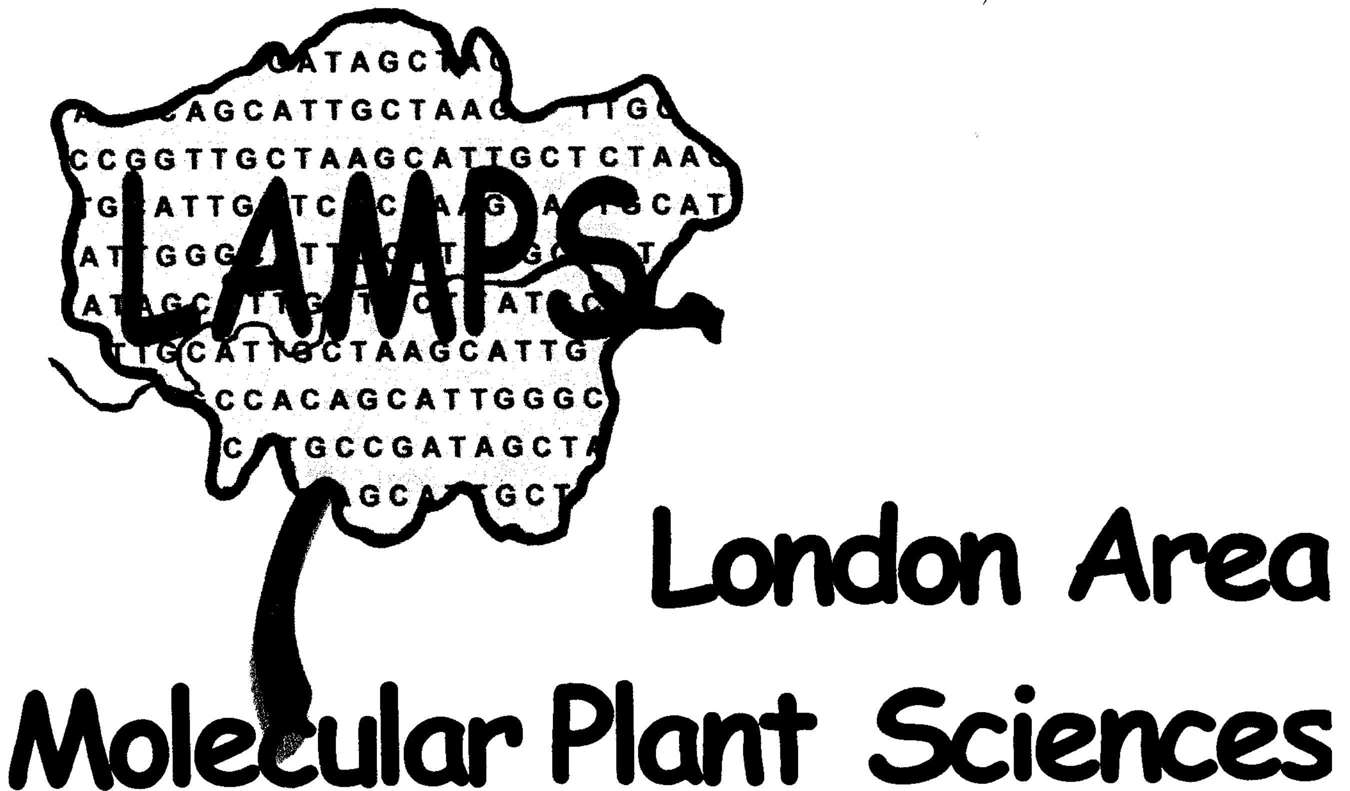

London Area Molecular Plant Sciences

Organisers:
Royal Holloway, University of London
University College London
12 September 2002

Abstracts

London Area Molecular Plant Sciences Symposium – Programme

12th September 2002, University College London

8.45 am	Registration desk opens – North Cloisters	
9.20 am	Opening comments – John Bowyer, Chadwick Lecture Theatre	

