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Abstracts / Comparative Biochemistry and Physiology Part A 137 (2004) S221–S228

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**CBP**

## Society for Experimental Biology Annual Main Meeting 29th March–2nd April 2004, Heriot-Watt University, Edinburgh, UK

### **P6–METABOLISM AND TRANSPORT OF BIOACTIVE PRODUCTS IN PLANTS**

Organised by **R. Edward (Durham)** and **R. Theodoulou (Rothamsted)**

#### **P6.1 A new era in Phytochemistry**

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We are beginning to see a spectacular impact of new technologies on the quality and significance of publications since 2000. The reporting of the structural elucidation of natural compounds continues at a healthy level and many of these have demonstrable bioactivity. This enterprise is truly global and some interesting new lead compounds for various applications will be described. Many new terpenoids, phenolics, alkaloids, glucosinolates and other phytochemicals have been reported and addition of such mass spec data into the information resources has been essential for developing and interpreting metabolomic studies. Metabolomics as a technology has shown spectacular development both in terms of sensitivity and range of application. Advances have been made in understanding pathways and their regulation through the identification of structural genes, transcription factors and protein kinases. Functional genomics programmes, mostly through collaborative initiatives such as GARNet, Arabidopsis 2010 and various European networks, has been a major driving force and the structural genes for some pathways are now known in their entirety. Similar focus on gene families, such as those being discussed at this meeting, has also been a valuable complementary approach. Proteomics has been useful in correcting gene annotations however and there have been a surprising number of instances of this. Equally surprising has been the impact of engineering pathways, not only in discovering alternative routes but also discovering redundancy in metabolism. Furthermore, applications of metabolomics has revealed cross talk between unrelated pathways. This has implications not only for unintended effects of transgenesis but also suggests that natural products research will need to be integrated into modern systems biology to understand global regulation in the era of post-genomic plant science.

#### **P6.2 Regulation of flavonoid metabolism and transport**

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Molecular genetic analysis in maize established the biosynthetic enzymes and regulatory transcription factors for tissue-specific and condition-induced synthesis of diverse flavonoids. Colored anthocyanin pigments (found in vacuoles) require a basic helix-loop-helix (R=B) plus a myb (C1=PI) transcription factor for expression of the biosynthetic pathway. The standard alleles are expressed in anther, seed, and seedling (R+C1) or adult somatic organs (B+PI). *bz2*, the terminal known step, is a glutathione S-transferase that functions as an anthocyanin carrier protein; GFP-BZ2 is cytoplasmically localized. Because vacuolar sequestration of anthocyanin was very sensitive to vanadate, we inferred that an ABC transporter was required. Analysis of maize ESTs identified a suite of multi-drug resistant associated proteins (MRP), this ABC class was the most likely to transport a bulky organic molecule based on drug studies in mammalian cells. Based on significant up-regulation of transcripts in purple compared to green tissue, only *Mrp3* was co-regulated with anthocyanin synthesis; the promoter region contains putative ‘anthocyanin’ boxes. GFP-MRP3 fusion protein localizes to the tonoplast, the first demonstration of a specific MRP in this membrane in planta. Transgenic plants expressing a *Bz1* promoter: antisense MRP3 construct were significantly impaired in anthocyanin accumulation in adult tissues, compared to isogenic control plants; aleurone coloration was unaffected, and a second MRP was identified that is expressed only in this tissue. We hypothesize that BZ2 hands its anthocyanin cargo off to MRP3 and that the glutathione bound to BZ2 may prime the pump. Supported by the NSF.

### P6.3 Plant P450s-roles in natural product and herbicide metabolism

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There are 271 P450 genes in *A. thaliana* and 458 in the rice genome. Some of these genes are conserved in all vascular plants, while others appear specific to some plant taxa. The function of more than 80% of these P450s, some of which are highly conserved during evolution, is so far unknown. Systematic approaches are thus undertaken to understand their function. They include construction of collections of recombinant enzymes, their high-throughput screening for activity, and dedicated transcriptome analysis. More focused functional analysis of selected P450 families such as CYP98s and CYP76s has been undertaken and reveals their metabolic plasticity and selectivity with regard to metabolism of phenolic esters and amides, other phenolics, or with regard to herbicides. Analysis of KO mutants of CYP98A3 from *A. thaliana* which is involved in the 3'-hydroxylation of p-coumaroyl esters confirms that it is a gene essential for plant development, with a major impact on the synthesis of cell-wall and cell growth. The mutants also accumulate unusual concentrations of flavonoids. Herbicide metabolism seems to be restricted to some plant P450 families such as CYP76. The structural basis of this relaxed substrate specificity is so far not understood. In addition to herbicide metabolism, such P450 families seems to play a role in interaction and incompatibility between agrochemicals. Supported by Genoplante.

