influence on what products cell wall degrading enzymes form from digestion of the pectins. (Supported in part by DOE grant DE-FG05-86ER 13496 and the Oklahoma Agricultural Experiment Station.)

Y 229 RESISTANCE IN MUSKMELON TO WATERMELON MOSAIC VIRUS 2 PATHOGENESIS, S. M. Gray and J. W. Moyer, USDA/ARS, Cornell University, Ithaca, NY and North Carolina State University, Raleigh, NC.

Resistance of the Cucumis melo accession 91213 to watermelon mosaic virus 2 (WMV 2) was expressed as a reduction in the amount of virus antigen accumulating in leaf tissue. WMV 2 symptoms on resistant plants were discrete chlorotic lesions unlike the uniform mottle expressed in susceptible plants. Differential temperature manipulation of the systemic infection of resistant plants increased the initial number and uniform distribution of the chlorotic lesions. As a result capsid protein accumulated to similar levels in resistant and susceptible tissue, although, infectious virus was 25-56% lower in resistant plants. Virus particle counts and aphid transmission efficiency were correlated with infectious virus levels. The kinetics of cylindrical inclusion protein and capsid protein accumulation were similar in susceptible tissue. Cylindrical inclusion protein was detected, but did not accumulate in resistant tissue. The resistance appears to involve several distinct mechanisms that reduce the production of infectious virions, limit cell-to-cell movement, and differentially affect production or stability of viral proteins.

Y 230 LOCALIZATION OF NITROGENASE AND THE 32kD PROTEIN OF PSII IN A. CAROLINIANA WITH IMMUNOELECTRON MICROSCOPY, Ellen B. Braun-Howland and Sandra A. Nierzwicki-Bauer, Plant Science Group, Dept. of Biology, Rensselaer Polytechnic Institute, Troy, N. Y. 12180-3590.

Azolla is a small water fern which is symbiotically associated with the filamentous nitrogen-fixing cyanobacterium Anabaena azollae. The Anabaena species, which occurs as an endosymbiont in the leaf cavity of Azolla, can fix enough atmospheric nitrogen to satisfy both its own requirement for combined nitrogen and that of its host. Results that have been reported previously showed that the transcript levels of nitrogenase and the 32kD protein of photosystem II were higher in symbiotic versus free-living Anabaena. In light of these results, it was of interest to localize these proteins within individual cells of symbiotic Anabaena filaments with immunoelectron microscopy. Nitrogenase was located exclusively within mature heterocysts of the A. caroliniana endosymbiont. The labeling intensity however, was decreased in older (aged) heterocysts. The 32kD protein was detected in both vegetative cells and heterocysts. The thylakoid membranes of vegetative cells labeled very heavily, while the heterocysts labeled only slightly above background levels. The intensity of labeling varied in vegetative cells within leaf cavities of different ages. These ultrastructural results will be correlated to molecular studies on the transcription of genes encoding these proteins in symbiotic Anabaena. This work was supported by NIH Grant No. RO1 GM37051-01.

Y 231 STUDIES ON ALTERNARIA SOLANI-SOLANUM TUBEROSUM IN VITRO INTERACTIONS, G.Nascari, M. Broggio, C.Montanelli, A.Garbuglia*, M.G.Pellegrini*, C.Simeti*, M.Buiatti*, Istituto Agronomico per l'Oltremare, Firenze - *Dipartimento di Biologia Animale e Genetica, Firenze, Italy.

In this work the behaviour of resistant and susceptible potato cvs. to A.solani, the etiological agent of early blight, was analyzed following two different in vitro approaches: the possible hypersensitive response to the pathogen or its component, and the ability to grow in presence of the fungal toxic filtrate. When calli of one susceptible and one resistant cv. were challenged with A.solani cell wall components released at room temperature or after heat treatment, some differences between the two were noted in terms of UV-detectable compounds, but no production of rishitin was observed. Differences and number of induced compounds were higher in the case of heat released elicitors. An active response is also suggested from the data obtained in dual culture, in which only the resistant calli showed an increase of oxydative metabolism in the presence of the fungus. On the other hand,differences between the cvs. were noted also in terms of ion leakage after treatment with A.solani culture filtrate, and survival on toxic substrates; in fact only the resistant calli don't release ions in the culture medium after adding the toxic filtrate at the tested concentrations. Finally, the plating of susceptible cells on toxic media allowed the isolation of tolerant cell lines.

Y 232 ROLE OF FUSARIC ACID IN THE SYMPTOM DEVELOPMENT IN FUSARIAL WILT OF TOMATO, Hisako Hashimoto, Hideyoshi Toyoda and Seiji Ouchi, Kinki University, Higashi-Osaka, Osaka 577, Japan

Fusaric acid,5-n-butylpicolinic acid(FA), one of the common metabolites of Fusarium species was once implicated to play a role in wilting of tomato plants infected by Fusarium oxysporum f.sp. lycopersici. Its real function in the syndrome, however, has been questioned by some researchers. To verify the role of FA in the development of wilting, we mutagenized an avirulent isolate of Pseudomonas solanacearum to obtain a fusaric acid resistant mutant(A-16) and tested it for its ability to protect tomato plants from FA treatment and fungal inoculation. The mutant A-16 grew to a similar extent in the presence of 100μg/ml FA to those contaning no FA. When A-16 was cultured in various media containg 100μg/ml FA, the filtrates became non-toxic to tomato callus cells indicating detoxification. Excised tomato leaf cuttings were protected from wilting even if they had been treated with 300μg/ml FA, provided they had been administered with A-16 from the cut end prior to FA treatment. The parent isolate U-10A did not protect the tomato cuttings under the same conditions. Leaf cuttings were completely protected when they had been administered with 10^9 cells of A-16. The bacteria were detected in petioles as well as in compound leaves as soon as the suspension was sucked up by the cuttings. A-16 protected also the whole plants from race J3 of the wilt fungus when it was inoculated through injured roots one week before the fungal inoculation. A-16 had no antifungal activity against races of the wilt fungus. These results suggest that FA may play a significant role in the development of wilt symptom in ifected tomato plants. Application of this mutant to the control of Fusarium wilt is under way.

Y 233 **STRUCTURE AND PROMOTER ANALYSIS OF PR-1 GENES FROM TOBACCO**
Artur J. P. Pfitzner, Howard M. Goodman[1] and Ursula M. Pfitzner, Botanisches Institut, Menzingerstr. 67, D-8000 München 19, FRG, [1]Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

Pathogenesis-related proteins (PR-proteins) are a heterogeneous group of host encoded proteins, which are induced in plants by different external stimuli such as pathogen attack or exposure of the plants to certain chemicals. We have characterized 3 tobacco genomic clones encoding PR-1 genes. Two of these correspond most presumably to pseudogenes, whereas the 3rd gene is essentially identical to the PR-1a cDNA clones. The 5' flanking region of this gene contains direct repeat elements and a heat shock element like motif, which might be involved in transcriptional regulation of PR-1 genes. We have now assayed promoter constructs containing the 5' upstream regions of the putative PR-1 pseudogenes and deletion mutants of the PR-1a flanking sequence fused to the *E. coli* β-glucuronidase by transient expression. The results of these experiments will be presented and implications for the regulation of PR-1 genes will be discussed.

Y 234 PLANT-INDUCED CYTOKININ BIOSYNTHESIS IN *AGROBACTERIUM TUMEFACIENS* IS DIRECTLY CONTROLLED BY THE *VIRA/VIRG* REGULATORY SYSTEM. Gary K. Powell and Roy O. Morris, The University of Missouri, Columbia, MO 65211.

Considerable attention has recently been focused on the action of Ti plasmid encoded virulence functions on the process of *Agrobacterium tumefaciens* mediated plant transformation. Expression of several virulence loci was found to be induced by plant phenolics through the action of a two component positive regulatory system consisting of VirA and VirG [Stachel, S. et al. (1985) Nature, 318:624-629]. We show here that cytokinin biosynthesis, catalyzed by the *tzs* gene product in nopaline strains, is also regulated by the VirA/VirG system. In the presence of acetosyringone zeatin secretion is induced more than 100-fold, a result which suggests that cytokinins may play a crucial role in the virulence process.

Although the details of the molecular mechanism by which this regulatory system acts are largely unknown, we have found that the *virA* and *virG* loci are the only factors essential for phenolic induced cytokinin production. Although no other Ti plasmid encoded factors are necessary to obtain induced *tzs* expression, it does appear that other *Agrobacterium* encoded factors are essential since phenolic induction does not occur in *E. coli* strains carrying the *tzs*, *virA* and *virG* loci. (This work was supported by USDA grant 85 CRCR-1-1645 and by Abbott Laboratories)

Y 235 THE INVOLVEMENT OF THE COAT PROTEIN mRNA, THE COAT PROTEIN, AND THE tRNA-LIKE STRUCTURE IN CROSS PROTECTION OF TRANSGENIC TOBACCO, Patricia A. Powell, Patricia R. Sanders, Robert T. Fraley and Roger N. Beachy, Washington University, St. Louis, MO 63130. Having obtained cross protection against TMV infection in trangenic plants expressing a cDNA for the coat protein cistron, we attempted to determine the molecule(s) responsible for the protection. Constructs were made which transcribe mRNA for the coat protein but do not translate the protein, as well as the control construct which produces both RNA and protein. Another construct from which the tRNA-like structure at the 3' end of the viral genome has been deleted was also used. Seedlings of plant lines expressing these different constructs were inoculated with TMV to determine whether protection occurred. The results of these experiments will be presented.

Y 236 SAM:6A-HYDROXYMAACKIAIN 3-O-METHYLTRANSFERASE, PURIFICATION AND CHARACTERIZATION, Carol L. Preisig, David E. Matthews and Hans D. VanEtten, Cornell University, Ithaca, NY 14853.

The isoflavonoid phytoalexin pisatin is synthesized by *Pisum sativum* in response to microbial infection and certain other forms of stress. An enzyme that synthesizes pisatin by methylating the 3 hydroxyl of (+)6a-hydroxymaackiain (HMK) was extracted from $CuCl_2$-stressed pea seedlings. The enzyme was specific for (+)HMK, methylating (+)maackiain, (–)HMK and (–)maackiain less than 5% as rapidly. The methyltransferase has a pH optimum of 7.9, no apparent divalent cation requirement, and a high affinity for the phenolic substrate (K_m 1 to 2 μM).

The enzyme has been purified 300-fold by ammonium sulfate precipitation, DEAE chromatography and chromatofocusing, with a 16% yield and final specific activity of 5.8 μkat/g protein. Further purification by HPLC utilized hydrophobic interaction chromatography (HIC) followed by gel filtration. Enzyme activity profiles of chromatofocusing and HIC columns suggest the presence of two or more isozymes. Non-denaturing gel filtration indicates an M_r of 64,000. Methyltransferase-containing fractions from gel filtration showed two bands upon SDS gel electrophoresis, of M_r 62,000 and 39,000. Both bands were most intense in chromatographic fractions containing peak enzyme activity.

Y 237 IN VITRO TRANSCRIPTION AND TRANSLATION OF THE COAT PROTEIN GENE OF PLUM POX VIRUS, Michel Ravelonandro, Rene Delbos, Geneviève Tavert, Marie Monsion and Jean Dunez, Station de Pathologie végétale, INRA, BP 131, 33140 Pont-de-la-Maye, FRANCE.

The coat protein cDNA clone of plum pox virus (PPV) contains 936 bp of coding sequence followed by 215 bp of 3'untranslated sequence. The 1.2 Kb cDNA insert was ligated to the leader sequence cDNA of alfalfa mosaic virus RNA 4 and cloned into pSP64.
The cDNA insert was transcribed by the SP6 polymerase to yield a translatable RNA. In vitro translation of the transcript RNA encodes a polypeptide which comigrates with PPV capsid protein and reacts with specific antisera.
These studies show that PPV coat protein gene could be tested to produce transgenic plants that will express coat protein of the plant virus.

Y 238 COMPARATIVE MOLECULAR STUDIES OF CHALCONE ISOMERASES, Julio Reinecke, E. Richard Blyden and Richard A. Dixon, Dept. of Biochemistry, Royal Holloway & Bedford New College, Univ. of London, Egham Hill, Egham, Surrey, TW20 0EX, U.K.
Chalcone isomerase (CHI) catalyses the isomerization of chalcones to their corresponding flavanones, a key step in the biosynthesis of flavonoid pigments in most plant and also in the synthesis of microbially-induced antifungal phytoalexins in legumes [1]. In bean and alfalfa cell cultures exposed to elicitor polysaccharides, CHI activity is induced as a result of transient increases in its mRNA. In Petunia, CHI activity and its mRNA are highly expressed during floral development [2], but not in response to elicitor.

Enzyme inhibition and western blotting studies using antisera directed against purified bean or Petunia CHIs indicate an apparent lack of antigenic relatedness between CHI proteins from bean, Petunia and alfalfa. cDNA clones encoding CHI have recently been isolated from bean [3] and Petunia [2] λgt11 expression libraries, and we have isolated a number of putative alfalfa CHI cDNAs from a λgt10 library of elicitor-induced sequences. These cDNAs have been used in comparative northern blot analyses of bean, alfalfa and Petunia total RNA, with the cross-hybridisation results given below. We are currently investigating the considerable differences between the CHIs in relation to nucleic acid sequences and genomic organization.

Probe	RNA source		
	Bean	Alfalfa	Petunia
Bean	+	-	-
Alfalfa	-	+	+
Petunia	-	+	+

[1] Robbins, M.P. and Dixon, R.A. (1984) Eur. J. Biochem 145, 195
[2] van Tunen, A.J. et al. In Preparation.
[3] Mehdy, M. and Lamb, C.J. (1987). EMBO J. 6, 1527.