Photosynthesis and photobiology Chair – Saul Purton

9.30 am	Dr Conrad Mullineaux (UCL)	*Role(s) of the FtsH protease in assembly and turnover of the photosynthetic apparatus*
10.00 am	Dr Alec Forsyth (UCL)	*Electron transport through photosystem 1*
10.15 am	Ms Alison Hills (RHUL)	*Light control of chloroplast development: did phytochrome hitchhike a pre-existing pathway?*
10.30 am	**Coffee – North Cloisters**	

Plant responses to abiotic stress Chair – Paul Devlin

11.00 am	Ms Suki Kasinathan (UCL)	*Effects of mutations in polyamine metabolism on salt tolerance*
11.15 am	Mr Nicholas Fourrier (RHUL)	*Sequence and activity of homologs of the SFR2 freezing tolerance gene*

Plant pathogen interactions Chair – Paul Devlin

11.30 am	Dr Murray Grant (IC Wye)	*The difference between disease or resistance; interdiction of pathogen effectors*
12.00 pm	Dr Richard Thwaites (IC Wye)	*Regulation of the type III secretion system in Pseudomonas syringae PV. Phaseolicola*
12.15 pm	Ms Jane Hollywood (UCL)	*Biological control of late blight of potatoes*
12.30 pm	Dr James Whiteford (IC)	*Function and localisation of hydrophobins in the tomato pathogen Cladosporium fulvum*
12.45 pm	**Lunch – North Cloisters**	
1.30 pm	Discussion about future LAMPS activities – Chadwick Lecture Theatre	

Cell and developmental biology Chair – Murray Grant

2.00 pm	Professor Jeff Duckett (QMUL)	*On the many ways to making water-conducting cells: novel insights from immunocytochemistry*
2.30 pm	Dr Lazlo Bögre (RHUL)	*Spatial regulation of plant mitosis studied by GFP fusions*
2.45 pm	Dr Lisa Harper (QMUL)	*How do gallwasps reprogramme plant development?*

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Plant natural products and chromosome structure Chair – Murray Grant

3.00 pm	Dr Geoff Kite (RBG Kew)	*Hyphenated mass spectrometry techniques in natural product research at RBG Kew*
3.30 pm	Dr Eva Sykorova (QMUL)	*Non-classical telomeres in plants*
3.45 pm	**Tea – North Cloisters**	

Plant Biotechnology Chair – Tony Stead

4.15 pm	Professor Paul Bolwell (RHUL)	*Engineering secondary cell walls for novel fibre resources*
4.45 pm	Dr Patricia Obregon (KCL)	*Expression of a novel HIV-1/IgA recombinant fusion protein in plants*
5.00 pm	Dr Pascal Drake (KCL)	*Expression strategies and potential remediation applications of antibodies in plants*

5.15 pm Closing remarks followed by reception in North Cloisters

ROLE(S) OF THE FTSH PROTEASE IN ASSEMBLY AND TURNOVER OF THE PHOTOSYNTHETIC APPARATUS

Conrad Mullineaux and Elinor Thompson

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FtsH proteases are membrane-anchored, zinc-dependent ATPases which may also act as molecular chaperones. They are very widespread in bacteria, mitochondria and chloroplasts. *Arabidopsis* has at least 9 nuclear-encoded FtsH proteins (of which around 6 are probably in the chloroplast) and the cyanobacterium *Synechocystis* 6803 has 4. In collaboration with the groups of Nick Mann (Warwick University) and Peter Nixon (Imperial College) we have been exploring the roles of two very homologous FtsH proteins: the slr0228 gene product in the cyanobacterium *Synechocystis* 6803 and the chloroplast Var2 FtsH in *Arabidopsis thaliana*. Our strategy has been to look at the phenotypes of *fts*H null mutants. Both the *Arabidopsis* and the *Synechocystis* mutants show very poor recovery from photoinhibition and are extremely impaired in turnover of the D1 protein of Photosystem II, indicating that Var2 and slr0228 are the key proteases required for degradation of photodamaged D1 in their respective organisms. In *Synechocystis* slr0228 is also required for a slow turnover of fully-functional Photosystem II in the dark, and preliminary results suggest this may also be the case with Var2 in *Arabidopsis*. In *Synechocystis* the slr0228 null mutant is also impaired in the assembly of Photosystem I, suggesting that slr0228 functions as a chaperone as well as a protease. However, there is no indication of this in the *Arabidopsis* Var2 mutant. Maybe in *Arabidopsis* this function is performed by another FtsH protein.