### P6.4 Biotransformation of secondary metabolites and xenobiotics by peroxidases

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Heme containing peroxidases are ubiquitous proteins that use H<sub>2</sub>O<sub>2</sub> as the primary oxidant and in plants they are associated with polymerisation reactions leading to lignin formation. However they are capable of oxidising a wide range of organic and inorganic compounds, often with unpredicted outcomes. Three examples involving an anti-cancer drug (mitoxantrone); a polymeric xenobiotic (Poly R478), and NADH serve by means of illu-

stration. These compounds are not readily oxidised by peroxidases, yet in the presence of a second substrate or 'co-factor' their oxidation can be extensive. Using pre-steady-state and steady state kinetic methods, a set of underlying principles for these reactions can be described. Initial two-electron oxidation of native (ferric) peroxidase with H<sub>2</sub>O<sub>2</sub> forms Compound I (P<sup>+</sup>Fe(IV)=O), whose fate depends on the availability of electron donors, their redox potential, hydrophobicity and their binding proximity to the heme iron. With small electron donors that penetrate the heme pocket, fast two-electron reduction of Compound I back to the ferric state accompanied by oxygen transfer from the oxyferryl group to the substrate is possible. For bulkier electron donors, an electron tunnelling mechanism may operate, resulting in step-wise reduction of Compound I to form Compound II (P Fe(IV)=O) then the native state. But since Compound II may also rearrange with H<sub>2</sub>O<sub>2</sub> to form the catalytically dead-end intermediate, Compound III, the outcome of catalysis with bulkier electron donors can be modulated. In the examples described, this is achieved through the supply of suitable of electron donors, nitrite in the case of mitoxantrone, veratryl alcohol in the case of Poly R or lignin and salicylhydroxamic acid in the case of NADH. The biological implications of these reactions are discussed.

### P6.5 Serine hydrolases; the missing enzymes of phase 1 metabolism in plants

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In plants the hydrolyses of carboxyesters and amides is frequently the first step in xenobiotic biotransformation and in the case of herbicides can result in either their bioactivation or detoxification. Surprisingly, unlike the other enzymes of plant secondary metabolism involved in xenobiotic metabolism we know surprisingly little about the hydrolases involved. However, our recent studies have shown that the majority of the hydrolases of interest in cereals and grass weeds utilise an active site serine in catalysis, as demonstrated by their selective inhibition by organophosphate and fluorophosphonate suicide substrates (Cummins et al., (2002) *Phys Plant.* **113**, 477-485). Several of these hydrolases are species-specific and appear to be important in determining the selectivity of several herbicides which are formulated as esters. Using a combination of protein purification, directed proteomics and genomics we are currently functionally characterising several classes of serine carbox-

yesterases in Arabidopsis. One hydrolase of interest has been esterase D (S-formylglutathione hydrolase; AtSFGH), an enzyme which has conserved roles in formaldehyde detoxification across the phyla, but which has a surprisingly diverse range of activities toward xenobiotic carboxyesters, thioesters and amides. Although classical inhibition experiments have classified AtSFGH as a cysteine hydrolase, x-ray crystallography studies have revealed the enzyme to be a serine hydrolase with an unusual regulatory cysteine. Further serine hydrolases with distinct substrate preferences have also been identified, cloned and functionally characterised from Arabidopsis. The potential functions of these enzymes in endogenous and secondary metabolism and their diverse evolutionary origins will be presented.

### **P6.6 Glycosyltransferases of Arabidopsis for metabolic engineering of bioactive products in vivo and synthesis in vitro**

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It is well recognised that glycosyltransferases play a major role in metabolic pathways leading to the synthesis, storage and degradation of bioactive products in plants. Glucosylation of hormones, secondary metabolites and xenobiotics is a well known phenomenon and many enzymes from many different plant species undertaking these reactions have been identified in a number of laboratories.