Y 239 CHARACTERIZATION OF RAPIDLY ACTIVATED DEFENSE-RELATED PARSLEY GENES, Imre E. Somssich Wolfgang Schulz, Elmon Schmelzer and Klaus Hahlbrock, Max-Planck Inst. Köln, W. Germany
We are investigating the hypersensitive nonhost response of parsley to the soybean fungus Phytophthora megasperma f.sp. glycinea (Pmg) to better understand the molecular mechanisms of disease resistance in plants. For practical reasons, a plant/pathogen system of reduced complexity consisting of cultered parsley cells in combination with Pmg elicitor is beneficial in the identification and biochemical study of early induced plant cell responses. It has been shown, that the same coumarin derivatives, the putative phytoalexins of parsley, and their biosynthetic enzymes are synthesized de novo at infection sites in intact tissue as in cell cultures ater elicitor treatment.
From cDNA libraries made with RNA from elicitor treated cells, numerous clones corresponding to rapidly and transiently activated "defense-related" genes have recently been isolated and characterized. Many of the cDNAs encode for pathogenesis related PR1 proteins. Some of the clones were used for in situ hybridization experiments with whole infected plant tissue to demonstrate rapid and massive accumulation of their respective mRNAs at individual infection sites.
Beyond this, the structural organization of a few of the transcritionally activatable genes have been studied in detail and some potentially interesting structural elements found within their promoter regions.

Y 240 cDNA CLONING, SEQUENCING, AND EXPRESSION OF THE SOYBEAN MOSAIC VIRUS COAT PROTEIN CODING SEQUENCE, Alan L. Eggenberger, David M. Stark, and Roger N. Beachy, Biol. Dept., Washington University, St. Louis, MO 63130

DNAs complementary to the RNA of soybean mosaic virus (SMV), a member of the potyvirus group, were made and cloned into the expression vector pEMBL 18+. One of these, designated pSM 2-21, made a fusion protein in E. coli that reacted with antibodies against SMV coat protein. This cDNA was sequenced and was found to include the coat protein coding sequence. The N-terminus of the SMV coat protein was blocked but sequencing of a 28 kD trypsin resistant core gave internal sequence which confirmed the reading frame. The coat protein coding sequence was modified using oligo-directed mutagenesis for introduction into transgenic plants for genetically engineered resistance studies. This transformation is currently underway.

Y 241 MODIFICATION OF CYTOKININ PRODUCTION IN BRADYRHIZOBIUM CULTURES. Barbara J. Taller and Dawn Sturtevant, Department of Biology, Memphis State University, Memphis, TN 38152.

An early event in nodule development is the induction of cell divisions in the legume root. We have recently confirmed that Bradyrhizobium japonicum 61A68 produces the cytokinin zeatin and its derivatives. Because plant factors induce some bacterial genes, it was of interest to determine whether plant factors affect cytokinin production. Cytokinins were isolated from culture filtrates by Amberlite XAD-2 trace enrichment followed by fractionation on Sephadex LH-20. Pooled fractions were analyzed for cytokinin activity in the tobacco bioassay. B. japonicum in defined medium produced three cytokinin-active fractions, corresponding to the elution volumes of ribosylzeatin (ZR), zeatin (Z) and methylthiozeatin (msZ). ZR was the most active fraction; msZ was the least active. When cells were grown in the presence of soybean seed extract, activity attributable to msZ increased about 35-fold and no Z activity was found. Bioassay of the fractionated seed extract showed only ZR and a trace of Z. Bradyrhizobium grown in a comparable amount of ZR showed an increase in msZ activity, but not equivalent to that seen with seed extract. The effect of the flavonoids daidzein and genistein, recently identified as inducers in soybean root extracts, is being investigated. The ability to modify cytokinin production by the addition of seed extract, flavonoids or cytokinins suggests that the cytokinins found in Rhizobium culture filtrates are not merely the result of nonspecific tRNA autolysis.

Y 242 MOLECULAR CHANGES IN PLANT CELLS INITIATED BY ELICITOR TREATMENTS, Anne J. Anderson, Craig S. Tepper and Kim R. Rogers, Utah State University, Logan, UT 84322-4500.

Necrosis in suspension cultured bean cells was initiated between 6-8 hours after treatment with purified and crude elicitors from the α race of Colletotrichum lindemuthianum, a bean pathogen. The onset of necrosis was accompanied by accumulation of phenolics and phytoalexins in the cells and in the cell medium. Free radicals, detected by their EPR signal, and a product of lipid peroxidation, lipofuscin, also began to accumulate at this time. These data suggest that a complex set of responses including radical formation are involved in elicitor induced plant cell necrosis.

The mRNAs specifying enzymes concerned with phytoalexin synthesis, phenylalanine ammonia-lyase and chalcone synthesis accumulated earlier in the elicitor treatments. Two peaks of synthesis of these mRNAs were observed at 3 and 6-7 hours in elicitor treated cotyledon tissues. The relative magnitude of the two peaks was dependent on the elicitor used.

Y 243 CLONING OF CHALCONE ISOMERASE (CHI) cDNA FROM PETUNIA HYBRIDA: COORDINATE AND LIGHT REGULATED INDUCTION OF FLAVONOID SPECIFIC GENE EXPRESSION.
Arjen J. van Tunen, Ronald E. Koes, Antoine R. Stuitje and Joseph N.M. Mol, Free University, Dept. of Genetics, Amsterdam.

CHI is one of the key enzymes of the flavonoid biosynthesis route which results in the synthesis of flower pigments in higher plants. Furthermore flavonoid biosynthesis plays an important role as part of the plant defence system and flavonoid genes are induced after various stress treatments. In order to be able to study regulation of flavonoid gene expression at the molecular level CHI cDNA from corolla flower of Petunia hybrida was cloned using a λgt11 expression library. A nearly full length cDNA was used as a probe to analyze CHI genomic organization. Southern blot analysis in combination with genomic cloning reveals the presence of only two distinct non-allelic CHI genes. Both genes were characterized and the promoter regions were compared with other cloned flavonoid genes (CHS, DFR). Furthermore flavonoid gene expression was studied using CHI and CHS cDNA probes. First, in developing floral tissue both CHI and CHS mRNA's are coordinately expressed. Second, both CHI and CHS mRNA is present in flower tissue of normal light-grown plants but a dark treatment eliminates both mRNAs completely. Third, when Petunia hybrida seedlings are irradiated with continuous UV light this stress situation results in the coordinate induction of CHS and CHI mRNA, probably as part of the plant UV defence mechanism. As an exception to the principle of closely coordinated regulation of CHI and CHS expression the accumulation of a 0.3 kb larger CHI transcript in mature anthers is observed which, in contrast to the small CHI transcript, is not light regulated nor UV inducible. The nature of this mRNA and possible biological functions will be discussed.

Y 244 A GENE CONFERRING RESISTANCE TO ROOT KNOT NEMATODES, Valerie M. Williamson, Department of Nematology, University of California, Davis, CA 95616.

Mi is a single, dominant locus in tomato that confers resistance to three species of root knot nematode. The resistance is highly effective in the field and has been reported to involve a hypersensitive response. We find that root explants of susceptible and resistant cultivars growing on agar plates can be clearly differentiated after addition of nematode juveniles. Within a few days swellings or galls are visible on susceptible roots where the endoparasitic nematodes have entered, whereas no galls are visible on roots from resistant cultivars. Often localized browning is visible near the root tips of nematode resistant cultivars.

Mi is tightly linked to a gene encoding acid phosphatase, and an electrophoretic variant of this enzyme is present in many nematode resistant cultivars. This linkage is used by tomato breeders who score for nematode resistance by starch gel or cellulose acetate electrophoresis of leaf extracts followed by staining for acid phosphatase activity. As a step toward cloning Mi, we are purifying the enzyme acid phosphatase from tomato cell suspension culture. Our purification procedure will be presented. Purified enzyme will be used as an aid to cloning the corresponding gene, Aps-1. We plan to use "chromosome walking" techniques to clone the Mi DNA using the Aps-1 clone as a starting point. Candidate clones will be tested after introduction into susceptible tomato for their ability to confer nematode resistance.

Y 245 THE USE OF GRAFTING TO STUDY A MECHANISM OF CROSS PROTECTION IN TRANSGENIC TOBACCO PLANTS CONTAINING THE TMV COAT PROTEIN GENE. L.A. Wisniewski and R.N. Beachy Biol. Dept., Washington Univ., St. Louis, MO 63130. In order to gain a better understanding of the mechanism of protection against the systemic movement of tobacco mosaic virus (TMV) in plants expressing the TMV coat protein (CP) gene a series of plant grafting experiments were done. Grafted plants were produced by grafing a stem section into a systemic host of TMV, Nicotiana tabacum cv. Xanthi. Stem sections were taken from transgenic plants that were CP(+), CP(-) or from non-transgenic plants. Some sections used included a leaf while others did not. The Xanthi leaf below the grafted section was inoculated with TMV and signs of disease development were scored on the leaves above the grafted section. Enzyme linked immunoabsorbsant assays (ELISA) were also completed to determine TMV accumulation in the leaves on the grafted section and above the grafted section. These experiments allowed us to determine the role of the stem and leaf from the transgenic CP(+) plants on systemic movement of virus and lead to a better understanding of the resistance in transgenic plants that express TMV-CP.

Y 246 VICTORIN BINDING TO PROTEINS IN SUSCEPTIBLE AND RESISTANT OAT GENOTYPES, T.J. Wolpert and V. Macko, Boyce Thompson Institute, Cornell University, Ithaca, NY 14853

Cochliobolus victoriae Nelson (Helminthosporium victoriae Meehan and Murphy) produces the host-specific toxin, victorin. Cultivars of oats with the dominant Hv allele are susceptible to the fungus and sensitive to victorin, but victorin does not affect the recessive (resistant) genotypes. Recently, we have isolated and characterized victorin as a group of closely related peptides. Through chemical modification of victorin several derivatives were prepared. These derivatives include compounds which are less active than native toxin but still host-specific, non-toxic compounds, and a non-toxic protective compound (i.e. pre-incubation with the protectant prevents the toxic effects of subsequent addition of native toxin). These chemical manipulations have provided the means to radioiodinate victorin to specific activities of > 2000 Ci/mmole for use as a probe of victorin binding, and to produce other probes of varying biological activities. By treating near-isogenic lines of oats differing in the Hv gene, with radiolabelled victorin, we have identified several proteins which bind victorin in a genotype-specific manner.

Y 247 A SPECIFIC RESISTANCE PROTEIN ON FUSARIUM WILT OF COTTON, Yishen Zeng, Liangpeng Yang and Guangyu Zhou, Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China

We are the first to identify and purify a specific resistance protein R from resistant cotton cultivars which responds directly the resistance to certain physiological races of Fusarium oxysporum but not to the others. From the data of hapten inhibition, the protein also show its specificity. These results showed that protein R is a specific resistance protein. On the other hand, a protein r from wilt susceptible cotton has also been purified. Protein r showed very similar biochemical behaviours as protein R, as the pI, the M.W. and the quaternary structure etc., but no resistance function . Both of them are glycoprotein and constitutive expression.

We are going to compare the structure and furcture of protein R and r, to synthesize the probes and to isolate the genes.

Molecular Genetics of Fungi; RNA Viruses

Y 300 DETAILED CHARACTERIZATION OF A GENOMIC CLONE SPECIFICALLY EXPRESSED DURING DIFFERENTIATION OF BEAN RUST GERMLINGS, Srirama Bhairi[1], Pauline Freve[1], Olen C. Yoder[2] and Richard C. Staples[1], [1]Boyce Thompson Institute, Tower Road, Ithaca, NY 14853 and [2]Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The germ tube of the bean rust fungus, Uromyces appendiculatus, differentiates to form a series of infection structures in response to surface signals provided by the lip of the stomatal guard cell. The structures may also be induced by a 0.5 µm ridge in a plastic surface. We have isolated twenty differentiation-specific clones using cascade-hybridization technique. One of the clones, clone 24, encodes a 1.2 kb transcript. Using the hybrid-arrest cell free translation technique, we have shown that the clone 24 encodes a 23 kDa polypeptide. A detailed characterization of clone 24 in terms of its nucleotide sequence and transcription start site will be presented.

Y 301 GENOME ORGANIZATION OF COCHLIOBOLUS HETEROSTROPHUS, C. R. Bronson, Tzy-Hwa Tzeng, Hsin-Ru Chang, and Clark Ford, Iowa State University, Ames, IA 50011.
We are in the process of constructing a genetic map of Cochliobolus heterostrophus, causal agent of southern leaf blight of maize. The map is being generated using restriction fragment length polymorphisms (RFLPs) and conventional phenotypic markers. Probes for detecting RFLPs are being made from random fragments of Cochliobolus nuclear DNA and from cloned genes of known function from Cochliobolus and related organisms. Crosses and progeny have been chosen to permit the mapping of centromeres and characterization of a putative chromosome rearrangement tightly linked to the virulence locus Tox1. CHEF gel electrophoresis is being used to separate whole chromosomes; this should allow rapid detection of differences in chromosome organization between strains and confirmation of linkage relations.

Y 302 DEVELOPMENTAL CYTOLOGY OF USTILAGO MAYDIS IN MAIZE LEAVES. Allen D. Budde[1], William A. Russin[2], and Sally Leong[1]. USDA/NCR-ARS[1] and Department of Plant Pathology[2], University of Wisconsin-Madison, Madison, WI 53706

Aspects of disease interactions between Ustilago maydis and maize, including potential sites of penetration, hyphal progress through leaves, and host responses were examined. Haploid sporidia, diploid solopathogens, and compatible or incompatible combinations of haploid sporidia of the fungus were injected near the apical meristem of young maize plants. Leaves were sampled and prepared for light and electron microscopy. Haploid sporidia and incompatible combinations resulted in limited penetration, usually near the injection wound. Solopathogens or mixtures of compatible sporidia resulted in hyphal penetration through various sites. These hyphae progressed to the vascular bundles and then longitudinally within the lamina. Host responses to infection included aberrant arrangement of organelles in cells having intimate contact with hyphae and neoplastic growth initiation in bundle sheath cells.