INVESTIGATION OF ELECTRON TRANSPORT THROUGH PHOTOSYSTEM I USING SITE-DIRECTED MUTAGENESIS OF PsaA and PsB

Alec Forsyth[1], Mike Evans[1], Wendy Fairclough[1+2], Peter Heathcote[2] and Saul Purton[1]

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Photosystem I (PSI) uses light energy to transfer electrons from plastocyanin to ferrodoxin. A crystal structure of a cyanobacterial PSI complex reveals that the co-factors for electron transport are arranged symmetrically within the core proteins PsaA and PsaB (Jordon *et al.* 2001). Spectroscopic analysis of wild-type (Joliot and Joliot 1999) and mutant (Purton *et al.* 2001, Muhiuddin *et al.* 2001) PSI complexes suggest that both possible pathways of electron transport (A and B) can operate. Clear evidence for electron transport via co-factors that constitute pathway A has been obtained by analysis of *Chlamydomonas reinhardtii* cells expressing point mutations within the *psaA* gene (Purton *et al.* 2001, WF *et al.* unpublished). To confirm electron transport via both pathways we are constructing *C. reinhardtii* mutants that express PSI complexes that should have altered electron transport along pathway B. Mutations predicted to affect the accessory chlorophyll and phylloquinone binding pocket of pathway B have been introduced into the *psaA* and *psaB* genes of *C. reinhardtii*. The results of spectroscopic analysis of the mutant complexes are consistent with electron transfer through both A and B pathways.

Joliot and Joliot (1999) Biochemistry 38:11130-11136.
Jordon et al. (2001) Nature 411:909-917.
Muhiuddin et al. (2001) FEBS Letters 503:56-60.
Purton et al. (2001) Biochemistry 40:2167-2175.

LIGHT CONTROL OF CHLOROPLAST DEVELOPMENT: DID PHYTOCHROME HITCHHIKE A PREEXISTING PATHWAY?

Alison Hills and Enrique López-Juez

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Between one quarter and half of all phytochrome-regulated genes, when seedlings are first exposed to light, encode proteins targeted to plastids. The same genes are expressed only when the plastids are functional, and are thus under the control of a chloroplast-to-nucleus communication signal (plastid signal). We have examined to what extent those two signals may be related. In most gymnosperms, like pine, there is only a minor control of chloroplast development by light (greening occurs in the dark), and so we have analysed whether this may help separate light and plastid signals. A pine *PtLhcb* promoter is active in the dark in pine, and also in transgenic tobacco, driving GUS. We have found that treatments that result in chloroplast bleaching (in the light) or disrupt plastid translation (in the dark or the light) inhibit the expression of *PtLhcb*::GUS in tobacco, measured as both activity or mRNA levels. Equivalent treatments have the same effect in pine on the expression of the native promoter. It appears that the 'plastid signalling' pathway is ancestral and predates the appearance of phytochrome control of greening in angiosperms. We propose a model, according to which angiosperm photosynthetic promoters have acquired the ability to have the preexisting plastid response repressed in the dark. Light would, through the same promoter elements, allow the plastid response to be expressed, so the gene is activated. Only in the presence of functional plastids would the light response be possible.

