

# **YSM ABSTRACTS**

**Manipulation of plant phenylpropanoid metabolism by the expression of a bacterial enzyme which diverts hydroxycinnamoyl-CoA thioesters to p-hydroxybenzaldehydes.**

M.J. Mayer, A.J. Parr, M.L. Parker, N.J. Walton and A.J. Michael.  
Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA,  
UK.

The plant phenylpropanoid pathway is responsible for the synthesis of a range of compounds which are central to the structure and protection of the plant and have important effects on texture, flavour, and processing traits. A bacterial hydratase/lyase enzyme (HCHL) which converts p-coumaroyl-CoA, caffeoyl-CoA and feruloyl-CoA to the corresponding hydroxybenzaldehydes has been expressed in tobacco. Two generations of transgenic plants from four lines exhibited a severe phenotype which was associated with striking biochemical and molecular changes. Significant quantities of p-hydroxybenzaldehyde and vanillin derivatives were accumulated in the form of acid and alcohol glucosides and glucose esters. The levels of chlorogenic acid and the major flavonoids were decreased. An examination of phenylpropanoid biosynthetic gene expression revealed up-regulation of messages for PAL, C4H and 4CL in transgenic plants. Strong expression of HCHL coincided with leaf interveinal chlorosis and necrosis, reduced petal anthocyanin, male sterility in the primary transformants and delayed elongation of internodes in progeny of one line, while lower-expressing lines appeared normal. Microscopic examination of the stem vascular ring of transgenic plants indicated changes in cell wall structure, with an orange/red colouration, distorted cells and reduced phloroglucinol staining.

## **A Complete Arabidopsis GST Database**

Mark Crowe and Martin Trick

John Innes Centre, Norwich Research Park, Colney, Norwich

The BBSRC GARNet microarray project was established to take full advantage of the recently completed *Arabidopsis* genome sequence to produce a microarray with probes to all *Arabidopsis* genes. We have now joined with the European ‘Complete *Arabidopsis* Transcript MicroArray’ (CATMA) consortium to help us to provide the most complete and specific microarray that we can.

An important initial role of bioinformatics in a project of this size, especially one distributed over several sites, is the tracking of samples and the co-ordination of information between the participating groups. Therefore we have developed a database, accessible through the web, to store data on the approximately 30,000 probe sequences expected to be produced. This poster describes the database and, more importantly, how users of the microarray service will be able to access the data.

Information about CATMA is available on our website at: <http://jic-bioinfo.bbsrc.ac.uk/CATMA>. Once the microarrays have been fully validated and quality-controlled, expected to be by early next year, the full database will also be made publically available at this site.

## **Proteomic analysis of lactococcal flp mutants**

Ismail Akyol, Claire Shearman, Francis Mulholland and Mike Gasson  
Institute of Food Research, Norwich Research Park, Norwich

FNR-like proteins act as regulators in both gram positive and gram negative bacteria. Two FNR-like proteins FlpA and B are identified in *Lactococcus lactis* but their role is unclear. flpA gene expression is regulated in response to oxygen level and this expression needs both FlpA and FlpB proteins. A comparative two-dimensional electrophoresis gel study was carried out using flp mutants and wild type strains under anaerobic and aerobic conditions at mid exponential and stationary phase. Gel images were compared using 2D-analysis software and unique spots identified. Individual protein spots were excised from the gel, trypsin-digested, the peptide masses determined by MALDI-tof MS and the \_peptide fingerprint\_ data used in a Mascot database search to identify proteins. The most interesting spot absent in the flpA- and flpAB- mutants was a Glutaredoxin like protein (NrdH). Glutaredoxin acts as a hydrogen donor for ribonuclease reductase and several oxidative stress regulators which switch between an active/inactive form (e.g. FNR). The NrdH protein appears to be regulated by FlpA implying a link with the oxidative stress response.