Y 303 CHARACTERIZATION OF THE RED CLOVER NECROTIC MOSAIC VIRUS 35 KDA POLYPEPTIDE, L.A. Calvert, Z. Xiong, and S.A. Lommel, Dept. Plant Pathology, Kansas State University, Manhattan, KS 66506.
Red clover necrotic mosaic virus (RCNMV), a member of the dianthovirus group, is composed of two nonhomologous RNAs of 4.1 kb (RNA-1) and 1.4 kb (RNA-2) and a single 39 kDa capsid protein component. The genome organization of RCNMV was determined by in vitro transcription and translation analyses from full length cDNA clones. The capsid protein is encoded by RNA-1 and is expressed in vivo from a sub-genomic RNA. A unique 35 kDa protein is the only in vitro translation product directed by RNA-2. The position of the 35 kDa open reading frame was determined by sequence analysis and confirmed by translation of deletion transcripts derived from RNA-2 cDNA clones. RNA-1 is able to replicate in initially inoculated cells of host plants but is unable to move systemically. Consequently, the 35 kDa polypeptide encoded by RNA-2 is presumed to be the cell-to-cell movement protein. To further characterize the function of the 35 kDa polypeptide, a fusion protein consisting of the staphylococcal protein A IgG binding region and 33 kDa of the carboxy terminus of the 35 kDa polypeptide was constructed. A polyclonal antisera to the 60 kDa fusion protein is being used as a probe in immunocytological studies to determine the intracellular location of the RNA-2 gene product.

Y 304 **REGULATION OF EXPRESSION OF THE YEAST ENOLASE GENE *ENO1*,** Andrew A. Carmen, Alan E. Pepper and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California; Davis, CA 95616

There are two enolase genes (*ENO1* and *ENO2*) per haploid genome of the yeast *Saccharomyces cerevisiae*. *ENO2* expression is induced 20-fold in cells grown in the presence of glucose versus gluconeogenic carbon sources while *ENO1* is constitutively expressed in these carbon sources. Both genes contain a complex enhancer-like region (upstream activation sites, UAS) located approximately 500bp upstream from their respective initiation codons. These UAS regions are required for expression of each gene and contain sequences which mediate glucose dependent induction. The *ENO1* gene also contains an upstream repression site (URS) located approximately 210bp upstream from the initiation codon. This URS region prevents glucose dependent induction of *ENO1* and is essential for the constitutive expression of the *ENO1* gene. Sequences required for URS function have been localized to a 20-30bp region by deletion mapping analysis. We do not observe URS-dependent repression of transcription when the UAS region of *ENO1* is replaced with the UAS region from *ENO2* or the UAS region from the glyceraldehyde-3-phosphate dehydrogenase gene *TDH3*. Similarily, we do not observe repression of transcription when the URS sequences from *ENO1* are inserted between the UAS region and TATAAA box of *ENO2*. We have shown by deletion analysis that the UAS regions of the *ENO2* and *TDH3* genes are functionally redundant (contain multiple UASs). When portions of the UAS regions from *ENO2* or *TDH3* are deleted, URS-dependent repression of transcription is observed. The ability of an *ENO1* URS element to repress transcription is, therefore, highly dependent on the UAS region. These observations suggest that the URS represses transcription by interfering with UAS activity.

Y 305 TRANSFORMATION OF *NECTRIA HAEMATOCOCCA* WITH A GENE FOR PISATIN DEMETHYLATING ACTIVITY, AND THE ROLE OF PISATIN DETOXIFICATION IN VIRULENCE , L.M. Ciuffetti, K.-M. Weltring, B.G. Turgeon, O.C. Yoder and H.D. VanEtten, Cornell University, Ithaca, NY 14853.

The filamentous Ascomycete *Nectria haematococca* (anamorph: *Fusarium solani*) possesses a specific, inducible enzyme (pisatin demethylase) that detoxifies the phytoalexin pisatin, produced by one of its hosts, *Pisum sativum* (pea). Previous genetical studies have supported the hypothesis that pisatin demethylating ability (pda) is necessary for pathogenicity, by showing that isolates lacking pda are non-pathogenic while all highly virulent isolates have high levels of pda.

A *PDA* gene from a Pda$^+$ isolate of *N. haematococca* has been cloned by expression in *Aspergillus nidulans*. We have transformed a Pda$^-$, non-pathogenic isolate of *N. haematococca* with a vector containing this isolated *PDA* gene and the selectable gene *hyg*B, which confers resistance to the antibiotic hygromycin B. Southern analysis of the three hygromycin B resistant transformants indicated that the vector had integrated into the genome of the fungus. The three transformants were able to demethylate pisatin and were tolerant to the phytoalexin. Pea epicotyls were inoculated with mycelium from each of the Pda$^+$ transformants and the Pda$^-$ recipient. Two of the three Pda$^+$ transformants were pathogenic on pea. The ability of the Pda$^+$ transformants to cause disease is direct evidence that pda is a necessary factor for the pathogenicity of this fungus on pea.

Y 306 USTILAGO MAYDIS : ATTEMPTS TOWARDS A MOLECULAR UNDERSTANDING OF PATHOGENICITY; Marlis Dahl, Burkhard Schulz and Regine Kahmann, Institut für Genbiologische Forschung Berlin GmbH, D-1000 Berlin 33 (West).

Corn smut is caused by the heterothallic fungus Ustilago maydis, a parasitic Basidiomycete. We are interested in a molecular analysis of genes involved in pathogenicity. As a step towards this goal we have characterized the complexity of the U. maydis genome by pulsed-field gel-electrophoresis. The haploid genome consists of at least 20 DNA molecules ranging in size from 250 to more than 2000 kb. Chromosomes show extensive size heterogeneity in different field isolates. Several U. maydis genes have been identified by heterologous DNA probes and were mapped to specific chromosomes. In attempts to develop an effective transformation protocol we describe the construction of plasmid vectors carrying selectable markers and transformation results using the spheroplast technique.

Y 307 METABOLISM OF THE POTATO PHYTOALEXIN LUBIMIN BY GIBBERELLA PULICARIS, Anne E. Desjardins, Harold W. Gardner and Ronald D. Plattner, USDA/ARS, Northern Regional Research Center, Peoria, IL 61604.

The potato phytoalexin lubimin displayed a complex pattern of metabolism by strains of the potato pathogen Gibberella pulicaris (anamorph: Fusarium sambucinum). The metabolites 15-dihydrolubimin and isolubimin were common to a lubimin-sensitive strain and to two lubimin-tolerant strains. In the lubimin-tolerant strains, 15-dihydrolubimin and isolubimin disappeared within 2 days. In one lubimin-tolerant strain, several unique metabolites, including the tricyclic compounds cycloketolubimin and cyclolubimin and their epoxides, accumulated at the expense of 15-dihydrolubimin and isolubimin. The tricyclic compounds were not toxic to the lubimin-sensitive strain. To further investigate the metabolic pathway, several putative intermediates were purified and incubated with cultures of the two lubimin-tolerant strains. The results of these studies suggested that a likely pathway for lubimin detoxification in G. pulicaris involves cyclization of isolubimin to cycloketolubimin via an unsaturated intermediate. Further evidence to support this hypothesis was obtained from a study of lubimin metabolism in the presence of deuterium-labeled water. All of the highly virulent strains tested were tolerant of lubimin and were able to convert lubimin to apparently nontoxic products. These preliminary results suggest that lubimin detoxification contributes to virulence of G. pulicaris on potato tubers.

Y 308 H PROTEIN OF TOBACCO MOSAIC VIRUS IS UBIQUITINATED COAT PROTEIN, David D. Dunigan, Donna Hazelwood and Milton Zaitlin, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The H protein of tobacco mosaic virus (TMV) is 1) a structural protein, 2) 26,000 molecular weight, and 3) isolated from virus particles at an average frequency of one per virion. It consists of TMV coat protein (MW = 17,500) and a covalently linked host-derived moiety, Mr = 8,500. From peptide fragmentation analysis of the coat protein moiety of H protein, the N- and C-termini are intact, and thus the structure is branched, suggesting a possible linkage through an isopeptide bond. The coat protein of the common strain (U1 strain) is acetylated at the N-terminus; with this knowledge, the N-terminal 17 amino acids of the host-derived moiety were microsequenced by Edman degradation analysis. The sequence was a perfect match to the 17 N-terminal amino acids of ubiquitin. When antibodies to human ubiquitin were used in immunoblots of H protein concentrates from various TMV strains, a strong signal was observed at monovalent conjugated coat protein, as well as minor signals at polyvalent conjugated coat protein. Structural implications will be discussed. Type members from various plant virus groups are being analyzed for the presence of ubiquitinated structural proteins to determine if this phenomenon occurs in other plant\virus systems.

Y 309 EMBRYOGENIC MAIZE CALLUS LINES EXPRESSING HC-TOXIN RESISTANCE OR SUSCEPTIBILITY: A MODEL SYSTEM FOR STUDY OF THE HM1 LOCUS Jonathan Duvick, Joyce Maddox (Pioneer Hi-Bred, International, Inc. Johnston, IA 50131); Steve Briggs, Beth Elliot (Cold Spring Harbor Laboratories, Cold Spring Harbor, New York 11724).

Maize plants that are homozygous recessive at the hm1 locus on chromosome 1L are highly susceptible to Race 1 of Cochliobolus carbonum and to a host-specific toxin (HC-toxin) produced by this pathogen, whereas Hm1/- confers resistance to both toxin and pathogen. Since available hm1\hm1 germplasm did not give stable callus cultures for in vitro studies, we crossed hm1/hm1 lines with an inbred (Pioneer (R) T66) which confers a high tissue culture response on progeny. F1 plants were selfed, and a total of 2069 immature F2 embryos were cultured. Out of 42 stable, type II (friable, embryogenic) callus lines that were obtained, 9 showed a rapid necrotic response at toxin levels of 0.44 ug/ml or greater (scored as susceptible or S), while 33 lines showed little or no response at 4.4 ug/ml toxin (resistant or R). A total of 35 plants were regenerated from 5 R and 4 S lines, and all showed a leaf response to the pathogen that correlated with the callus response to the toxin. We tentatively conclude that Hm1 is expressed in tissue culture. We are using these lines to investigate the early effects of HC-toxin on cellular metabolism and gene expression in susceptible or resistant maize.

Y 310 cDNA CLONING, SEQUENCING, AND EXPRESSION OF THE SOYBEAN MOSAIC VIRUS COAT PROTEIN CODING SEQUENCE, Alan L. Eggenberger, David M. Stark, and Roger N. Beachy, Biol Dept. Washington University, St. Louis, MO 63130

DNAs complementary to the RNA of soybean mosaic virus (SMV), a member of the potyvirus group, were made and cloned into the expression vector pEMBL 18+. One of these, designated pSM 2-21, made a fusion protein in E. coli that reacted with antibodies against SMV coat protein. This cDNA was sequenced and was found to include the coat protein coding sequence. The N-terminus of the SMV coat protein was blocked but sequencing of a 28 kD trypsin resistant core gave internal sequence which confirmed the reading frame. The coat protein coding sequence was modified using oligo-directed mutagenesis for introduction into transgenic plants for genetically engineered resistance studies. This transformation is currently underway.

Y 311 THE DNA OF BREMIA LACTUCAE , David M. Francis and Richard W. Michelmore University of California, Davis, CA 95616.

Bremia lactucae is a biotrophic fungal pathogen causing downy mildew of lettuce. Extensive genetic studies on both the host and pathogen have shown the gene - for - gene hypothesis to be a remarkably accurate model for predicting the outcome of the interaction between B. lactucae and lettuce. We are now investigating the molecular basis of disease specificity and pathogenic variation in the downy mildew-lettuce system. Our present strategy for cloning the genes conditioning avirulence involves a chromosome walk from RFLP markers which flank avirulence loci. An understanding of genome structure and organization is a prerequisite for this approach. The chemical composition of nuclear DNA is 49% G+C as determined by buoyant density centrifugation and by thermal denaturation. Genome size has been estimated to be 5×10^7 bp using single copy clones to probe dot blots of genomic reconstructions. This technique was confirmed using Arabidopsis thaliana and Aspergillus nidulans. Randomly selected lambda clones probed with genomic DNA were used to investigate genome organization. Repetitive sequences appear to be interspersed with single copy sequences throughout much of the genome. Overlapping clones will have to be carefully screened during chromosome walking as a result of the interspersed repetitive DNA. Pulse field gel electrophoresis is being used to develop an electrophoretic karyotype which will facilitate the selection of clones by providing a rapid method of determining their chromosomal location.

Y 312 A MOLECULAR ANALYSIS OF MATING TYPE IN HETEROTHALLIC AND HOMOTHALLIC SPECIES OF NEUROSPORA. N.L. Glass, J. Grotelueschen and R.L. Metzenberg, Physiological Chemistry, University of Wisconsin, Madison, WI 53705. C. Staben and C. Yanofsky, Biological Sciences, Stanford University, Stanford, CA 94305.

The fungal genus Neurospora comprises species that are self-sterile and exist in two mating types (heterothallic) and species that are self-fertile (homothallic). The mating type loci control a complex series of developmental events that culminate in the production of ascospores. The A and a mating type alleles from N. crassa have been cloned. The A mating type region comprises a 4.4kbp region of the N. crassa A genome that is not homologous to any sequences in the a mating type genome. DNA digests from six homothallic species were probed with the N. crassa A and a mating type clones. Four species hybridized to the A probe only, one hybridized to the a probe only, and one hybridized to both of the A and a probes. Thus, for five of the six homothallic species of Neurospora, this result eliminates models in which homothallism is explained by the presence of genetic information for both mating types, with facile switching of expression of the mating type genes. The DNA sequences from the homothallic species, N. africana, that hybridized to the N. crassa A mating type clone were isolated. Transformation of the N. africana clone into a sterile N. crassa mutant partially restores fertility. Restriction mapping of the N. africana clone and comparison to the N. crassa clone reveals multiple restriction site differences. By the physical and functional characterization of the N. crassa and N. africana mating type clones, we hope to develop a model explaining heterothallism and homothallism in filamentous fungi.

Y 313 MUTATIONAL ANALYSIS OF TOBACCO RATTLE VIRUS RNA-1.

P.J. Guilford, V. Ziegler-Graff, W.D.O. Hamilton and D.C. Baulcombe, Institute of Plant Science Research (Cambridge Laboratory), Cambridge CB2 2LQ, England.