EFFECTS OF MUTATIONS IN POLYAMINE METABOLISM ON SALT TOLERANCE

Suki Kasinathan and Astrid Wingler

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Polyamines have been suggested to be involved in the protection of plants against salt stress, although their exact role is under dispute. In order to determine the importance of polyamine synthesis for salt tolerance, four *Arabidopsis* mutants of the polyamine biosynthetic pathway (*mto1-1*, *mto2-1*, *spe1-1* and *spe2-1*) were compared to their background lines. The *mto* mutants both over-produce methionine, an important substrate in the production of spermine and spermidine, while both *spe* mutants have reduced levels of the arginine decarboxylase, a key enzyme in polyamine synthesis. Seedlings were grown on MS medium at concentrations of 0 or 100 mM NaCl. After 13 days, the plants were transferred to Magenta Vessels containing either 0 or 150 mM NaCl until bleaching was observed (usually after one week). The fresh weight and overall chlorophyll content in *spe1-1* was significantly less than in its background line, whereas both fresh weight and chlorophyll content were significantly increased in *mto1-1*. No clear relationship was observed with the level of salt tolerance and fresh weight or chlorophyll content in *spe2-1* or *mto2-1*. F_v/F_m, which reflects maximum photosynthetic efficiency, was measured both on the 13th day and the final day of growth. Results indicated that the *spe* mutants had a lower F_v/F_m than their background lines, whereas the *mto* mutants showed no significant difference. Overall results suggest that polyamines may play an important role in the protection of plants against salt stress. Further work will examine the polyamine content of these mutants and their background lines under stress conditions.

SEQUENCE AND ACTIVITY OF HOMOLOGS OF THE *SFR2* FREEZING TOLERANCE GENE

Nicolas Fourrier, Glenn Thorlby and Gary Warren

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The *AtSFR2* gene of *Arabidopsis thaliana*, which encodes a novel beta-glycosidase, is essential for freezing tolerance. *SFR2* homologues are detectable in the rice genome and in EST databases derived from a number of other freezing-sensitive species. A full-length homologue (*GmSFR2*) has been obtained from soybean and we are in the process of obtaining and characterising the full *SFR2* homologues from maize and pine.

Sequence comparisons among the various *SFR2* homologues indicate strong conservation of key protein structural features and absolute conservation of the active site residues. This implies that there has been continued selection for enzymatic function even in the freezing-sensitive species. Thus *SFR2* is likely to have a different role, which might be additional or alternative, in the freezing-sensitive species.

We are testing the ability of the various *SFR2* homologues to complement the *sfr2-* mutation in *Arabidopsis*, to determine whether they have the functionality of the *AtSFR2* gene in freeze-protection. We hope to be able to report on the first such test with *GmSFR2* at the LAMPS meeting.

THE DIFFERENCE BETWEEN DISEASE OR RESISTANCE; INTERDICTION OF PATHOGEN EFFECTORS.

Murray Grant, Marta de Torres Zabala, Antonius Al-Doaude, Isabelle Fernandez-Delmond. Department of Agricultural Science, Imperial College at Wye, Wye, TN25 5AH E-mail m.grant@ic.ac.uk

During the transition from infection to induced resistance following challenge of *A. thaliana* leaves carrying the *RPM1* disease resistance gene with *P. syringae* carrying the cognate avirulence gene, *avrRpm1*, we relate key landmark events involving transcriptional (*in planta* avirulence gene induction), biochemical (increases in host $[Ca^{2+}]_{cyt}$) or macropathological (leaf collapse) processes to changes in host transcript levels. We show the host plant responds rapidly, dynamically and discriminately to infection by phytopathogenic bacteria at two distinct levels corresponding to basal and race specific responses respectively. Type III effector proteins do not contribute to immediate early events in host transcriptome reprogramming. The timing of induction for specific transcripts is reproducible, hierarchical and modulated at least in part through EDS1 function. *R* gene specific signatures are first observed 180 min post infection and are predicted to be targeted to diverse cellular addresses indicative of an immediate global impact on cellular homeostasis.

To be able to react rapidly and specifically to its cognate avirulence gene product we predict RPM1 exists in a multiprotein complex. We have isolated two members of this signalling complex or "resistosome" , RIN12 and RIN13, that act to modulate RPM1 function. Transgenic plants ectopically expressing *RIN12* or *RIN13* are both hyper-resistant **and** significantly delay or abolish hypersensitive cell death elicited by challenge with bacteria expressing *avrRpm1*. Plants expressing antisense of either *RIN* gene significantly enhance bacterial growth in the presence of *avrRpm1*. In all transgenic lines the timing of the HR, and restriction of bacterial growth in the *avrRpt2/RPS2* interaction is identical to wild type plants.