In this context, our laboratory at York is focussing on the multigene family of enzymes in Arabidopsis thaliana involved in the metabolism of small lipophilic molecules. The subset of Family 1 glycosyltransferases contains 107 open reading frames, divided phylogenetically into 14 groups. We are studying the potential of these genes for metabolic engineering and for their use in fermentation systems as novel biocatalysts.

### **P6.7 Molecular and biochemical approaches to elucidate the specificity and mechanism of plant natural product glycosyltransferases**

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In higher plants, many secondary metabolites are converted to their glycoconjugates. In coordination with

hydroxylation, acylation and methylation, this contributes to an unsurpassed variety of phenolic compounds in almost all plant species, but also to a species specific set of individual sets of secondary metabolites, like glucosinolates, or cyanogenic, terpenoid, and cardiac glycosides. Glycoside formation is catalyzed by soluble glycosyltransferases, which transfer nucleotide-diphosphate activated sugars to low molecular weight substrates. Enormous progress has been made during the last decade on identification, annotation, and classification of a variety of the corresponding plant glycosyltransferases (UGTs), although characterized for a limited set of plant species with the focus on mostly phenolic compounds. In contrast to the bacterial and animal enzymes, data on structural features of these plant UGTs are extremely rare. This communication summarizes current efforts to elucidate the structural motifs and suggest a models of potential active sites of the plant enzymes from the NRD (nucleotide recognition domain)1 $\beta$ -UGT subgroup, characterized by an inversion of the sugar configuration from an  $\alpha$  in the sugar donor to a  $\beta$ -configuration in the final product. The individual protein domains essentially provide the molecular scaffold for the observed substrate and position specificities of this large superfamily of plant enzymes.

### **P6.8 Saponins as plant bioactive metabolites: a gene cluster for the synthesis of defence-related triterpenes in oat**

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Secondary metabolites are associated with the scents, colours and flavours of plants and provide chemical defence against pests and pathogens. The evolution of different biosynthetic pathways in distinct taxa is likely to have been key for survival and diversification. Oats (*Avena* spp.) produce antimicrobial triterpenoids (avenacins) that protect against disease [1]. Here we report that genes required for distinct steps in avenacin biosynthesis in oat are clustered in a region of the genome that is not conserved in other cereals. Although paralogous gene clusters are common in plants (e.g. genes for globin, rRNA and specific disease resistance), there are very few reports of clusters of genes that do not share sequence relatedness and whose products contribute to

a single selectable function. A well-known example is that of the gene cluster for benoxazinoid biosynthesis in maize [2]. Our data indicate that *Sad1*, the gene encoding the first committed enzyme in the avenacin biosynthetic pathway in oat [3], has arisen by duplication and divergence of a cycloartenol synthase-like gene and that the properties of this gene have been refined since the divergence of oats and wheat. Collectively our evidence suggests that gene clusters for complex functions can be created by shuffling and accelerated evolution of existing genetic components, and that clustering confers selective advantages. We are utilising a variety of complementary approaches to investigate the nature and functional significance of this gene cluster. Progress in this area will be presented.

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### P6.9 Conjugation and transport of xenobiotics and natural products

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Plants employ a vast array of biotransformations in the synthesis of secondary metabolites. Similarly, foreign compounds (xenobiotics) undergo a wide variety of chemical modifications in plants, and many enzyme and transporter families are common to secondary product and xenobiotic metabolism. Classically, xenobiotic processing is considered to consist of four phases: activation, conjugation, sequestration and further metabolism. Phase II conjugation reactions serve a number of related purposes, including the protection of functional groups, the generation of biologically inactive forms and the direction of metabolites to intracellular compartments or to the apoplast via specific transporters in Phase III. Thus, the chemical modifications to both xenobiotics and natural products can profoundly influence their transport properties and hence intracellular location. This has implications for food and environmental safety, and impacts on attempts to engineer the synthesis and accumulation of high-value plant products in plants and cell cultures. This paper will review current understanding of the relationships between conjugation and transport of bioactive molecules, using the examples of malonylation and glutathionylation to illustrate emerging general principles.