**Abrogation of upstream open reading frame-mediated translational regulation of *Arabidopsis* S-adenosyl-L-methionine decarboxylase leads to severe growth abnormalities in transgenic plants.**

Colin Hanfrey, Marina Franceschetti, Melinda Mayer and Anthony J. Michael  
Institute of Food Research

S-adenosyl-L-methionine decarboxylase (AdoMetDC) catalyses a key step in the biosynthesis of the essential polyamines spermidine and spermine. All plant AdoMetDC mRNAs identified to date are characterised by an unusually long 5' leader, within which there is a highly conserved arrangement of two overlapping upstream open reading frames (uORFs). Using tobacco plants stably transformed with reporter gene constructs, we have established that the presence of the second ('small') uORF in the *Arabidopsis* AdoMetDC1 5' leader represses translation of a downstream cistron. Heterologous expression of the *Arabidopsis* AdoMetDC1 5' leader in *Saccharomyces cerevisiae* indicated that removal of the first ('tiny') uORF results in small uORF-mediated blocking of downstream translation, but that the tiny uORF alone exerts no repressive effect. In vitro wheat germ translation experiments with mutated AdoMetDC1 mRNA templates largely confirmed in planta results, and established that the small uORF is translated. Elimination of the intact small uORF permitted over expression of AdoMetDC in transgenic *Nicotiana tabacum* plants, due to an increase in translational efficiency, resulting in severe growth abnormalities.

## **Links between protein secretion and membrane synthesis in the filamentous fungus *Aspergillus niger***

Anoushka Dave, David Jeenes and David Archer  
Institute of Food Research

Filamentous fungi such as *Aspergillus niger* are ideally suited for use as cell factories due to their inherent ability to secrete large amounts of protein. However, secretion levels of heterologous proteins are greatly reduced compared to native proteins. To improve the secreted yields of heterologous proteins, it is necessary to identify bottlenecks in the secretion pathway and use the knowledge to overcome the bottlenecks. Secretion involves vesicle trafficking, which requires membrane synthesis. A link between protein secretion and membrane synthesis has been established in the yeast, *Saccharomyces cerevisiae* and we are investigating the possible existence of such a link in *A. niger*. The effects of over-expression of a membrane-bound protein, cytochrome P450 reductase and secretion of a soluble protein, hen egg white lysozyme, on genes involved in the Unfolded Protein Response (UPR) and lipid synthesis are being studied. Electron micrographs suggest that increased membrane synthesis occurs in response to over-expressed membrane-bound protein. Overproduction of cytochrome P450 reductase increases transcription of the lipid biosynthetic genes, *accA* and *inoA* as well as the chaperone, *bipA*, which is known to be upregulated in the UPR. However, no UPR-related motifs have been identified on the *accA* promoter during initial sequence homology studies.

## **Can Suspension Cultured Cells Provide A Rich Source Of Plasmodesmata For Molecular Characterisation?**

Emmanuelle Bayer, Celine Laporte, Christophe Ritzenthaler, and Andrew Maule.  
John Innes Centre, Norwich Research Park, Norwich, NR4 7TJ

Plasmodesmata are channels within the plant cell wall that create symplastic continuity between cells. They are complex highly regulated structures that control the passage of large molecules between cells, and have a profound influence on plant development. Despite their importance, we still understand very little about their structure or the nature of their constituents. This poster reports some preliminary observations on the suitability of suspension culture cells as sources of plasmodesmata for molecular characterisation. The emergence of the proteomic technology linked with complete genome sequence has created new opportunities for a deeper understanding of plasmodesmal composition and function. In an attempt to fill the gap in our understanding, we are exploring new approaches for the isolation and characterisation of plasmodesmal proteins. Suspension culture cells form simple, primary plasmodesmata during the formation of the cell plate. To test whether large molecules can interact with them in a manner akin to that seen in intact tissues, a viral movement protein fused to GFP (MP-GFP) was inducibly expressed in BY-2 cells and its incorporation into plasmodesmata recorded. In parallel, plasmodesmata in *Arabidopsis* suspension cells were also observed using electron microscopy and immunofluorescent techniques. Both sources of cells were used for cell wall purification and preliminary assessments of protein profiles established.

## **Testing the structural model of maize streak virus: identification of domains important for encapsidation and infection of maize**

Carolina G. Casado, Lee Faulkner, Robert McKenna, Huanting Liu, Jeffrey W. Davies and Margaret I. Boulton

John Innes Centre, Norwich Research Park, Norwich, NR4 7UH

The Nigerian strain of Maize streak virus (MSV-N) has geminate (twinned) particles containing a single stranded (ss) DNA genome. The particle consists of two joined incomplete  $T = 1$  icosahedra and a total of 110 coat protein (CP) subunits, organised as 22 pentameric capsomers. The CP is the only protein detected in particles. A predicted structural model of the MSV CP, based on alignment of the MSV CP with the CP of the satellite of Tobacco necrosis virus, has been reported (1). The MSV-N CP is predicted to have an eight-stranded antiparallel  $\beta$ -barrel motif with an N-terminal 'Q-helix that is likely to be involved in DNA binding (2).