REGULATION OF THE TYPE III SECRETION SYSTEM IN *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA*

Richard Thwaites[1], Pietro Spanu[2], Conrad Stevens[3] and John Mansfield[1]

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The plant pathogen *Pseudomonas syringae* pv. *phaseolicola,* in common with other Gram-negative bacterial pathogens, delivers proteins directly into host cells via a type III secretion system (TTSS). TTSSs are an absolute requirement for pathogenicity in many plant-pathogenic bacteria, where they are encoded by *hrp* gene clusters. The system consists of a surface appendage, the type III pilus, and a complex arrangement of proteins located within the bacterium at the base of the pilus. The structure and functions of some of these proteins are conserved between Gram-negative bacterial pathogens of both animals and plants. Relatively little is known about the precise regulatory mechanisms governing the TTSS in *P. syringae* pv. *phaseolicola,* or indeed in any plant-pathogenic bacterium. However, similarities between elements of the TTSS regulatory system and other control mechanisms, notably that governing flagellar biosynthesis, can be observed. Furthermore, the complexity of TTSSs implies that some degree of coordinated expression of genes encoding both TTSS components and secreted proteins is necessary.

In an effort to elucidate the nature of such a control mechanism in *P. syringae* pv. *phaseolicola,* and in particular to discover the order in which TTSS genes are transcribed, we have employed quantitative reverse-transcriptase polymerase chain reaction amplification techniques. Primers targeted to genes representing all operons within the *hrp* cluster have revealed that there is indeed some ordering amongst the expression of TTSS genes, which in *P. syringae* pv. *phaseolicola* are likely to be controlled by more than one pathway. This probably involves regulatory elements that remain, as yet, unknown. Analysis of genes encoding effector proteins suggests that expression of these genes may be triggered by a number of different cues. Real-time polymerase chain reaction amplification using the Taqman system is proving a useful tool in assaying differences in both the timing and levels of expression of TTSS genes.

BIOLOGICAL CONTROL OF LATE BLIGHT OF POTATOES

Jane Hollywood and Richard Strange

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Phytophthora infestans, the causal agent of late blight, is a devastating pathogen of potatoes and tomatoes world-wide. Present control measures are expensive, environmentally undesirable and increasingly ineffective. Biological control represents a realistic solution. This investigation adapts an *in vivo* bioassay for the selection of soils antagonistic to late blight of potatoes. Of the eight soil samples screened, four demonstrated reproducible antagonism to the development of infection of potato tubers by *Phytophthora infestans* as determined by the volume of rotted tissue. Microorganisms were then isolated from suppressive soils using a variety of selective and non-selective media. In total 292 bacterial samples and 20 fungal samples were recovered from the soil samples. When they were tested individually against *P. infestans* using the same assay as above 15 bacterial isolates suppressed tuber rotting by >85% in at least three out of four assays. These isolates were characterised to strain level as *Pseudomonas* spp. (3 strains), *Enterobacter* spp. (7 strains), *Bacillus* spp. (1 strain), *Pantoea* spp. (2 strains), *Citrobacter* spp. (1 strain) and *Buttiauxella* spp. (1 strain) by routine bacteriological tests, fatty acid profiling and partial sequencing of the gene encoding for 16S ribosomal RNA. Further analysis of the antagonists included determination of mode of action and population dynamics. Work is in progress towards developing a consortium of biological agents with different modes of action for application to potatoes since this strategy would avoid placing a single selection pressure on the pathogen population and would therefore promote longevity of the protectants.

FUNCTION AND LOCALISATION OF HYDROPHOBINS IN THE TOMATO PATHOGEN *CLADOSPORIUM FULVUM.*

James Whiteford and Pietro Spanu.

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Hydrophobins are small secreted proteins which perform a range of functions in fungal biology. To date six hydrophobin genes have been isolated from the tomato pathogen *Cladosporium fulvum* and these are called HCf-1 to HCf-6. With the exception of HCf-4 all of these genes are highly expressed. Gene deletion studies have shown that HCf-1, HCf-2 or HCf-6 are not essential for infection. However, HCf-1 has been found to be essential for water mediated spore dispersal. Here we report the immunolocalisation of HCf-1 using an approach whereby a copy of the HCf-1 gene was engineered to contain the viral V5 epitope and reintroduced into the ΔHCf-1 deletion mutant. Transformants were tested for the production of modified HCf-1 using an anti-V5 antibody in an immunoflourescence assay. Spore hydrophobicity assays on strains producing HCf1-V5 revealed a restoration of the HCf-1 phenotype. Using immunoflourescence and confocal microscopy we show that HCf-1 localises to *C. fulvum* conidia, is secreted into culture medium and coats aerial hyphae. We also show that although HCf-1 gene expression increases during sporulation it is not essential for this process.