Tobacco rattle virus (TRV), the type member of the tobravirus group, is a single stranded, bipartite RNA virus. TRV RNA-1 (SYM strain) has been cloned and sequenced. Transcripts derived from the constructed full-length cDNA clone are infectious.

RNA-1 encodes proteins of 134K, 194K, 29K and 16K and is able to infect and spread systemically through tobacco plants even in the absence of RNA-2. Mutations have been introduced at the DNA level into the genes encoding the 29K and 16K proteins and RNA transcripts have been inoculated onto tobacco plants. In each instance, the effect of the mutation was to destroy the infectivity of the RNA. The infectivity could not be recovered either by inoculation of the RNA onto transgenic plants expressing the 16K or 29K genes or by co-inoculation of the mutant transcripts with the transcript of the full-length genome. These results suggest that there are several internal sequences which act in *cis* to support the replication, translation, stability or intercellular transport of TRV RNA-1. This hypothesis is being investigated by the creation of further mutations in the cDNA and infectivity studies with the mutant transcripts on plants and protoplasts.

Y 314 ANALYSIS OF THE MECHANISM OF PROTECTION IN TRANSGENIC PLANTS EXPRESSING THE PVX COAT PROTEIN GENE, Cynthia Hemenway, Rong-Xiang Fang*, Wojciech K. Kaniewski, Nam-Hai Chua*, and Nilgun E. Tumer, Monsanto Company, St. Louis, MO , *The Rockefeller University, New York, NY

Transgenic plants expressing either the PVX CP gene (CP+) or the corresponding antisense transcript (CP-antisense) were challenged with PVX and PVX RNA. The best-expressing CP+ plants were protected over a 3 log range in virus inoculum concentration, as evidenced by reduced lesion numbers on inoculated leaves, delay or absence of systemic symptoms, and reduction in virus accumulation in inoculated and systemic leaves. These characteristics also were observed in protected CP-antisense plants and CP+ plants expressing lower levels of CP, but only at the lowest virus inoculum concentration. When CP+ plants were inoculated with comparable viral RNA concentrations, lesions on inoculated leaves were absent or reduced in number. In addition, virus levels in inoculated and systemic leaves were reduced to similar extents in both virus and RNA inoculated plants. As reported previously, protection observed in transgenic plants expressing the TMV or AlMV CP was overcome by inoculation with the corresponding viral RNA, indicating that presence of CP on the challenge virus was necessary for optimum protection. Our data indicate that CP-mediated protection is applicable to PVX in response to inoculation with both virus and viral RNA.

Y 315 TRANSFORMATION OF *GAEUMANNOMYCES GRAMINIS*, Joan M. Henson, Nancy K. Blake and Alice Pilgeram, Montana State University, Bozeman, MT 59717

Gaeumannomyces graminis is a soilborne filamentous Ascomycete that parasitizes many members of the Gramineae plant family. Although *G. graminis* var. *graminis* colonizes wheat roots, it does not cause root damage as does *G. graminis* var. *tritici*, which is the etiologic agent of take-all disease of wheat and barley. We transformed *Gaeumannomyces graminis* with pBT3, a vector with a benomyl resistance gene (*tub-2*) from *Neurospora crassa* (1). Using the transformation procedure of Wang et al. (2), the frequency of transformation was ~10 transformants/ug linearized pBT3, and a similar frequency of transformation was observed with circular plasmid DNA. Most transformants (8/12) apparently had a single copy of pBT3 integrated at a site distinct from the resident *tub-2* gene. We have also been characterizing the linear, dsDNA mitochondrial plasmids of *G. graminis*. They ranged in number from 0-3 and were 2-11 kb in size. We cloned one 4 kb fragment from one of the plasmids which displayed homology with almost all of the other linear plasmids in different strains. Hybridization experiments indicated that at least 2 of the plasmids displayed slight homology with the mitochondrial genome, but this may have been due to a high A-T content of both the mitochondrial genome and the linear plasmids.

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2. Wang, J., Holden, D.W., and S.A. Leong. 1987. Proc Nat Aca Sci USA, in press.

Y 316 BIOLOGICAL ACTIVITY OF IN VITRO TRANSCRIPTS FROM cDNA CLONES OF BARLEY STRIPE MOSAIC VIRUS, I. T. D. Petty, B. G. Hunter, and A. O. Jackson, University of California, Berkeley, CA 94720.

Barley stripe mosaic virus (BSMV) is the type member of the hordeivirus group and has a tripartite plus-sense RNA genome. The genomic RNA components are designated α (ca. 3.8kb), β (ca. 3.3kb), and γ (size variable). The size and number of γ-specific RNA species varies among strains of BSMV. We have developed a novel cDNA cloning strategy which has allowed us to isolate a number of independent full-length clones of the three genomic components from both the Type and ND18 strains of BSMV. The cloning strategy relies on a specialized plasmid vector which allows full-length viral cDNA to be directionally inserted behind a modified T7 promoter. After linearization of the recombinant plasmids, run-off in vitro transcripts can be generated which have the same 5'-terminal sequence as native virion RNAs and up to 4 additional nucleotides at the 3'-terminus. Data demonstrating biological activity of in vitro transcripts derived from cDNA clones of BSMV will be presented.

Y 317 STRUCTURE AND EXPRESSION OF A *B. LACTUCAE* HSP70 GENE AND CONSTRUCTION OF VECTORS FOR FUNGAL TRANSFORMATION, Howard S. Judelson and Richard W. Michelmore, Univ. of California, Davis, CA 95616

Our goal is to develop methods for the DNA-mediated transformation of *B. lactucae*. This technology will be used to clone avirulence genes from the fungus which determine specificity in the lettuce-*B. lactucae* interaction (lettuce downy mildew). We have constructed vectors for transformation which contain selectable drug resistance genes fused to promoter and polyadenylation sequences from a *B. lactucae* hsp70 gene. This gene was cloned from a genomic library using a hsp70 gene from *Ustilago maydis* (provided by Dr. S. Leong, Univ. Wi.). Southern blot analysis indicates that few other hsp70 genes or alleles are present in *B. lactucae*. The cloned gene contains canonical hsp70 regulatory elements and polyadenylation signals but no discernable upstream "TATA" or "CAT" sequences. The major 5' and 3' ends of the transcript were located by primer extension and S1 protection analysis to 60 bases upstream and 152 bases downstream of the coding sequence, respectively. These experiments and Northern blot analysis indicate that transcription occurs during non-heat shocked growth and germination conditions (15C) and is induced 10-fold by germination at 4C, 28C, and 35C, and during sporulation. A series of plasmid vectors have been constructed that contain hygromycin B or G418 phosphotransferase genes, respectively, inserted between upstream hsp70 sequences (for transcriptional or translational fusions) and hsp70 polyadenylation sites. Methods for the insertion of these vectors into *B. lactucae* and the selection of transformants are being developed.

Y 318 STABILITY OF FOREIGN DNA IN THE COCHLIOBOLUS HETEROSTROPHUS GENOME. N. P. Keller, G. C. Bergstrom and O. C. Yoder. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The Escherichia coli hygromycin B phosphotransferase gene fused to a fungal promoter was integrated into the genome of the southern corn leaf blight fungus, C. heterostrophus. Transformed colonies were selected on hygromycin amended medium. The foreign gene was stable in the genome when the fungus was sequentially subcultured five times times on a complete growth medium. However, when the fungus was passaged for five generations on corn, a high frequency of hygromycin-sensitive conidia were observed. Southern blotting of these revertants indicated that the E. coli gene was lost from the fungal genome. These findings suggest that stability of foreign DNA in fungi may be a stress-related phenomenon subject to environmental pressures affecting fungal replication. Investigations are underway to determine the effect of insert size, position and copy number on stability of foreign DNA .

Y 319 ISOLATION OF METABOLIC AND PATHOGENICITY GENES FROM USTILAGO MAYDIS. J. Kronstad[1] J. Wang[2] S. Covert[2], and S. Leong[1], USDA-ARS, Plant Disease Resistance Unit[1] and Department of Plant Pathology[2], University of Wisconsin-Madison, Madison, WI 53706.

Ustilago maydis cDNA clones have been isolated which complement mutations in the URA3, LEU2, and TPI genes of yeast. The cDNA which complements mutations in the yeast URA3 gene has been sequenced and used to isolate the genomic pyr3 gene of U. maydis. The genomic pyr3 gene has been employed as a marker for transformation and disrupted with a hygromycin B marker to demonstrate gene replacement in U. maydis.

A cosmid library containing DNA from a haploid strain with the mating factors a1 b1 was transformed into an a1/a2 b2/b2 diploid strain. Cells of this diploid have a yeast-like morphology and are non-pathogenic on Zea mays. A 39 Kb cosmid has been identified which transforms the a1/a2 b2/b2 diploid to a pathogenic mycelial phenotype. The cosmid does not alter the phenotype of an a1/a2 b1/b1 diploid and may carry a b1 allele.

Y 320 GENERATION OF SEQUENCE HETEROGENEITY IN CLONED CUCUMBER MOSAIC VIRUS SATELLITE RNAs DURING PASSAGE IN PLANTS, Gael Kurath and Peter Palukaitis, Cornell University, Ithaca, New York 14853.

The presence of satellite RNAs in cucumber mosaic virus (CMV) infections results in a decrease in virus yield and various modifications of the CMV-induced disease symptoms. Populations of CMV satellite RNAs exhibit sequence microheterogeneity, a phenomenon common to most RNA virus populations. In the past, study of the generation of RNA sequence heterogeneity has been complicated by the difficulty in obtaining inocula of uniform sequence. To overcome this problem, we have used infectious transcripts of a CMV satellite RNA clone as an inoculum comprised of a single RNA sequence. This inoculum was serially passaged in five host plant species, and the satellite RNA from each passage was assessed for heterogeneity by using an RNA protection assay with the minus-sense transcript of the satellite RNA clone as a probe. After annealing the probe to a test RNA, differences in sequence were detected by RNase digestion. Our results indicate that heterogeneity is generated in a rapid, non-random manner, with most of the detectable mutations occurring in a hypervariable region between bases 224-227 of the 335-base satellite RNA sequence. Heterogeneity in this region is visible within three passages in some plant species, and accumulates with further passage. In natural satellite RNA populations it reaches a level of 50-65% of the RNA. This rapid generation and maintainance of heterogeneity in satellite RNA populations provides insight into both the evolution and survival mechanisms of satellite RNA populations.

Y 321 THE USE OF AN INFECTIOUS cDNA CLONE OF COWPEA MOSAIC VIRUS MIDDLE-COMPONENT RNA TO INVESTIGATE GENE FUNCTIONS, G. P. Lomonossoff, C. Holness, A. Maule and *D. Evans, Dept. of Virus Research, John Innes Institute, Norwich, U.K. and *Dept. of Biological Sciences, University of Warwick, Coventry, U.K.

A full-length cDNA copy of cowpea mosaic virus (CPMV) middle-component RNA has been inserted next to the E. coli RNA polymerase promoter in the transcription vector, pPM1. In vitro transcription of the linearised plasmid results in production of full-length, virion-sense RNA. When co-inoculated into cowpea protoplasts together with bottom component RNA, these transcripts are capable of being replicated resulting in a full virus infection in up to 30% of the inoculated protoplasts. Using this clone, we have constructed a series of deletion, insertion and frameshift mutants and have tested the ability of the transcripts to replicate in cowpea protoplasts. In this way we hope to elucidate those factors on the RNA which enable it to be replicated and determine which gene product(s) are important for virus movement within a plant.

Y 322 MESSENGER RNA AND DNA SEQUENCE ANALYSIS OF A VIRULENCE GENE FROM A FUNGAL PATHOGEN. Alan P. Maloney and Hans D. VanEtten, Cornell University, Ithaca, NY 14853. Pisatin demethylase of the pathogenic fungus *Nectria haematococca* is an inducible enzyme that detoxifies pisatin, a phytoalexin synthesized by peas in response to fungal infection. The enzyme is a cytochrome P-450 monooxygenase. There is a family of pisatin demethylase genes which encode enzymes with different activity levels. We are conducting molecular analysis of a highly inducible pisatin demethylase gene, which we have cloned from the fungus, to help us understand the specific role it plays in pathogenicity. The gene encodes an approximately 1800 nt mRNA. RNAse protection assays demonstrate that the mRNA is heterogeneous at its 5' end and contains a small intron approximately 200 nt downstream of the 5' end. Sequence analysis has located the open reading frame, exon-intron boundaries, and sequences in the gene which are homologous to other cytochrome P-450's. Transcriptional studies indicate a biphasic induction of transcription after pisatin stimulation: a low level of transcript appears within one hour after exposure to the phytoalexin, followed 6 to 9 hours later by a surge of mRNA synthesis which corresponds to the detection of high levels of enzyme activity.

Y 323 mRNA INDUCTION IN CEREALS BY FUNGAL PATHOGENS, J.M. Manners*, A.D. Davidson, W. Jutidamrongphan, G.M. Mackinnon and K.J. Scott, Department of Biochemistry and *CSIRO-UQ Plant Pathology Unit, Department of Botany, University of Queensland, St. Lucia 4067, Australia.

Six cDNA clones have been obtained for infection-related (IR) mRNAs of barley that are induced during early stages of infection by *Erysiphe graminis* f.sp. *hordei*. Two of the IRmRNAs are induced in greater amounts and at earlier stages of infection of resistant cultivars of barley carrying either the Mla, Mlp or Mlk genes when compared to near-isogenic susceptible lines during infection by the powdery mildew fungus. This suggests a role for these IRmRNAs in monogenic resistance to powdery mildew in barley. A comparison of mRNA induction in barley during infection by a number of compatible and incompatible fungal pathogens has also suggested that these two IRmRNA may have a role in non-host resistance. The IRmRNAs are induced by necrotrophic fungi e.g. *Pyrenophora teres* but we have not been able to induce the IRmRNAs by infection with viruses and bacteria. The IRmRNAs are readily induced by certain types of wounding and fungal elicitors. The IRmRNAs have homologous counterparts in wheat, rice and sorghum which are also induced by pathogenic fungi. Because of the possible importance of the IRmRNAs in the resistance of cereals to fungal pathogens current research is aimed at determining their primary sequence which may enable the assignment of a function for the proteins they encode.