We are using the predicted structure of the CP to identify residues likely to be important for encapsidation or CP-CP interaction and residues likely to be on the surface of the particle. Mutagenesis of these residues allows us to assess the ability of the mutant genomes to infect maize plants and cell cultures. The ability of the mutants to form stable particles and to be transmitted by the insect vector is also evaluated. To date, we have identified mutants producing altered symptom phenotypes, and mutants unable to spread systemically in plants. All of the mutants that are unable to infect plants are competent for replication in single cells, although they can be further differentiated by their ability to direct the accumulation of ss DNA in infected cells.

We will describe the phenotypic, molecular and biochemical characteristics of the mutants and attempt to explain the characteristics with relevance to the predicted MSV CP and particle structure.

1. Zhang, W., Olson, N. H., Baker, T. S., Faulkner, L, Agbandje-McKenna, M, Boulton, M. I., Davies, J. W. & McKenna, R. (2001). Structure of the maize streak virus geminate particle. *Virology* 279, 471-477. 2. Liu, H., Boulton, M. I. & Davies, J. W. (1997). Maize streak virus coat protein binds single- and double-stranded DNA in vitro. *J. Gen. Virol.* 78, 1265-1270.

## **Molecular analysis of Avra12 in barley powdery mildew fungus *Blumeria graminis* f.sp.hordei.**

Paraskevi Skamnioti, Chris Ridout, James Brown  
John Innes Centre, Norwich Research Park1, Norwich, NR4 7UH

*Blumeria graminis* f. sp *hordei* causes powdery mildew in barley. Over 30 avirulence genes have been described for this pathogen, and these are uniquely recognised by race-specific resistance (R) genes in the host. We have initiated a programme to isolate Avra12, recognised by the R gene Mla12 which has recently been cloned from barley. Extensive bulk segregant analysis in the cross CC52xDH14 with AFLP markers resulted in obtaining markers only proximal to the gene, with the closest being 10cM. Since, presumably this is a region of high recombination, attempts are being made to establish the physical/genetic distance. As the gene is more likely located in the subtelomeric region of the linkage group III, in order to find markers distal to the gene, we initiated telomere-mapping, to identify telomere-associated sequences linked to Avra12. Furthermore, a new cross CC148xDH14 was set up, in order to map Avra12, in relation to other avirulence genes that segregate in this cross. FLP analysis provided us with a closer marker (approximately 3 cM) which is being used for physical mapping. To complement the mapping approaches, we look for differences in expressed genes, consistent with Avra12 phenotype, by conducting high throughput suppression subtractive hybridisation.

## **Genetic Analysis of cajal body mutants in *Arabidopsis thaliana***

Sarah Wastell Peter Shaw Liam Dolan

John Innes Centre, Norwich Research Park, Norwich, NR4 7UH

Cajal (CBs) are small spherical structures located in the cell nucleus. They are found in both plant and animal cells, and are dynamic, mobile structures. They contain small nuclear ribonucleoproteins, small nucleolar ribonucleoproteins, nucleolar proteins, as well as coilin – a protein regarded as diagnostic for CBs. CBs frequently associate with the nucleolus and were first identified by Ramon y Cajal a century ago, and termed nucleolar accessory bodies. Their precise function remains unknown, although it has been suggested that CBs may play a role in spliceosome assembly or may control the transport of splicing components within the nucleus. We have developed an *Arabidopsis* line transformed with a U2B<sub>1</sub>::GFP construct, which enables the visualization of the CBs by fluorescence microscopy in living plants. This line was mutagenised and the resulting populations were screened by microscopy for CB mutants. At present four distinguishable mutants have been identified; no cajal body, small cajal body, cap cajal body and poly cajal body. We initially decided to map and characterize the ncb mutant. This mutant appears to have completely lost all observable CBs in all cell types examined. Mapping and sequence analysis have shown that the mutation occurs within a splice junction of coilin.