ON THE MANY WAYS OF MAKING WATER CONDUCTING CELLS: NOVEL INSIGHTS FROM IMMUNOCYTOCHEMISTRY

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Though generally considered much simpler than vascular plants, bryophytes contain a wide range of cells with specialized walls that conduct water preferentially and are dead at maturity. Calobryalean and pallavicinean liverworts possess water-conducting cells (WCCs) with walls perforated by large plasmodesmata-derived pores whereas peg rhizoids act as internalized external water-conducting channels in the Marchantiales. Small plasmodesmata-derived pores also occur in the isolated genus *Takakia* but the hydroids of bryoid mosses are imperforate. Dye tracers, used in conjunction with wall-degrading enzymes, show that the structural and functional integrity of the lateral interconnecting walls between hydroids depends on both carbohydrate and protein moities that render these cells highly resistant to cavitation. Monoclonal antibodies against a range of wall polysaccharide and glycoprotein carbohydrate epitopes reveal remarkable diversity in the immunocytochemical characteristics of WCCs on a par with the complexity in the cell walls of higher plants. Together the ultrastructural, physiology and immunocytochemical data indicate that the perforate WCCs in *Takakia*, Calobryales and Metzgeriales and the imperforate hydroids of bryoid mosses evolved independently. The discovery of several carbohydrate epitopes in hydroid walls runs strongly counter to the notion that their maturation involves the hydrolysis of non-cellulosic carbohydrates. The compelling evidence that none of the WCCs found in bryophytes are likely homologues with the tracheids of vascular plants but may have counterparts amongst Devonian mesofossils of uncertain affinities has major implications for land plant phylogeny.

SPATIAL REGULATION OF PLANT MITOSIS STUDIED BY GFP FUSIONS

Magdalena Weingartner and Laszlo Bogre

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Mitotic progression is timely regulated by the accumulation and degradation of A- and B-type cyclins and thus by the activation and localisation of cyclin-dependent kinases (CDKs). We studied the localisation of CDK-A and two B-type cyclin classes (B1 and B2) in tobacco cultured cells using GFP fusions. We found that CDK-A is associated with mitosis-specific cytoskeleton structures, B2-cyclins are functioning early in mitosis and degraded at pro-metaphase, while the B1 class of mitotic cyclin is degraded at meta to anaphase transition. Moreover, the degradation of B1-cyclin is required for phragmoplast formation.

HOW DO GALLWASPS REPROGRAMME PLANT DEVELOPMENT?

Lisa J. Harper[1], Karsten Schönrogge[2] and Conrad P. Lichtenstein

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A major problem in developmental biology is to identify morphogenetic signals controlling plant development. Cynipid wasps must be able to actually re-programme plant development to produce the most fascinating and elaborate galls. Adult cynipids lay their eggs in plant tissue, chiefly of oak or rose, eliciting the formation of a gall, which incorporates various cell types, around the developing larva(e). Morphogenetic signals emanating from the insect must be responsible for this complex plant response. We are performing a detailed molecular study of this captivating biological problem to identify the morphogenetic signals and characterise patterns of gene expression in cynipids and their plant galls. We have identified a number of proteins which are over-produced in the inner gall plant tissue surrounding the developing wasp larvae (Schönrogge *et al* 2000). We have also discovered that the wasp larva induces polyteny –multiple duplication leading to polyploidy– of cells in this surrounding plant tissue (Harper *et al.*, in prep.). This feature, together with the signature of proteins produced that are required for the production of nutrients, suggest that this inner gall tissue that feeds the larva embarks upon a developmental programme reminiscent of that of other secretory and feeding tissues of plants such as suspensor cells in the seed that feed the embryo. The cynipids may be recruiting endogenous plant developmental pathways in novel combinations to make a plant gall.