Y 324 FUNGAL PATHOGENS OF THE COCOA TREE, Theobroma cacao, P. J. Fritz, J. A. Couch, L. McHenry, and T. Snyder, The Pennsylvania State University, University Park, PA16802. T. Cacao is the tropical tree from which chocolate and cocoa products are derived. Cacao seeds are a major export of some 40 developing nations, accounting for nearly $2 billion annual cash income to these countries. Crop losses from disease consistently prevent the cash in-flow from being at least 25% higher and in serious seasonal outbreaks may entirely destroy a crop. Bacterial diseases are unknown in cacao and viral diseases are mostly limited to West Africa, but fungal diseases are a world-wide problem, the most serious caused by species of Phytophthora, Crinipellis, Monilia, Ceratocystis, and Oncobasidia. Traditionally, control of cacao fungal diseases has been through agronomic practices and anti-fungal sprays, but recently our laboratory has begun to investigate the problem at the molecular level with the goal of producing disease-resistant cocoa trees through genetic changes. Currently we are studying Phytophthora and Crinipellis. The approaches we are using and preliminary results will be presented.

The Penn State greenhouse plantation contains 27 five-year old trees of Ecudorian and Costa Rican origin, 25 of which are flowering and 10 of which have born fruit after hand pollination. Screening of these trees by detached leaf assay has shown a variable response to Phytophthora infection, some trees appearing to be resistant, as judged by limited lesion size after fungal inoculation. Reasons for resistance are being explored and it has been established that resistance is not related to leaf polyphenols, either constitutive or fungal induced.

Y 325 ELUCIDATION OF THE RESPONSIBLE SUBSTITUTIONS IN THE TMV GENOME FOR OVERCOMING THE RESISTANCE OF THE Tm-1 GENE, T. Meshi, F. Motoyoshi, Y. Watanabe, N. Takamatsu and Y. Okada, Dept. Biophys. & Biochem., Fac. Sci., Univ. Tokyo, Tokyo 113, JAPAN.

The Tm-1 gene of tomatoes confers the resistance to tobacco mosaic virus (TMV) infection. The resistance of the Tm-1 gene is expressed in single cells as well as in whole plants. We spontaneously isolated a resistance-breaking strain (TMV Ltal) from a tomato strain, TMV L, and compared their genomic sequences in order to know the viral factor involved in the virus-host interaction. Two base substitutions causing amino acid changes were found in the replicase gene. To examine the importance of these substitutions, we constructed a strain (T1) using the in vitro transcription system, whose genomic sequence had the same as TMV L except the two base substitutions. Results showed that T1 multiplied in tomatoes with the Tm-1 gene causing typical mosaic symptoms as TMV Ltal. Further, two strains were prepared, each of which had one substitution out of the two. One strain (T3) multiplied in the Tm-1/Tm-1 tomatoes with symptoms as Ltal. The other strain (T2) also multiplied but the multiplication was greatly decreased. Progeny viruses isolated from T2- and T3-inoculated Tm-1/Tm-1 tomatoes were in most cases a mixture of viruses having detectably the same sequences as the inoculated viruses and those with the second base substitutions causing amino acid changes. Sequencing studies of other independently isolated resistance-breaking strains showed the same two substitutions. These observations imply that the two substitutions, possibly amino acid changes of the replicase, are important for breaking the resistance. The mode of interaction between the Tm-1 gene product and TMV could be considered.

Y 326 Meiotic loss of a gene for pisatin demethylase from *Nectria haematococca* MP VI. V. Miao, and H.D. VanEtten, Cornell University, Ithaca, NY 14853.

Phytoalexins are induced compounds of host origin which have antimicrobial activity. The phytoalexin pisatin is produced by garden pea (*Pisum sativum*) in response to challenge by the pea pathogen *Nectria haematococca* MP VI. The ability of *Nectria* strains to detoxify pisatin is encoded by a family of *PDA* genes; a positive allele at any of these loci confers a pisatin detoxifying phenotype (Pda+). Conventional genetic crosses between strains with single Pda+ alleles and non-detoxifying (Pda-) strains sometimes give a higher than expected proportion of Pda- progeny. Tetrad analysis suggested the loss of the Pda+ allele, and a subsequent test cross supported this hypothesis. Loss of the Pda+ phenotype is correlated with loss of a 9-10kb fragment of genomic DNA which cross-hybridizes with a cloned *PDA* gene.

Y 327 INTEGRATION OF TRANSFORMING DNA BY HOMOLOGOUS RECOMBINATION ALLOWS GENE REPLACEMENT IN COCHLIOBOLUS HETEROSTROPHUS, P.G. Mullin, B.G. Turgeon, R.C. Garber and O.C. Yoder, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

C. heterostrophus can be transformed to hygromycin B resistance at an efficiency useful for gene cloning experiments. Transforming DNA integrates stably into the fungal chromosome. When the fungus is transformed with a plasmid containing a cloned trifunctional tryptophan-synthesis gene (TRP1) from *C. heterostrophus*, the plasmid frequently integrates at the chromsomal TRP1 locus by homologous recombination. Several different types of integration event occur, including double-crossover homologous recombination between chromosomal DNA and the transforming plasmid. These events occur with high enough frequency to make gene replacement an easily used tool for the study of *C. heterostrophus* genes. The biological function of any cloned single-copy gene from this fungus can be definitively assessed by replacing the wild-type allele with a copy that has been specifically altered *in vitro*.

Y 328 WHEAT STREAK MOSAIC VIRUS - CEREAL INTERACTION
Annette Nassuth and Annalisa Bottacin. Botany Department, University of Guelph, Guelph, N1G 2W1 Canada.

Wheat Streak Mosaic Virus (WSMV) is naturally disseminated by the wheat curl mite in Canada, USA and Europe. Like other mite-transmitted viruses it is also sap-transmissible and infects only members of the family Gramineae, causing mosaic symptoms and stunting. WSMV has filamentous particles about 700 nm long consisting of single-stranded RNA encapsidated by a coat protein with an apparent molecular weight (MW) of 45 kD. It is assumed that the genome of WSMV consists of only one RNA species of plus-strand polarity with a MW of 2.8 x 10^6. A 66 kD virus-encoded protein seems to form the major component of virus-induced pinwheel inclusions and laminated aggregates. For potyviruses, similar inclusion bodies have been suggested to adapt the cytoskeleton for virus synthesis and/or translocation.

Our study on WSMV has two main objectives:
(1) The determination of the genome composition and expression strategy of WSMV.
(2) The identification of host products that are induced upon WSMV infection and play a role in WSMV multiplication or the defence reaction of the plant.
With these goals in mind, we are preparing a cDNA library from WSMV RNA and from mRNA from WSMV-infected wheat plants. The results of this work will be presented.

Y 329 MOLECULAR COMPARISON OF STRAINS OF CUCUMBER MOSAIC VIRUS USING AN RNA PROTECTION ASSAY, Judith L. Owen and Peter Palukaitis, Dept. of Plant Pathology, Cornell University, Ithaca, N.Y. 14853.

Cucumber Mosaic Virus is a cucumovirus with a tripartite, positive-sense RNA genome of which RNAs 1 and 2 are involved in replication. RNA 3 encodes two proteins: a 34K protein thought to be involved in cell-to-cell movement of the virus, and the virus coat protein. cDNA was prepared to the Fny strain of CMV RNA and transferred to transcription vectors between bacterial promoters T_3 and T_7. Labeled, anti-sense RNA transcripts prepared from all or part of the cDNA of RNA 3 of Fny-CMV were annealed to the RNA of a number of strains of CMV and digested with RNAses. The sizes of RNA fragments which were protected from digestion revealed the extent and location of heterogeneity among the CMV strains. This allows differences in host range and disease processes to be correlated with variations in the genomic RNA. Moreover, digestion patterns are diagnostic for each CMV.

Y 330 BIOLOGICAL ACTIVITY OF *IN VITRO* TRANSCRIPTS FROM cDNA CLONES OF BARLEY STRIPE MOSAIC VIRUS, I. T. D. Petty, B. G. Hunter, and A. O. Jackson, University of California, Berkeley, CA 94720.

Barley stripe mosaic virus (BSMV) is the type member of the hordeivirus group and has a tripartite plus-sense RNA genome. The genomic RNA components are designated α (ca. 3.8kb), β (ca. 3.3kb), and γ (size variable). The size and number of γ-specific RNA species varies among strains of BSMV. We have developed a novel cDNA cloning strategy which has allowed us to isolate a number of independent full-length clones of the three genomic components from both the Type and ND18 strains of BSMV. The cloning strategy relies on a specialized plasmid vector which allows full-length viral cDNA to be directionally inserted behind a modified T7 promoter. After linearization of the recombinant plasmids, run-off *in vitro* transcripts can be generated which have the same 5'-terminal sequence as native virion RNAs and up to 4 additional nucleotides at the 3'-terminus. Data demonstrating biological activity of *in vitro* transcripts derived from cDNA clones of BSMV will be presented.

Y 331 TRANSFORMATION OF GAEUMANNOMYCES GRAMINIS, Joan M. Henson, Nancy K. Blake and Alice Pilgeram, Montana State University, Bozeman, MT 59717

Gaeumannomyces graminis is a soilborne filamentous Ascomycete that parasitizes many members of the Gramineae plant family. Although G. graminis var. graminis colonizes wheat roots, it does not cause root damage as does G. graminis var. tritici, which is the etiologic agent of take-all disease of wheat and barley. We transformed Gaeumannomyces graminis with pBT3, a vector with a benomyl resistance gene (tub-2) from Neurospora crassa (1). Using the transformation procedure of Wang et al. (2), the frequency of transformation was ~10 transformants/ug linearized pBT3, and a similar frequency of transformation was observed with circular plasmid DNA. Most transformants (8/12) apparently had a single copy of pBT3 integrated at a site distinct from the resident tub-2 gene. We have also been characterizing the linear, dsDNA mitochondrial plasmids of G. graminis. They ranged in number from 0-3 and were 2-11 kb in size. We cloned one 4 kb fragment from one of the plasmids which displayed homology with almost all of the other linear plasmids in different strains. Hybridization experiments indicated that at least 2 of the plasmids displayed slight homology with the mitochondrial genome, but this may have been due to a high A-T content of both the mitochondrial genome and the linear plasmids.

1. Orbach, M.J., Porro, E.B., and C. Yanofsky. 1986. Mol Cell Biol 6:2452-2461.
2. Wang, J., Holden, D.W., and S.A. Leong. 1987. Proc Nat Aca Sci USA, in press.

Y 332 LINEAR, MITOCHONDRIAL PLASMID (pFOXC2) SEQUENCES UNIQUE TO FUSARIUM OXYSPORUM F.SP. RAPHANI TRANSFERRED TO F. OXYSPORUM F.SP. CONGLUTINANS AND F. OXYSPORUM F.SP. LYCOPERSICI, William A. Powell, Ulla Benny, H. Corby Kistler, University of Florida, Gainesville, FL 32611, USA.

Linear mitochondrial plasmids, pFOXC1 and pFOXC2, are characteristic to strains of Fusarium oxysporum f.sp. conglutinans and F. oxysporum f.sp. raphani respectively. A 1.8kB fragment of the 1.9kB pFOXC2 has been cloned into pUC12 with a hygromycin B resistance (Hyg^R) gene. This chimeric plasmid (pFT1) was used to transform F. oxysporum f.sp. conglutinans and F. oxysporum f.sp. lycopersici to determine if the pFOXC2 sequence would be transcribed and also to determine if the pFOXC2 sequence could allow the plasmid to replicate autonously. Transformation occurred by integration of pFT1 into the nuclear genome. Transformants with intact pFT1 produced no detectable RNA with sequence similarity to pFOXC2. Two transformants displayed a rearrangement of the pFT1 sequence in which part was integrated into the genome while part existed free of the high molecular weight, chromosomal DNA. A plasmid (pFT1+) of approximately 8.7kB was recovered from the total, uncut DNA of these transformants by transformation of E. coli. The recovered pFT1+ had sequence similarity to both pFT1 and the fungal genome. The Hyg^R gene and most of the pFOXC2 sequence are not present on pFT1+. The Hyg^R gene has been cloned into a unique SphI site in pFT1+. This modified plasmid will be tested for its effects on transformation of F. oxysporum.

Y 333 THE NUCLEOTIDE SEQUENCE OF cDNA CLONES FROM RNA 3 OF CUCUMBER MOSAIC VIRUS STRAINS C AND WL, Hector Quemada[1], Chris Kearney[2], Dennis Gonsalves[2], and Jerry L. Slightom[1], [1]Department of Molecular Biology, Upjohn Company, Kalamazoo, MI 49007; and [2]Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

We have sequenced clones from the 3' portion of CMV-C and CMV-WL RNA 3, and compared them with the corresponding regions of CMV-Q RNA 3. There is a 76% similarity between the 3' end of the CMV-C and CMV-Q 3A protein coding region, but the predicted 3A protein of the C strain is 54 amino acids shorter than that of the Q strain. The intergenic region of CMV-C is 177 b.p. longer than that of CMV-Q; excluding this difference, the intergenic regions of CMV-C and CMV-Q share a 62% sequence similarity. The coat protein region of CMV-C shares a 77% similarity with CMV-Q, while the corresponding region of CMV-WL is almost identical to CMV-Q. The predicted CMV-C and WL coat proteins are 19 amino acids shorter than that of CMV-Q. There is a 69% similarity between the 3' untranslated region of CMV-C and CMV-Q, while CMV-WL and CMV-Q share a 97% similarity in this region.

Y 334 IDENTIFICATION OF GENE PRODUCTS ENCODED BY DOUBLE-STRANDED RNA GENETIC ELEMENTS ASSOCIATED WITH BIOLOGICAL CONTROL OF ENDOTHIA PARASITICA, Brendan Rae, Bradley Hillman, James Tartaglia and Donald L. Nuss, Roche Institute of Molecular Biology, Nutley, N.J.

cDNA clones corresponding to the heteropolymer and homopolymer ends of double-stranded (ds) RNA associated with the European hypovirulent strain of E. parasitica, Ep 713, have been characterized. Sequence analysis revealed a number of large open reading frames (ORFS). For example, the sequence at the heteropolymer end of the largest RNA consists of a 497 nt noncoding region followed by a 956 nt ORF which specifies a 29 kd polypeptide, a 108 nt intercistronic region and a 1,172 nt ORF which specifies a 43 kd polypeptide. Portions of cDNA clones corresponding to individual ORFs were subcloned into transcription vectors and expressed *in vitro*. Antisera prepared against synthetic oligopeptides and bacterially expressed polypeptides were used to demonstrate the *in vivo* expression of these ORFS.