## **Infrared Spectroscopic Analysis Of The *Chara Corallina* Cell Wall Under Deformation**

Geraldine A. Toole, Marta Kahurakova, Andrew C. Smith, Keith W. Waldron, Reginald H Wilson  
Institute of Food Research, Norwich Research Park, Norwich

Fourier Transform Infrared microspectroscopy was used to investigate both the chemical composition, and the effects of an applied strain on the structure of the *Chara* cell wall, both in its native state and following the removal of various biopolymers by a sequential extraction. FT-IR microspectroscopy with polarised radiation (infrared dichroism) was used to examine the orientation of different biopolymers in cell wall samples subjected to longitudinal strain. In the *Chara* system, the microfibrils that were initially aligned predominantly transverse to the length of the cell became increasingly transverse in orientation as longitudinal strain increased. Simultaneously, the pectic polysaccharide matrix underwent molecular orientation parallel to the direction of strain. Following extraction in CDTA, the microfibrils were again oriented transversely to the strain direction however the pectic polysaccharides of the matrix were not detected in the dichroic difference spectra. After a full sequential extraction, the cellulose microfibrils, now with greatly reduced crystallinity, were responsible for longitudinal load bearing, and were oriented parallel to the direction of strain. A shift in frequency and the conversion to a symmetric bipolar band, were observed as the sample was strained, due to a molecular deformation of the glycosidic bond.

## **The Effect Of Physical And Chemical Treatment On The Mechanical Properties Of The Cell Wall Of The Algae *Chara Corallina***

Geraldine A Toole, Andrew C. Smith, Keith W. Waldron  
Institute of Food Research, Norwich Research Park, Norwich

The mechanical properties of the cell wall are of great importance. By imposing a series of physical and chemical treatments on plant cell wall samples, then determining the changes in tensile strength and modulus experienced, it is possible to show the relative impact of the various components of the cell wall on its overall mechanical properties. The extremely large size of the individual cells of Charophytes, such as *Chara corallina* makes it possible to obtain material for a useful model system for studying the mechanical properties of plant cell walls. The aim of this research was to determine the stiffness and fracture strength, in tension, of intact cell-wall material derived from single cells of *Chara corallina*, following physical and chemical extraction treatments designed to remove classes of cell wall components, in other words to sequentially deconstruct the chemical structure of the cell wall. The carbohydrate composition of the cell walls was determined following each treatment. The tensile strength, modulus and notch sensitivity of the *Chara* cell wall were determined previously (Toole et al. 2001) and this method was used here to determine the changes in the mechanical properties of the cell wall following selected physical and chemical treatments. Tensile tests, using prepared test pieces of extracted *Chara* cell wall with a single-edge notch, were carried out on a universal testing machine. The tensile strength and the Young's modulus were calculated from the data obtained, and compared with the cell-wall carbohydrate composition.

Toole GA, Gunning PA, Parker ML, Smith AC, Waldron KW (2001) Fracture mechanics of the cell wall of *Chara corallina*. *Planta* 212: 606-611

## **Physical and genetic mapping of transgenes in barley**

Lorelei Bilham, Wendy Harwood, Haroldo Salvo, Silvia Travella, Judith Harden, Lesley Fish and John Snape.

John Innes Centre, Norwich Research Park, Norwich, NR4 7UH

The most important part of the transformation process over which, at present, we have no control is the site of transgene integration. It is this area we are working to address, by developing and applying methodologies to determine the physical and genetic location of transgenes.

Fluorescent in situ hybridisation (FISH) has allowed us to locate transgenic DNA in barley by hybridisation of a labeled DNA plasmid probe, made from the introduced transgene, to DNA in a chromosome preparation. Characterised rDNA marker probes were used to give distinct and specific chromosomal patterns to ambiguously identify the individual barley chromosomes. FISH was used as a starting point for fine interchromosomal genetic mapping using Restriction Fragment Length Polymorphisms (RFLP) and Simple Sequence Repeat (SSR) markers to determine the genomic map location of the introduced DNA.

The physical location of transgenes has been determined at the chromosomal level by FISH in 14 independent transgenic barley lines. Genetic mapping has confirmed the genomic location of transgenes in 8 of these lines.

FISH has proved to be a very efficient method for the physical mapping of transgenes. Our results suggest that in barley, following transformation, transgene integration may not be random since transgenes were found in only 4 of the 7 barley chromosomes.