Refs: Schönrogge, K., Harper, L.J. and Lichtenstein C.P. (2000) The protein content of cynipid galls (Hymenoptera: Cynipidae): Similarities between cynipid galls and seeds. *Plant, Cell and Environment* **23**: 215-222.

HYPHENATED MASS SPECTROMETRY TECHNIQUES IN NATURAL PRODUCT RESEARCH AT RBG KEW

Geoffrey Kite, Renee Grayer, Nigel Veitch, Melanie Howes and Monique Simmonds

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Hyphenated mass spectrometry techniques, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), are suited to analysing complex mixtures of natural products, as occur in crude plant extracts. These techniques are used at RBG Kew in comparative phytochemical research and the chemical authentication of plant extracts.

Small fragments of herbarium material can be analysed by GC-MS and LC-MS enabling the occurrence of some unusual plant metabolites to be examined in more obscure relatives of the original source species. Such studies often support current systematic hypotheses obtained from DNA sequence analysis. Examples of this approach in the family Leguminosae will be shown.

Concern about the misidentification or prohibited use of plants in commercial products has prompted investigations into means of chemical authentication. Direct thermal desorption-GC-MS can be used to distinguish fruits of Chinese Star Anise (*Illicium verum*), used as a spice, from the morphologically-similar Japanese Star Anise (*Illicium anisatum*), which is poisonous. Instigation of published LC-MS methods to distinguish Asian ginseng (*Panax ginseng*) from CITES-restricted American ginseng (*Panax quinquefolius*) is revealing issues that need to be considered in standardising ginseng extracts. Targeted LC-MS methods have also been developed to analyse herbal remedies for *Aristolochia*, which is now banned from medicinal use in the UK.

UNUSUAL TELOMERES IN PLANTS

Eva Sykorova [1,2], Kar Yoong Lim [1], Mark Chase [3], Sandra Knapp [4], Ilia Judith Leitch [3], Andrew Rowland Leitch [1] and Jiri Fajkus[2]

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The ends of eukaryote chromosomes frequently have T_nG_n rich minisatellite sequences occurring in tandem arrays of many hundreds or thousands of repeats. However in plants the highly conserved minisatellite telomeric sequence are not so universal as it was once believed to be. It is known that the monocot genus *Allium* and many other relatives in Asparagales lack this sequence. Now, using slot-blot and fluorescent *in situ* hybridization (FISH), we found no evidence for the presence of the *Arabidopsis*-type telomeric sequence $(TTTAGGG)_n$ at the chromosome termini in the *Cestrum* species (Solanaceae) we investigated. Probing for the human-type telomere $(TTAGGG)_n$ also revealed no signal. However, polymerase chain reaction experiments indicated that there are short lengths of the sequence TTTAGGG dispersed in the genome, but these sequences are almost certainly too short to act as functional telomeres even if they were at the chromosome termini. An analysis of related genera *Vestia* and *Sessea* indicated that they too lack the *Arabidopsis*-type telomere, and the sequences were lost in the common ancestor of these genera. We found that *Cestrum* species investigated had particularly large mean chromosome sizes. We discuss whether this is a consequence of alternative telomere end-maintenance systems.