Y 335 TOWARDS AN UNDERSTANDING OF THE MECHANISM OF PROTECTION AGAINST TMV INFECTION IN TRANSGENIC TOBACCO PLANTS, James C. Register, III and Roger N. Beachy, Washington University, St. Louis, MO 63130.

Constitutive expression of the tobacco mosaic virus (TMV) coat protein (CP) gene in transgenic tobacco plants results in inhibition of disease symptom development following inoculation with TMV (Powell Abel, P. et al. 1986 Science 232, 738-743). Here we describe experiments designed to address the mechanism of this protection using protoplasts from these transgenic plants. It is shown that protection against TMV infection is expressed in protoplasts, but that inoculation with either TMV RNA or TMV which has been treated at pH 8.0 overcomes the protection. The protection is largely virus-specific, as inoculation of protoplasts with viruses not closely related to TMV can overcome the protection. Analogous results have been obtained in this laboratory using whole plants. It has been proposed that brief treatment of TMV at pH 8.0 removes approximately one turn of CP subunits from the 5' end of the virus, allowing initiation of translation of the viral RNA and cotranslational disassembly of the virus (Wilson, T.M.A. 1984 Virology 137, 255-265). Thus, the results presented here suggest that expression of TMV CP in transgenic plant cells prevents TMV from uncoating. A model which relates these results to early events in plant virus infection is presented.

Y 336 GENETIC ANALYSIS OF RACE SPECIFICITY AND TRANSFORMATION EVENTS IN *GLOMERELLA CINGULATA* F. SP. *PHASEOLI* (*COLLETOTRICHUM LINDEMUTHIANUM*), R.J. RODRIGUEZ AND O.C. YODER, Department of Plant Pathology Cornell University, Ithaca, NY 14853.

Two field isolates of *Glomerella cingulata* f.sp. *phaseoli*(Gcp) differing in mating type and race specificity were sexually mated and the resulting ascospore progeny analyzed for segregation of race specificity and a restriction fragment length polymorphism(RFLP). The RFLP segregated as a single genetic element whereas race specificity appeared to be multigenic.

Transformation of Gcp was accomplished by chromasomal integration of a hygromycin B resistance gene fused to a fungal promoter. DNA flanking the integration site from one transformant was found to contain two RFLPs and one repeated sequence. The repeated element is dispersed throughout the genome and is highly conserved in a number of different fungal and plant genera, suggesting that this element may provide insight on the molecular evolution of these organisms.

Y 337 GENETIC MAPPING OF REPLICATION FUNCTIONS IN CUCUMBER MOSAIC VIRUS, Marilyn J. Roossinck and Peter Palukaitis, Cornell University, Ithaca, NY 14853.

Cucumber Mosaic Virus (CMV) is a plus-stranded, RNA virus with a tripartite genome. CMV infects a large number of plant species, with various strains displaying differential host ranges and severity of symptoms. Such variation is due to differences in virus:host interactions, including those specifying virus replication and virus cell-to-cell movement in a given host. In this study, two strains of CMV, isolated 15 miles apart, but displaying markedly different symptoms and viral titers in both squash and muskmelon hosts, were compared. In muskmelon protoplasts, the two strains showed differences in the level of viral RNA synthesis after 24 hours of incubation. By infecting protoplasts with pseudorecombinant viruses made from these two strains, we can delineate which RNA segments are involved in replication and/or cell-to-cell movement functions. We are using this strategy to map replication functions and to characterize the differences between a number of CMV strains.

Y 338 COMPLEMENTARY OLIGONUCLEOTIDE MEDIATED INHIBITION OF TMV RNA TRANSLATION, Don Roth, Arlen Nelson and Jerry D. Johnson, University of Wyoming, Laramie, WY 82071

Two different "antisense" oligodeoxynucleotides and their RNA analogues, each complementary to non-overlapping sequences of 51 bases near the 5' end of TMV RNA, inhibit *in vitro* translation of the genomic RNA in a rabbit reticulocyte lysate. Inhibition is dependent upon complementarity, concentration, and hybridization of the oligomers with TMV RNA. Inhibition is observed at molar ratios of TMV RNA to antisense oligomers as low as 1:1.5. A plateau of inhibition at which 10-25% of the control signal remains is achieved by molar ratios of TMV RNA:antisense DNA or RNA $\geq$ 1:15. The extent of inhibition is not increased by the simultaneous presence of both complementary fragments. Oligodeoxynucleotides and their RNA analogues identical to the same regions of TMV RNA have no direct effect on translation, however, they can block inhibition by the antisense fragments. Translation of BMV RNA is not affected by any of the oligodeoxynucleotides. Polyacrylamide gel electrophoresis shows translation of TMV p126 is selectively inhibited. We conclude that the observed inhibition of translation is due to direct interference with ribosome function. Each of the synthetic oligodeoxynucleotide pairs have been cloned into both the NOS gene and a CaMV 35S RNA promoter driven transcription unit in pMON530. These constructions have been used to transform *T. xanthi* nc leaf disks to kanamycin resistance. Analyses of plants regenerated from kanamycin resistant callus will be presented.

Y 339 ALLELOCHEMICALS: INDICATIONS OF CELL DISTURBANCE SIMILAR TO FUNGAL INFECTIONS, Matthew Ryuntyu, Department of Agronomy and Soil Science, University of New England, Armidale. N.S.W. 2351.

Recent workers suggest that interference with membrane function at the level of the cell can explain some secondary allelopathic phenomena:inhibition of mineral uptake. The primary allelopathic effects of Camelina upon Linum are associated with impaired mitochondrial function, increased vacuolar activity and phagocytosis (Lovett & Ryuntyu, 1987. Proc. 8 Austr. Weeds Conf., Artarmon: 179-183). TEM shows arrested development of linseed root tips. Organelles cannot be distinguished, in contrast to control. Lipid bodies are abundant in the cell contents and amyloplast may also be present.

These features suggest that metabolism of food reserves by affected cells has been disrupted.

Malformation of the primary and secondary cell walls is also apparent as compared with control. This may represent a response to 1000 µg/ml benzylamine, as allelochemical, similar to that following fungal infection (Leath & Powell, 1969. Phytopathology 59:1654).

Y 340 MOLECULAR MECHANISMS OF RESISTANCE IN BARLEY AGAINST POWDERY MILDEWS, Jan B. Andersen, Per L. Gregersen, Hans Thordal-Christensen and Viggo Smedegaard-Petersen, Dept. Plant Pathology, Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.

Investigations of induced resistance in barley against powdery mildews have provided a good insight into the dynamics of the resistance mechanisms during the first hours after inoculation with fungal pathogens (1,2,3). These investigations have provided a model system for further studies of the molecular mechanisms underlying the resistance. These studies are performed by constructing cDNA libraries of host lines expressing resistance and host lines not expressing resistance. These libraries are differentially screened in order to isolate clones that differ between libraries. Different combinations of host lines and pathogenic and non-pathogenic fungal races are also taken into consideration in order to distinguish between resistance induced by pre-treatment with another fungus and resistance governed by powdery mildew resistance genes. Differences in gene expression will be related to their significance in the expression of resistance providing additional information on the dynamics of resistance reactions in barley plants.

1. Cho, BH & V Smedegaard-Petersen (1986): Phytopathol. 76: 301-305.
2. Thordal-Christensen, H & V Smedegaard-Petersen. Plant Pathol. in press.
3. Thordal-Christensen, H & V Smedegaard-Petersen. J. Phytopathol. in press.

Y 341 DEVELOPMENT OF A TRANSFORMATION SYSTEM FOR FUSARIUM SAMBUCINUM, Yangkyo P. Salch and Marian N. Beremand, USDA/ARS, Northern Regional Research Center, Peoria, IL 61604.

Fusarium sambucinum is a soil saprophyte and a pathogen on a variety of plants, and a trichothecene producer. It can also metabolize phytoalexins such as rishitin, lubimin, and xanthotoxin. In order to isolate and to study the regulation and expression of the genes involved in the mechanism of pathogenicity, toxin production, and metabolism of various phytoalexins at the molecular level, a transformation system has been developed by introducing cloning vectors into protoplasts of F. sambucinum. A method was developed which allowed, for the first time, the isolation of protoplasts from F. sambucinum. We found that mycelia grown in medium containing yeast extract, peptone, and glucose and then treated in a potassium chloride solution containing Novozym 234, driselase, and chitinase yielded almost 100% protoplasts. These protoplasts were then mixed with either plasmid pDH25 or cosmid pCOS (both contain a resistance gene to hygromycin B as a selectable marker), incubated in a polyethylene glycol solution, and regenerated in an osmotic medium. Five to six transformants per 25 µg of DNA were obtained. The efficiency of transformation and the integration pattern and stability of the transforming DNA will be discussed.

Y 342 ASSOCIATION OF MITOCHONDRIAL PLASMIDS AND PATHOGENICITY IN FUSARIUM SOLANI, Deborah Samac[1] and Sally Leong[2], Dept. of Plant Pathology[1], USDA-ARS[2], Univ. of Wisconsin, Madison, WI 53706.

Two linear mitochondrial plasmids, pFSC1 and pFSC2, have been described in one isolate (FS37) of Fusarium solani f. sp. cucurbitae (teleomorph: Nectria haematococca MP I). This isolate is highly pathogenic on susceptible cucurbit hosts. The plasmid-containing isolate was cured of plasmid DNA by treatment of germinating microconidia with ethidium bromide. The plasmid-cured strains demonstrated lowered pathogenicity than FS37. Pathogenicity tests were carried out by inoculating dry seeds with a spore suspension at 100 spores/seed. Pathogenicity was measured as the percent plants infected 10 days post inoculation. Protoplast fusion was used to introduce plasmid-containing mitochondria into a weakly pathogenic isolate (FS3). The fusion-created strains were more pathogenic than FS3 on selected cultivars suggesting a role for pFSC1 and pFSC2 in phytopathogenicity.

Y 343 TOWARDS A MOLECULAR GENETICAL ANALYSIS OF PATHOGENICITY AND OTHER MUTANTS OF PYRENOPEZIZA BRASSICAE, Maria K. Sawczyc, Alison M. Ball, Keith Johnstone and David S. Ingram, University of Cambridge, Cambridge, England.

The hemibiotrophic, heterothallic ascomycete Pyrenopeziza brassicae is the causal agent of light leaf spot of brassicas. The overall objective of our project is to isolate and study genes involved in the pathogenicity of P. brassicae to brassicas. This objective will be achieved by the complementation of "pathogenicity" mutants with DNA from a gene library prepared from a wild-type strain. Progress on the following aspects of the project will be reported:

1. The development of an in vitro pathogenicity test for P. brassicae using excised cotelydons of Brassica napus.
2. The induction, isolation and characterisation of a range of mutants of P. brassicae, including mutants which cause altered symptom production in the in vitro pathogenicity test.
3. The development of methods for vector-mediated transformation in P. brassicae, including the production and regeneration of protoplasts from conidiospores.
4. The preparation of a P. brassicae genomic library in the fungal cosmid cloning vector pAN7-2.
5. Complementation studies of mutants of P. brassicae.

Y 344 TRANSFORMATION OF THE MAIZE PATHOGEN *COCHLIOBOLUS HETEROSTROPHUS* TO A PEA PATHOGEN BY INSERTION OF A PDA GENE OF *NECTRIA HAEMATOCOCCA*, W. Schäfer, H. D. Van Etten and O.C. Yoder, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

The product of the *Nectria haematococca PDA* gene demethylates the pea phytoalexin pisatin and is thought to be required by *N. haematococca* for pathogenicity to pea. A 3.2 kb fragment carrying the *PDA* gene and encoding a single transcript was cloned into a plasmid containing the hygromycin B phosphotransferase gene of *E. coli* fused to a *C. heterostrophus* promoter (Weltring *et al.*, Gene, submitted; Turgeon *et al.*, Mol. Cell. Biol., 1987, 7:3297-3305). This plasmid was transformed into *C. heterostrophus*; transformants were selected for very high levels of hygromycin tolerance (2-3 mg / ml). Enzyme assays showed that the *PDA* product in one of these transformants was as active as it is in the most virulent strains of *N. haematococca*. When inoculated on pea stems, transformed *C. heterostrophus* caused larger lesions than the wild type but smaller than those caused by highly virulent *N. haematococca*. On pea leaves, wild type *C. heterostophus* caused no symptons whereas the transformant caused lesions substantially larger than those produced by *N. haematococca*.

Y 345 MOLECULAR MAPPING AND ELECTROPHORETIC KARYOTYPING OF MAGNEPORTHA GRISEA. Daniel Z. Skinner[2], Hei Leung[3], and Sally A. Leong[1] USDA-ARS, Plant Disease Resistance Unit[1], Department of Plant Pathology[2], University of Wisconsin, Madison, WI 53706, and IRRI[3], the Philippines.

Magneportha grisea (anamorph = Pyricularia oryzae and P. grisea) parasitizes a wide variety of graminaceous hosts and causes rice blast. Most isolates virulent to rice are female sterile and can be crossed only to isolates virulent to grasses other than rice. We are developing a restriction fragment length polymorphism (RFLP) map of M. grisea to facilitate investigations of the genetic control of virulence. Random single copy fragments and heterologous cloned genes are being used as probes. About 50% of random fragments from a rice-pathogenic isolate detected polymorphisms between rice and non-rice isolates. About 10% of the same fragments detected polymorphisms among rice-pathogenic isolates. We are also developing an electrophoretic karyotype of M. grisea using clamped homogeneous electric field electrophoresis (CHEF). RFLP linkage groups will be assigned to chromosomes by probing CHEF gel blots with representative clones.