## **Post-translational processing of BldN, a sigma factor required for aerial mycelium formation in *Streptomyces coelicolor* A3(2)**

Bibb, Maureen J. and Buttner, Mark J.  
John Innes Centre, Norwich Research Park, Norwich,

BldN is a sigma factor in *Streptomyces coelicolor* and is involved in aerial mycelium formation. bldN point mutants exhibit a whi phenotype, forming aerial hyphae but unable to undergo normal levels of sporulation, whereas the null mutant has a bld phenotype, unable to produce aerial hyphae. bldN is also part of a complex transcriptional cascade involving other, previously defined, bld mutants. BldD functions as a repressor of bldN transcription, and bldG and bldH indirectly repress the transcription of bldN. bldN in turn directs the transcription of bldM, a response regulator, via one of its two promoters. BldN is an unusual sigma factor. As well as showing end-to-end homology to other ECF sigma factors, it has an 86 amino acid N-terminal extension. Polyclonal antibodies have been raised against BldN and used, together with mutational analysis, to investigate the post-translational processing of the sigma factor in *Streptomyces coelicolor*. Evidence for this post-translation regulation will be presented.

## **Using oat mutants to investigate starch synthesis in the endosperm**

Tamara Verhoeven and Kay Denyer

Starch is used as a major storage compound in plants and is one of the most abundant and important components of the human diet. Although the use of mutants in maize have improved our knowledge of starch synthesis in cereal endosperm, starch synthesis in cereals like rice and oats differs from maize because compound granules are formed due to multiple initiations per plastid. To investigate starch synthesis and in particular starch granule initiation in cereals, a mutagenised population of diploid oat (*Avena strigosa*) has been screened and two kinds of mutant in which starch synthesis, starch composition and starch structure are altered, are discussed.

## **DIR1 encodes a putative lipid transfer protein involved in systemic acquired resistance in *Arabidopsis thaliana***

Ana M.Maldonado<sup>1</sup>, Robin K.Cameron<sup>2</sup>, Peter Doerner<sup>3</sup>, Richard A.Dixon<sup>4</sup> and Chris Lamb<sup>1</sup>

<sup>1</sup>John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH

<sup>2</sup>Department of Botany, University of Toronto, 25 Willcocks Street, TORONTO, Ontario, M5S 3B2

<sup>3</sup>ICMB, University of Edinburgh, Mayfield Road, EDINBURGH, Scotland, EH9 3JR

<sup>4</sup>The Noble Foundation, P.O. Box 2180, ARDMORE, Oklahoma, OK 73402, USA

Systemic acquired resistance (SAR) is a broad-spectrum plant defense response induced after a local infection by a necrotizing pathogen. SAR has been observed in many systems, but the nature and transduction of the mobile signal in the distal leaves are not yet understood. We have screened *Agrobacterium tumefaciens*-DNA tagged lines of *Arabidopsis thaliana* for mutants specifically compromised in SAR. The *dir1-1* mutant (defective induced resistance) exhibits normal local resistance to both avirulent and virulent *Pseudomonas syringae* but fails to develop SAR to virulent strains of this bacterium or to an oomycete pathogen. Interestingly, this novel mutant exhibits wild type defense responses at the site of the initial inoculation, but the PR genes are not induced in uninoculated distant leaves. These data indicate that *dir1-1* is affected in the production or transmission of an essential mobile signal from the inoculated leaf to the rest of the plant. DIR1 encodes a putative apoplastic lipid transfer protein and we propose that the DIR1 protein may interact with a lipid-derived signal molecule.

## **The Rb-binding motif of maize streak virus RepA is required for interaction with Rb in yeast but not for infection of maize plants.**

David R. McGivern, Nicholas Montague, Jeffrey Davies and Margaret I. Boulton  
John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH

Maize streak virus (MSV) is a member of the genus *Mastrevirus* of the family *Geminiviridae*. It has a single-stranded DNA genome of 2.7 kb and despite its small genome size, the virus is able to replicate and infect most cereals and grasses. To do this, the virus uses many host enzymes, and must modify the host cell cycle in order to create an environment permissive for viral DNA replication. Of the four proteins encoded by the MSV genome, two, Rep and RepA, are produced early in infection. Rep and RepA are produced from the same transcription unit via an inefficient splicing event, with Rep being produced from the spliced transcript. Rep is required for viral rolling-circle replication and RepA is involved in host cell cycle modification. Together, Rep and RepA are able to activate viral late gene (coat protein, CP) expression. The RepA protein interacts with the maize retinoblastoma related protein (Rb) in the yeast two-hybrid system. This binding occurs via a conserved Rb binding domain (LxCxE) identified in RepA of most mastreviruses and, according to current models, leads to cells progressing into S-phase by a mechanism analogous to that used by some animal tumour viruses. Experiments were performed to determine the effects of mutations within the Rb binding motif of MSV RepA and Rep upon CP promoter (CPp) activation, virus replication and infectivity in plants, and Rb binding in the yeast two-hybrid system. Mutagenesis of LxCxE to IxCxE decreased binding of RepA to Rb in yeast, whilst mutation to LxCxK abolished the interaction. When the mutations were introduced into the MSV genome, both mutants were able to infect maize plants, and unlike a wheat dwarf virus LxCxK mutant, the MSV LxCxK-containing mutant was able to replicate in bombarded maize suspension cells. Thus, assay of the Rb-binding function of MSV RepA in yeast may not correlate with infectivity of the virus in plants. However, the LxCxK mutant did not produce wild type symptoms in maize plants, but gave a narrow streak phenotype. Although similar symptoms may be produced using MSV mutants affected in CP production, the LxCxK containing Rep and RepA were able to activate the CPp to wild type levels in transient gene expression assays. Therefore, a limitation of CP expression may not be responsible for the mutant phenotype. An alternative hypothesis is that the Rb binding activity is required to create a replication-permissive environment in mesophyll cells, but not in the vascular system, where the virus uses pre-existing DNA replication enzymes. Currently the extent of tissue invasion by the LxCxK mutant is being investigated by *in situ* hybridization in maize leaves.

## **Effect of sodium chloride and pH on the distribution of the lag times of individual cells of *Listeria innocua***

A. Metris, S. Georges, M. Peck, J. Baranyi

John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH

While the growth rate of pathogens in food can commonly be predicted as a function of the environmental factors, the lag time is difficult to model. Because of low infectious doses of pathogens, such as *Escherichia coli* O157:H7, stochastic models are more useful than deterministic models to describe the lag time within a population. It is well-known that both the environmental conditions and the history of the cells (e.g. heat or acid stress) influence the lag. In this study, the lag time distribution of individual *Listeria innocua* has been studied using automated turbidometric measurements (Bioscreen; Labsystems, Basingstoke, UK). The experiments were based on the principle that the distribution of the lag times should be reflected by the distribution of the detection times of the turbidity of the wells. The effect of different growth conditions, characterised by pH and NaCl concentrations, have been investigated while the inoculum was always prepared in the same conditions (same history). Gamma distributions were used to describe the lag time distributions. It was found that the more hostile the conditions for the micro-organisms, the higher the mean and the more spread the distributions were.

## **Crystallographic analysis of the molybdenum cofactor biosynthesis binding protein MobA from E.coli.**

C. E. M. Stevenson, A. Guse, F. Sargent, G. Buchanan, T. Palmer and D. M. Lawson.  
John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH

All mononuclear molybdoenzymes bind molybdenum in a complex with an organic cofactor termed molybdopterin. In many bacteria including *Escherichia coli*, molybdopterin can be further modified by the attachment of a GMP group to the terminal phosphate of molybdopterin to form molybdopterin guanine dinucleotide (MGD). This reaction requires GTP and MgCl<sub>2</sub> and is catalysed by the cellular enzyme MobA.

The crystal structure of MobA has been solved using MAD phasing with selenomethionine substituted protein and subsequently refined against native data to 1.35 Å resolution. The structure consists of a central predominantly parallel β sheet sandwiched between two layers of α helices and resembles the dinucleotide binding Rossmann fold. There is a wide depression lined by a number of strictly conserved residues on one face of the molecule where substrate binding and catalysis take place. Through comparisons with a number of structural homologs plausible functions have been assigned to several of these residues lining this substrate binding pocket.