ENGINEERING SECONDARY CELL WALLS FOR NOVEL FIBRE RESOURCES

G Paul Bolwell

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Among a number of EC programmes administered from RHUL is COPOL, which is an acronym for "Integrated control of polysaccharides and lignins to improve cellulose content and availability and fibre quality" (http://www2.rhbnc.ac.uk/%7Euhbc006/CopolWEB/copol.htm). COPOL is a consortium of Ten European labs funded under Framework V of the EC, and is a Quality of Life Key Action 5 project. Project number QLK5-CT-2000-01493. The programme is meant to comply with the Common Agricultural Policy in the diversification of Agriculture in the area of nonfood and forage crops and is designed around a core discovery programme using proteomics and leading to gene isolation involved in the biosynthesis of cell wall lignins and hemicelluloses in the model species tobacco. The influence of these components on fibre properties and cellulose availability will be determined in modified plants to define the limits to which they can be manipulated beneficially and improvement knowledge of cell wall assembly, which is at present seriously deficient, in order to direct future developments. Thus, this growing knowledge can be used to direct molecular breeding programmes and genetic modification to improve fibre in selected commercial species, poplar, for biomass energy and improved pulping and paper making, maize, for improved forage digestibility and flax for improved production of industrial fibre. Most progress so far has been made with tobacco and virtually all sites in the phenylpropanoid and monolignol biosynthesis have now been subjected to anti-sense down-regulation. This work will be described and some well understood case studies described in detail. Advances in understanding and targeting the polymerisation process will also be described. Progress in gene discovery in hemicellulose biosynthesis will be described. The approaches to transferring the technology will be discussed but information will be restricted due to propriety constraints.

EXPRESSION OF A NOVEL HIV-1/ IgA RECOMBINANT FUSION PROTEIN IN PLANTS

P. Obregon, D. Chargelegue, P. Drake and J. Ma

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It has been demonstrated that plants can be used to generate a variety of recombinant molecules and this expression system has many potential advantages. However, after several attempts to express vaccines in transgenic plants a persistent problem for some proteins has been the disappointingly low levels of expression. In contrast, for antibodies, levels of 1-5% total protein are found consistently. We have investigated the expression of novel fusion proteins based on a (Human Immunodeficiency Virus) HIV-1 p24 protein linked to an IgA fragment (p24/CH2-CH3) as the first stage toward producing multimeric, immune-complexes based on a secretory IgA molecule. We are focusing on the p24 core antigen of HIV as an example of an important viral antigen which has potential as a vaccine candidate.

HIV-1 p24 and p24 linked to CH2-CH3 heavy chain from a mab IgA and were cloned in the plant vector pMon 530. Both constructs were transfected in tobacco cells using *A. tumefaciens,* and transgenic plants were regenerated. Western blots analysis showed that HIV-1 p24 (24KD) and p24 linked to CH2-CH3 (MW 55KD) were expressed in plants. Both recombinant proteins were recognised by anti-p24 antibodies by Elisa. In addition, p24/CH2-CH3 recombinant protein was recognised by anti-human IgA antibodies. The recombinant p24/CH2-CH3 protein formed homodimers, and showed 13-fold higher expression level than that observed in transgenic plants carrying p24 expressed alone. Immunisation studies in animals to test p24/CH2-CH3 and p24 immunogenicity are in progress.

This is the first report on expression of recombinant protein in transgenic plants by fusion with a human immunoglobulin. The use of a fusion complex helped to increase the level of antigen expression, and may provide very effective immunogens for the development of mucosal vaccines.

EXPRESSION STRATEGIES AND POTENTIAL REMEDIATION APPLICATIONS OF ANTIBODIES IN PLANTS

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A model system consisting of hydroponic *Nicotiana* plant cultures expressing a murine monoclonal IgG1 antibody has been used to demonstrate the feasibility of using transgenic plants either to neutralise bioactive molecules in the rhizosphere, or to accumulate and concentrate the molecules in leaves. Two transgenic plant types were used in the assessment; in the first type, functional antibody was secreted from the roots and shown to bind with antigen in the surrounding medium to form an immune complex. In the second, monoclonal antibody was retained in the plant by the use of a transmembrane sequence which anchored it to the plasma membrane allowing antigen binding regions to protrude into the apoplasm. Antigen added to the nutrient medium around the roots of these plants was transported within 24 hr to the topmost leaves of the plant where it was sequestered as an immune complex by binding to antibody on the cell membrane. Concentration of immune complex in the leaf tissue remained constant over a 72 hr period after removal of antigen from nutrient medium. Free antigen was not detected in the leaves of wild-type plants. The two strategies of rhizosecretion-mediated binding and sequestration in leaf tissue could potentially be used in the phytoremediation of any pollutant for which it is possible to generate a monoclonal antibody. This technology may have further applications in the extraction and concentration of valuable soil components or in the engineering of resistance to plant pathogens.

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