Y 346 **MOLECULAR CHARACTERIZATION OF THE CUTINASE GENE FROM COLLETOTRICHUM GLOEOSPORIOIDES**, Munna L. Agarwal, Douglas Rice, Suresh S. Patil, and John I. Stiles, University of Hawaii, Honolulu, HI 96822.
The fungal pathogen Colletotrichum gloeosporioides is the causal agent in papaya anthracnose. For infection of undamaged fruit the fungus must secrete cutinase, an enzyme that hydrolyses cutin allowing penetration of the cuticle of the fruit. Mutants that do not secrete cutinase, or the application of chemicals or antibodies that inhibit cutinase, inhibit infection in undamaged fruit. A recombinant DNA library of C. gloeosporioides DNA in bacteriophage λ was differentially screened with radiolabeled cDNA synthesized from total mRNA isolated from glucose-grown C. gloeosporioides in which no cutinase activity is found and with radiolabeled cDNA synthesized from cells grown on cutin as the sole carbon source which express cutinase. Several clones were isolated that contained sequences transcribed only in cutin-grown cells. These clones were used to hybrid-select mRNA that was translated in vitro and challenged with an antiserum to cutinase. Two clones were isolated that contain sequences that select cutinase mRNA. The transcribed region of one of these clones has been subcloned and is being sequenced. A recombinant DNA library has been constructed for a mutant defective in cutinase. Clones containing the cutinase gene have been isolated and will be sequenced to determine the molecular basis for the mutation.

Y 347 ANALYSIS OF PROTECTION IN TRANSGENIC PLANTS EXPRESSING THE CUCUMBER MOSAIC VIRUS COAT PROTEIN OR ITS ANTISENSE RNA, Nilgun Tumer, Keith O'Connell, Maria Cuozzo[1], Wojciech Kaniewski, and Nam-Hai Chua[1], Monsanto Company, St. Louis, MO 63198, [1]The Rockefeller University, New York, NY 10021

Transgenic tobacco plants expressing the coat protein gene (+CP) of the cucumber mosaic virus D strain were protected from infection with CMV C strain on their inoculated and systemic leaves. After inoculation with 5 ug/ml of CMV, inoculated leaves of +CP plants contained 10 to 15% of the virus levels on the control plants, the systemic leaves of the +CP plants contained 2% or lower levels of virus detected on the systemic leaves of the control plants. Progeny of two different transgenic lines expressing antisense RNA to the viral coat protein, showed protection from infection on their inoculated and systemic leaves only at low inoculum concentrations (1 and 5 ug/ml). At inoculum concentrations greater than 5 ug/ml, virus levels on the inoculated and systemic leaves of the antisense plants parallelled the levels found on the control plants. These results indicate that its possible to obtain protection in transgenic plants through the expression of antisense RNA, however this protection is not as effective as that mediated by the coat protein.

Y 348 MOLECULAR ANALYSIS OF THE MATING LOCUS OF Cochliobolus heterostrophus, Gillian Turgeon, Lynda Ciuffetti, Willi Schafer, and Olen Yoder, Department of Plant Pathology, Cornell University, Ithaca, New York, 14853

Mating type in the heterothallic ascomycete Cochliobolus heterostrophus is determined by a single locus (MAT1) with 2 known alleles (MAT1-1 and MAT1-2). Opposite alleles are required for completion of the sexual cycle. We have cloned the MAT1-1 allele by transferring a cosmid library from a MAT1-1 strain to a MAT1-2 strain. Transformants were selected for resistance to hygromycin B and screened for the MAT1-1 allele by selfing. Since MAT1-1 x MAT1-1 or MAT1-2 x MAT1-2 crosses are infertile, formation of sexual structures (perithecia) was not expected unless a transformant carried both alleles. We identified one transformant which produced numerous perithecia with viable ascospores when selfed or when crossed with MAT1-1 and MAT1-2 testers. The transforming cosmid was recovered from the genome, cloned in E. coli and used to retransform MAT1-2 protoplasts to hygromycin resistance; most transformants were self-fertile, ie. homothallic. The MAT1-1 allele was subcloned on a 2 kb fragment and characterized.

Y 349 MOLECULAR GENETICS OF PHYTOPHTHORA MEGASPERMA f.sp. GLYCINEA

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The oomycetes, including Phytophthora, are a major group of fungal plant pathogens infecting a very wide range of hosts. Phytophthora megasperma f.sp. glycinea (Pmg) is a major pathogen of soybeans causing damping-off of seedlings and a root and stem rot of mature plants. There are at least 10 different soybean major resistance genes against Pmg and these are dominant .In the fungus which is diploid there is some genetic and biochemical evidence to indicate the presence of avirulence genes which correspond gene-for-gene with the host major resistance genes.

In preparation for trying to clone these presumptive avirulence genes we have characterised the genome of Pmg by Tm and Cot analysis. Our present evidence indicates that the Pmg genome contains 30-40% moderately repeated sequences and that the unique copy component has a complexity of 10-100 Mb.

We are also developing a transformation system for introducing cloned DNA into Pmg. We have constructed bleomycin resistance and hygromycin resistance genes for Pmg using the respective E.coli resistance genes in combination with transcriptional sequences from the hsp70 heat shock gene of Bremia lactucae. We are attempting to introduce these genes into Pmg protoplasts by treatment with PEG and $CaCl_2$

Y 350 CHARACTERIZATION OF THE GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE GENE IN *COCHLIOBOLUS HETEROSTROPHUS*

S. L. Van Wert and O. C. Yoder, Cornell University, Ithaca, NY 14853.

One approach to defining the role of a gene during disease development is to study altered expression of the gene by placing it under control of a strong constitutive promoter. In many species the glyceraldehyde-3-phosphate dehydrogenase (GPD) gene is known to have such a promoter. The goals of our research are to isolate the *GPD* gene (*GPD1*) from *C. heterostrophus*, a filamentous fungal pathogen of maize, and to characterize the regulatory sequences.

We have isolated, mapped and sequenced a 3300 bp piece of DNA that includes *GPD1* from *C. heterostrophus*. The *GPD1* sequence was compared to that of the same gene from other fungi; homology at the nucleotide level was between 60 and 80%. A 1300 nt *GPD1* transcript was detected by Northern analysis of poly A^+ RNA from *C. heterostrophus*. The 5' and 3' termini of the *GPD1* transcript were mapped. The steady state levels of *GPD1* mRNA expressed after growth of *C. heterostrophus* in several different media over time are currently being examined to determine whether or not expression of *GPD1* is constitutive. The quantity of *GPD1* mRNA relative to abundantly and weakly expressed genes in *C. heterostrophus* will indicate the strength of the *GPD1* promoter and/or stability of the mRNA. The DNA sequences required for transcription initiation are being analyzed by fusion to the reporter gene *lacZ*, from *E. coli*. Each promoter fusion will be tested for function in *C. heterostrophus* by transformation into a particular chromosomal locus via homologous recombination.

Y 351 INDUCTION OF SESQUITERPENOID-TYPE PHYTOALEXINS AND SUPPRESSION OF STEROL BIOSYNTHESIS UPON ADDITION OF FUNGAL ELICITOR TO TOBACCO CELL SUSPENSION CULTURES.

Urs Vögeli and Joseph Chappell, Plant Physiology/Biochemistry Program, University of Kentucky, Lexington, KY 40546

Addition of fungal elicitor to tobacco cell suspension cultures results in a rapid synthesis (within 15 hours) and secretion of large amounts (>10µg/hr x g fr wt) of sesquiterpenoid-type phytoalexins. Pulse-labelling experiments of the cell cultures with the isoprenoid precursors [14C]acetate and [3H]mevalonate have demonstrated that, the induction of the sesquiterpenoid biosynthetic activity was paralleled by a rapid and large decline of incorporation of radioactivity into sterols. Since farnesylpyrophosphate, FPP, is the last common intermediate for both sterol and sesquiterpenoid biosynthesis, it represents a branch point for the diversion of carbon into various classes of isoprenoids. Therefore sesquiterpene cyclase catalyzing the conversion of FPP into a bicyclic intermediate of the sesquiterpenoid biosynthetic pathway and squalene synthetase catalyzing the condensation of 2 FPPs into squalene, a committed intermediate of the sterol biosynthetic pathway, are potentially regulatory enzymes. The sesquiterpene cyclase is undetectable in control cell cultures and induced to a maximum level within 10 hours, persisting at that level thereafter, in elicitor-treated cell cultures. In contrast, squalene synthetase activity is rapidly suppressed to less than 25% of its control level within 7 hours of elicitor addition to the cell cultures. We conclude that the mechanism responsible for the channeling of isoprenoid intermediates, and especially FPP, into sesquiterpenoids occurs by a coordinated increase in sesquiterpene cyclase activity and a decrease in the squalene synthetase activity.

Y 352 BIOCHEMICAL AND MOLECULAR BIOLOGICAL STUDIES ON THE DEGRADATION OF PHYTOALEXINS BY ASCOCHYTA RABIEI, Klaus-M. Weltring, Martin Arnemann, Birgit Höhl, Hans C. Salmen and Wolfgang Barz, Lehrstuhl f. Biochemie der Pflanzen, Universität Münster, West-Germany.

Ascochyta rabiei is the causal agent of Ascochyta blight of chickpea (Cicer arietinum L.). During infection resistant cultivars of chickpea accumulate high levels of the pterocarpan phytoalexins medicarpin and maackiain. In the course of our studies on the involvement of phytoalexins in the interaction of chickpea with its pathogen we have demonstrated that Ascochyta rabiei is able to degrade the host phytoalexins with either a cleveage of the benzyl phenyl ether bond or a 1a-hydroxylation as the first steps. The pterocarpan: NADPH oxidoreductase, catalyzing the reductive cleveage of the pterocarpan phytoalexins to the corresponding 2'-OH isoflavans has been purified and partially characterized. The enzyme is specific for 3-OH pterocarpans and NADPH.

In order to study the regulation of genes involved in phytoalexin degradation and the possible role of this metabolism in the host parasite interaction we started to clone the genes for the reductase and the 1a-hydroxylase of Ascochyta rabiei. A genomic library of the Ascochyta genome was prepared in the cosmid vector pUCOSHI containing the hygromycin B resistance gene from E coli fused to a Cochliobolus promotor as a selectable marker. The library is used to transform the maize pathogen Cochliobolus heterostrophus, which cannot degrade medicarpin and maackiain. Hyg^r transformants are tested for expression of either gene.

Financial support by Deutsche Forschungsgemeinschaft.

Y 353 IDENTIFICATION OF TRANS-REGULATORS OF THE ENOLASE GENES OF SACCHAROMYCES CEREVISIAE, Catherine E. Willett, Janice P. Holland, Paul Brindle and Michael J. Holland, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616.

Several genes encoding enzymes of the glycolytic pathway are coordinately regulated in the yeast *Saccharomyces cerevisiae*. We are concerned specifically with transcriptional regulation of the two enolase genes, *ENO1* and *ENO2* as a model for this coordinate regulation. *Cis*-acting sequences required for transcription of *ENO1* and *ENO2* have been mapped by deletion analysis. Both genes have redundant upstream activation sequences (UAS's) or enhancers, located approximately 450 base pairs upstream from their respective transcriptional initiation sites. Mutations in the regulatory gene *GCR1* cause a 50-fold decrease in transcription of both *ENO1* and *ENO2*, as well as decreases in expression of many other glycolytic genes. Deletions have been constructed within the UAS regions of both *ENO1* and *ENO2* that render expression independent of *GCR1*; wild-type levels of expression are seen in *gcr1*$^-$ strains. This suggests that transcription of *ENO1* and *ENO2* is modulated by a repressor that binds within the UAS region of both genes, and that the *GCR1* gene product acts to antagonize this repressor. Gel mobility shift assays using the *ENO2* UAS region show *GCR1* - dependent binding patterns. In cell extracts from *GCR1* cells, a large complex (Complex III) is observed, however, in *gcr1*$^-$ extracts a smaller complex (Complex I) forms. DNAseI footprinting and methylation interference studies give identical protein-DNA interactions for Complex I and Complex III, implying that one DNA binding event gives rise to both complexes. A synthetic oligonucleotide spanning the footprinted region in *ENO2* effectively competes both complexes formed with the *ENO2* UAS, and also forms analogous complexes when incubated with each cell extract. Since *ENO1* is similarly regulated by *GCR1*, and deletions within the *ENO1* UAS region can confer *GCR1*-independence, it is expected that the same repressor is interacting with *ENO1* and *ENO2*. Gel mobility shift assays done with the *ENO1* UAS region show the same *GCR1*-dependent binding patterns as seen with the *ENO2* UAS. We suggest that the *GCR1* gene product modulates binding of the repressor to its operator within the UAS regions of the two enolase genes.

Y 354 TRANSFORMATION OF ASPERGILLUS FLAVUS BY THE OROTIDINE-5'-PHOSPHATE DECARBOXYLASE GENE OF NEUROSPORA CRASSA. C. P. Woloshuk, E. R. Seip, G. A. Payne, and C. R. Adkins, North Carolina State University, Raleigh, NC 27695-7616.

Aspergillus flavus was transformed with the orotidine-5'-phosphate decarboxylase gene (pyr4) from Neurospora crassa. Uracil requiring mutants of the aflatoxin non-producing isolate 650 (tan, leu$^-$, afl-2) were obtained by irradiating conidia with UV and selecting colonies resistant to 5-fluoro-orotic acid (FOA). Of 50 colonies resistant to FOA, four required uracil for growth. Protoplasts prepared from two mutants (650-27 and 650-33) were successfully transformed using a plasmid vector (pRG-3) containing the Neurospora pyr4 gene. The frequency of stable pyr^+ transformants was 2 per ug DNA whereas the frequency of abortive transformants was 50-100 times greater than that of the stable forms. Southern analysis of total DNA from six transformants indicated that the pyr4 vector intergrated into different regions of the 650-27 genome. Diploids were obtained from forced heterokaryons of the uracil auxotroph 650-33 with three other aflatoxin non-producing strains, 796 (white, arg$^-$, afl-15), 774 (white, pdx$^-$, afl-b2$^-$) and 656 (white, met$^-$, afl-9). These diploids produced aflatoxin indicating complementation of the aflatoxin loci. Parasexual recombination through haploidization of the diploids will allow transfer of the pyr$^-$ gene into these other strains. We are using this transformation system to investigate genes involved in aflatoxin biosynthesis and regulation.