**A small signalling molecule that stimulates antibiotic production in *Streptomyces coelicolor* A3(2)**

Takano, E., Nihira, T\*., Yamada, Y.,\* and Bibb, M.J.\*\*

John Innes Centre, UK

\*Osaka University, Japan

\*\* Diversa Corp., USA

To assess the role of diffusible signalling molecules in antibiotic production in *Streptomyces coelicolor* A3(2), we have identified  $\sigma$ -butyrolactones isolated from transition and stationary phase cultures and determined the structure of the most abundant factor (SCB1)(Takano *et al.*, 2000 J.Biol.Chem. 275;11010-16). A homologue (*scbA*) of *afsA*, an essential gene for A-factor synthesis in *S.griseus*, was cloned from *S.coelicolor* and found to be located divergently from a homologue (*scbR*) of *arpA*, which in *S. griseus* encodes a  $\sigma$ -butyrolactone binding protein. *ScbA* and *scbR* mutants produced no  $\sigma$ -butyrolactones, yet *scbA* mutant overproduced two antibiotics, while *scbR* mutant showed delayed production of Red. These phenotypes differ from those expected with the *S. griseus* A-factor system. Transcription analysis indicates that ScbR represses its own expression while activating that of *scbA*. Evidence also suggest that ScbA is a positive autoregulator in addition to being required for  $\sigma$ -butyrolactone production (Takano *et al.*, 2001 Mol.Microbiol. 41(5)1015-1028). Overall, these results indicate a complex mechanism for  $\sigma$ -butyrolactone-mediated regulation of antibiotic biosynthesis in *S. coelicolor*.

## **Characterisation of RIK1-Rar1 interacting kinase**

Maria Jodo Santos-Rosa and Ken Shirasu

Sainsbury Laboratory, John Innes Centre, Norwich Research Park, Colney, Norwich,  
NR4 7UH

The RAR1 gene is required for resistance against a wide range of pathogenic powdery mildew fungi, triggered by different R genes in barley. To understand how the RAR1 protein functions, the focus has been on identification of RAR1 interactors.

Recently, one serine/threonine kinase was isolated in a yeast 2-hybrid system using CHORD-I domain of the AtRAR1 protein as bait. Rik1- for rar1 interacting kinase- belongs to Raf-Mpk family.

It is possible that Rik1 is involved in RAR1 phosphorylation and subsequently be determinant in this signal transduction pathway.

## **Expression of a bacterial gene in tobacco plants affects phenylpropanoid metabolism, cell wall content and texture**

Zara M. Harrison, Melinda J. Mayer, Mary L. Parker, Andrew C. Smith, Anthony J. Michael and Keith W. Waldron  
Institute of Food Research, Norwich Research Park, Colney, Norwich

A previously isolated bacterial gene encoding the enzyme 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL), which has been shown to convert p-coumaroyl CoA, caffeoyl-CoA and feruloyl-CoA to the corresponding benzaldehydes (Narbad & Gasson, 1998, Gasson et al., 1998), has been expressed in tobacco plants (Mayer et al., 2001.). Plants expressing the HCHL gene exhibited a severe phenotype, including some features characteristic of plants with an altered phenylpropanoid metabolism. Stunted growth, interveinal chlorosis, curling of leaf margins and coloration of the xylem were observed in young transgenic tobacco plants and have been associated with the HCHL gene expression (Mayer et al., 2001). Cell wall material from plants expressing the phenotype (phe ) showed no significant changes in the carbohydrate composition of their cell walls, however a possible increase in uronic acid was observed in the stem tissues. A reduction in cell wall bound phenolics was shown in the leaves (trans-p-coumaric acid, trans-ferulic acid and p-coumaric acid), in less lignified stem tissue (trans-ferulic acid) and in highly lignified stem tissue (trans-ferulic acid and p-coumaric acid). All of these transgenic plants also showed a clear reduction in soluble phenolics such as chlorogenic acid and rutin and an accumulation of novel metabolites. Klason lignin showed a 50% reduction in the leaves from phe and using the DFRC method (derivatization followed by reductive cleavage) coniferyl and sinapyl peaks were also greatly reduced compared to the plants without the phenotype. HCHL sequesters the phenylpropanoid metabolites into an alternative pathway, affecting cell wall composition and architecture. Future work will entail analysing tobacco plants expressing the gene at lower levels, HCHL enzyme activity, sub cellular fractionation, comparison of cell wall biochemistry in cell suspension cultures and digestibility studies.

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