Y 355 PRINCIPLES INVOLVED IN REPLICATION AND PACKAGING OF WOUND TUMOR VIRUS GENOME RNAs, Zhengkai Xu, John V. Anzola and Donald L. Nuss, Roche Institute of Molecular Biology, Nutley, N.J. 07110.

Cloning and sequence analysis of defective interfering RNAs associated with the segmented double-stranded RNA genome of wound tumor virus (WTV) revealed the following: 1) Nucleotide sequence information required for replication and packaging of a genome RNA is located within the extreme terminal portions of the molecule. 2) Packaging of one pair of these terminal structures excludes the packaging of a second copy of the same pair of terminal structures. This means that each of the twelve WTV genome RNA segments must contain at least two recognition sequence domains (sorting signals): one which specifies that it is a viral and not a cellular RNA and a second which specifies that it is a particular RNA segment. Sequence analysis of the terminal portions of all twelve WTV genome RNA segments revealed a set of putative sorting signals. Significantly, the same general sequence organization was recently reported for the terminal portions of the segmented single-stranded RNA genome of influenza virus.

Y 356 ROLE OF DOUBLE STRANDED RNAs IN MYCOSPHAERELLA PINODES, Tetsuji Yamada, Masayuki Seno, Hisaharu Kato, Tomonori Shiraishi and Hachiro Oku, Faculty of Agriculture, Okayama University, Okayama, 700, Japan.

Studies on the structure of the extrachromosomal nucleic acids in the plant pathogenic fungus, Mycosphaerella pinodes OMP1, isolated from Okayama, Japan, indicate that at least three double stranded(ds) RNAs whose sizes are approximately 6.0kb(L), 4.0kb(M) and 2.4kb(S) are included in the cytoplasm. Some nonpathogenic or weak pathogenic mutants isolated from UV treatment show the different pattern of these dsRNAs; some contain higher level of S fragment, other contain no M fragment. Strong pathogen of other isolate E1 does not contain these dsRNAs. The role of dsRNA in fungi already elucidated are the killer factor in yeast and the hypovirulence factors found in chesnut blight fungus, Endochia parasitica, etc. The killer factor, however, has not been observed in the isolates of M. pinodes containing the dsRNA. The distinguished difference between the isolates which contain the dsRNAs and those do not is the growth rate on the artificial cultrure. It is likely that the presence of these dsRNAs results in inhibiting the cell growth. Electron microscopic observation did not clealy show the presence of mycovirus like particles. Southern hybridization analysis showed that RNA sequence homology does not exist between S fragment against M nor L. In order to elucidate the role of the dsRNAs observed in the isolates of M. pinodes, we constructed the cDNA of these dsRNAs and are currently investigating the mRNA products possibly transcribed from these dsRNAs in the pathogenic isolates and the nonpathogenic.

Viroids and DNA Viruses

Y 400 TRANSCRIPTIONAL ELEMENTS IN THE THE INTERGENIC REGION OF MAIZE STREAK VIRUS GENOME, Carmen Fenoll, Diane M. Black, Michel Schneider and Stephen H. Howell. Biology Department C-016, University of California San Diego, La Jolla, CA 92093.

Using a transient expression system in maize protoplasts, we have demonstrated that a fragment of the MSV genome including the intergenic region (IR) and the sequences upstream from the coat protein gene contains an active promoter. Within the IR, we have identified a 122 b fragment which is required for promoter activity and which acts as an upstream activating sequence (UAS). The UAS harbors a hairpin loop and a sequence within the loop common to all geminiviruses. The UAS can be substituted by a functionally similar element from the 35S promoter of cauliflower mosaic virus (CaMV), and, conversely, the MSV UAS is able to activate a truncated version of the CaMV 35S promoter. Fine deletion analysis of the UAS has shown that both the common geminivirus sequence and the hairpin loop are largely dispensible, and that the active elements within the UAS are GC boxes and the sequences immediately downstream. We have demonstrated that protein extracts from maize nuclei contain factors that bind specifically to the MSV UAS. Preliminary DNase I protection experiments indicate that the nuclear factors bind to the GC boxes present in the MSV intergenic region. The binding can be competed by the fragments of the UAS which contain the GC boxes and which activate the MSV promoter in the transient expression system.

Y 401 BINDING OF CELLULAR FACTORS TO A CONSERVED 35 BP SEQUENCE FOUND IN THE LARGE INTERGENIC REGION OF CAULIMOVIRUSES. Siddarame Gowda, Richard D. Richins, Karen-Beth Goldberg, Richard Kormelink and Robert J. Shepherd, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.
A highly conserved 35 base pair sequence located approximately 300 bp downstream of the probable 35 S promoter in the large intergenic region of several caulimoviruses (cauliflower mosaic virus, figwort mosaic virus, carnation etched ring virus, dahlia mosaic virus, and mirabilis mosaic virus) has been observed to bind cellular proteins. By using end-labelled oligonucleotide corresponding to the plus-strand of the conserved sequence and polyacrylamide gel mobility shift assay, at least two cytoplasmic factors were found to bind specifically to these intergenic sequences. These factors are present at comparable levels in both healthy and infected plants.

Y 402 THE COMPLETE NUCLEOTIDE SEQUENCE OF POTATO VIRUS X REVEALS HOMOLOGIES ON THE AMINO ACID LEVEL WITH VARIOUS PLUS-STRANDED RNA VIRUSES, Marianne J. Huisman, Huub J.M. Linthorst[1], John F. Bol[1], Ben J.C. Cornelissen, MOGEN International N.V., Einsteinweg 97, 2333 CB Leiden, The Netherlands; [1]Dept. of Biochemistry, State University of Leiden

Double-stranded cDNA of the genomic RNA of potato virus X has been cloned and sequenced. The primary structure (6,435 nucleotides excluding the poly(A) tail) reveals five open reading frames (ORFs) which are numbered 1 to 5 from the RNA's 5' end onwards and are coding for proteins of Mr 165,588 (166K), 24,622 (25K), 12,324 (12K), 7,595 (8K) and 25,080 (the coat protein), respectively. ORF 1 and 2 are in-phase coding regions: the stopcodon of ORF 1 and the start of ORF 2 are spaced by 30 nucleotides. Readthrough of the ochre stopcodon (UAA) of ORF 1 would result in the occurrence of a Mr 191,480 (191K) protein. The ORF 1 product contains domains of homology with the tobacco mosaic virus 126K and 183K products. The ORF 2 and 3 products show homologies with the barley stripe mosaic virus 58K and 14K proteins and the beet necrotic yellow vein virus 42K and 13K products, respectively. The significance of these homologies with respect to putative functions of the PVX open reading frames will be discussed.

Y 403 MOLECULAR CHARACTERIZATION OF A RETROVIRAL TRANSPOSABLE ELEMENT OF MAIZE, Young-Kwan Jin and Jeffrey L. Bennetzen, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.
By structural and functional criteria, most transposable elements can be separated into two distinct categories. Plant controlling elements, like prokaryotic insertion sequences and transposons, have terminal inverted repeats and transpose by excision and/or replication of the integrated element. "Retrotransposons," such as the proviral forms of animal retroviruses and the *Ty* elements of yeast, have terminal direct repeats and transpose through an RNA intermediate. Neither canonical retroviruses nor retrotransposons have been described in plants.

We have recently sequenced *Bs1*, an insertion isolated from an *Adh1* null mutation (1), and have found several retrotransposon hallmarks. The 3203 bp *Bs1* element has identical 302 bp long terminal direct repeats (LTRs), a 5 bp direct duplication of *Adh1* DNA flanking the insertion point, a tRNA primer site 2 bp downstream from the 5' LTR, and a polypurine tract upstream of the 3' LTR. *Bs1* has two long ORFs; these overlap for 58 bp and are in different reading frames. The longest ORF has homology to the protease moiety of other retrotransposons and retroviruses. *In vitro* translation systems produce a 95,000 dalton protein from *Bs1* RNA. A translational frameshift between ORF1 (740 aa) and ORF2 (168 aa) like that seen in retroviruses would produce a protein of about 95 kd. We have also sequenced the only RNA in maize which can prime retrovirus-like cDNA synthesis off *Bs1*. This molecule has the same sequence as the initiator $tRNA^{met}$ of wheat.

(1) Johns, Mottinger, and Freeling (1985) EMBO J. 4:1093.

Y 404 CAULIMOVIRUSES AND EVOLUTION OF PLANT VIRUSES, Ulrich Melcher, Oklahoma State University, Stillwater, OK 74078-0454

Caulimoviruses may have evolved at a slower rate than other plant viruses. Hydropathy profiles of the amino acid sequences of virally encoded proteins putatively responsible for cell-to-cell movement of infection were used to align the sequences of these proteins from several viruses. Highly significant similarity between caulimoviral sequences and those of all viruses tested was found. When sequences from viruses of different non-caulimoviral groups were compared, highly significant relations were infrequent. The relationships suggest that these proteins evolved from a common ancestor and that the sequences of the caulimoviral proteins have diverged from that ancestor more slowly than have the sequences from other viruses.

Analysis of the products of coinoculation of turnips with mutants and variants of cauliflower mosaic virus (CaMV) suggests that exchange of segments by homologous recombination, recombination by template switching during DNA synthesis, and a gene conversion-like mechanism may be responsible for a slow rate of divergence.

Comparison of nucleotide sequences of eight CaMV isolates in the vicinity of open reading frame II support this view. Positions at which only one isolate has a variant nucleotide are non-randomly distributed along the sequence. Multiply variant positions appear to exhibit genetic linkage. Research supported by the Herman Frasch Foundation and the Okla. Ag. Expt. Sta.

Y 405 STRUCTURAL FEATURES OF TWO NEW VIROIDS, M. Ali Rezaian and Anna M. Koltunow, CSIRO Division of Horticultural Research, GPO Box 350, Adelaide, S.A. 5001, Australia.

Two new viroids have been isolated from grapevines and named Australian grapevine viroid (AGV) and grapevine yellow speckle viroid (GYSV). The partial sequence of AGV which has about 370 nucleotide residues showed a sequence similar to the central conserved region of viroids in the potato spindle tuber viroid (PSTV) group. The 367 residue GYSV was completely sequenced and found to lack the central conserved sequence of the PSTV group. GYSV has 37% homology with the recently identified 330 residue apple scar skin viroid (ASSV) and these form a new viroid group. Comparison of structural elements conserved in GYSV and ASSV with sequences of other viroids identified domains which are also present in the PSTV group. There is a conserved sequence in the central region of this new viroid group which can form stable palindromic and stem loop structures similar to those found in other viroids. GYSV is associated with the yellow speckle disease of grapevines but the role of AGV is as yet unknown.

Y 406 VIRUS-LIKE PARTICLES AND DOUBLE-STRANDED RNAS OF ALTERNARIA ALTERNATA, Hurley S. Shepherd, USDA, ARS, SRRC, New Orleans, LA 70179.

The fungus Alternaria alternata produces a peptide toxin, tentoxin, which causes chlorosis in susceptible plants. In comparing isolates which produce tentoxin with those which do not produce tentoxin, the presence of one or more anomalous bands was noted in nucleic acid preparations from several of the producing strains. These bands were identified as double-stranded (ds) RNAs based on RNase digestion and CF-11 cellulose binding characteristics. The dsRNAs were pelletable from buffer by ultracentrifugation, banded in CsCl gradients, and were associated with 30 nm virus-like particles (VLPs). These characteristics are similar to those reported for VLPs in other fungi. Virus-like particles were found in 7 of 14 tentoxin-producing isolates, but in none of 15 non-producing strains. The sizes of dsRNAs ranged from 1.0 to 5.0 kbp, but were of similar size in only two isolates. The particles contained two major proteins which were also of different sizes in different strains. In three recently analyzed isolates, 2 of which produced tentoxin, 3 identical-sized dsRNAs were found which did not seem to be particle associated. They were not pelletable from buffer and were dispersed throughout CsCl gradients implying heterogeneous density. In another recently analyzed non-producing isolate, a dsRNA greater than 10 kbp in size was found which behaved like free nucleic acid, pelleting through a CsCl gradient but not through buffer.

Y 407 EXPRESSION OF VIRAL cDNA IN TRANSGENIC PLANTS AND CROSS-PROTECTION, Cees M.P. van Dun, Bert Overduin, Lous van Vloten-Doting[a] and John F. Bol, State University of Leiden, P.O. Box 9505, 2300 RA Leiden. [a]ITAL, P.O.Box 48, 6700 AA Wageningen The Netherlands. Transformation of plants with viral genes has led to promissing results with respect to crop-protection. Using the Agrobacterium tumefaciens binary vector system, we have transformed Nicotiana tabacum cv. Samsun NN with different chimaeric viral genes under control of the CaMV 35S promoter and nos polyadenylation signal. Expression of the CP genes of alfalfa mosaic virus (AlMV), tobacco streak virus (TSV) and tobacco rattle virus (TRV) resulted in a resistance of the plants to the homologous virus, resembling cross-protection. Though the CP molecules of AlMV and TSV are functional equivalent in genome activation, plants expressing AlMV CP did not resist TSV infection and plants expressing TSV CP did not resist AlMV infection. Apparently, the cross-protection function of the CP differs from the genome activation function. The transgenic plants expressing CP of TRV strain TCM resisted infection with the TCM strain but not with the PLB strain. The CP of the latter has a homology of 40% with the TCM strain. To investigate if the non-structural AlMV proteins are involved in cross-protection, plants were transformed with chimaeric cDNA1 and 2 genes. From complementation studies with transgenic protoplasts at least for the cDNA1 plants it could be concluded that functional viral protein is present. Progeny of the cDNA1 and 2 plants did not show resistance to AlMV or YSMV infection, indicating that AlMV P1 or P2 are individually not involved in cross-protection to the levels at which they are expressed.