### P6.10 The ABC of guard cell regulation

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Plants are exposed to biotic and abiotic toxins and produce themselves many potentially toxic compounds. Most of these compounds are conjugated to hydrophilic moieties and stored within the central vacuole. ATP-binding cassette (ABC)-type transporters especially of the subfamily of the multidrug resistance-associated proteins (MRP) are responsible for the vacuolar deposition of these substances. These transporters represent a novel class of pumps. Some members of the MRP family (14 genes in *Arabidopsis*) have been shown to be involved in cellular detoxification by mediating the transport of conjugated organic anions such as glutathione conjugates. An integrated approach using expression, localization and mutant analysis revealed that at least two MRP-type transporters are strongly expressed in guard cells. Both of these transporters are found on the plasma membrane. In spite of their homology, disruption of corresponding genes leads to opposite phenotypes: mutants in *AtMRP5* and *AtMRP4* are less and more susceptible to drought stress, respectively. Detailed analysis revealed that both genes are necessary for proper guard cell functioning and are presumably involved in different steps in stomatal regulation. Taken together, our recent results demonstrate that functions of MRPs may exceed vacuolar sequestration of toxins and possibly involve ion channel regulation.

### P6.11 Imaging the transport of bioactive compounds in plants

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There are a limited number bioactive compounds that have suitable fluorescence properties to image their uptake and distribution within plants directly. However for most compounds alternative strategies are required. Some progress can be made by imaging fluorescently tagged derivatives, but such modifications are likely to significantly alter the behaviour of the target molecule *in vivo*. An alternative approach is to use fluorescent probes to quantify important *in vivo* parameters, such as pH, or GSH-dependent detoxification rate, that can subsequently be incorporated into simulation models that predict the distribution of different xenobiotics based on

their physicochemical properties or transport characteristics measured *in vitro*. Although this approach is indirect it may provide a more generic solution for a wider diversity of molecules, although rigorous quantitative imaging techniques are required to ensure that the data are reliable, particularly when imaging deep into tissue. As an adjunct to such studies, we have developed new approaches to interrogate NMR spectra to yield information at a tissue level of additional parameters such as estimated potassium concentration and apparent ionic strength, as well as pH, that influence speciation of molecules *in vivo*. A third approach is to develop methods to track the dynamics of radio-labelled compounds that can be generated much more easily than fluorescent compounds. We have developed novel techniques to image dynamics of such molecules in fungal systems. Although this work is in its infancy it holds considerable promise to map uptake dynamics of any radiolabelled compound by roots.

### P6.12 Engineering plant bioactive metabolism with glutathione transferases

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Plant glutathione transferases (GSTs) are a superfamily of proteins with diverse functions in endogenous and xenobiotic metabolism. We are currently interested in characterizing plant GSTs with respect to their roles in herbicide metabolism and selectivity and oxidative stress tolerance, with the objective of using these adaptable proteins in engineering bioactive metabolism in plants. Tau (U) GSTs which are the most abundant class have specific members which detoxify diphenyl ether herbicides. We have generated mutant GSTUs showing enhanced activity toward diphenyl ether herbicides by a combination of gene shuffling and mutagenesis and shown that optimized mutant GSTUs confer increased tolerance to herbicides when expressed in *Arabidopsis*. In an alternative approach, the thiol metabolism of tobacco plants was modified to accommodate the alternative substrate usage of a transgenic GSTU originating from soybean. In soybean, the GSTUs involved in the detoxification of diphenyl ether herbicides show a marked preference for homogluthathione (hGSH) the major thiol in this species. By expressing a soybean homogluthathione synthetase in tobacco hGSH accumulated in the transgenic plants and co-expression of the

hGSH-dependent soybean GSTU resulted in elevated tolerance to diphenyl ether herbicides. With respect to engineering antioxidant metabolism we have determined that outlying members of the GST family in *Arabidopsis* cannot catalyse the detoxification of xenobiotics, but instead have evolved roles in redox homeostasis as dehydroascorbate reductases (DHARs) and thioltransferases. In addition to the DHARs a similar redox active site chemistry has been determined in the Lambda enzymes (GSTLs). Studies describing the functional characterization of the DHARs and GSTLs from *Arabidopsis* and their potential roles in counteracting oxidative stress will be described.

### P6.13 Glycosylation of xenobiotics and secondary metabolites in *Arabidopsis thaliana*

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*Arabidopsis thaliana* harbours more than 100 UDP-sugar dependent glycosyltransferase genes (UGT; see Bowles, this session). In addition to endogenous secondary metabolites, UGTs are able to glycosylate xenobiotic substrates as part of their detoxification. Using eight recombinantly expressed UGTs we tested whether they accepted the xenobiotic model compound 2,4,5-trichlorophenol (TCP) parallel to their putative endogenous substrates. Except the sterol-conjugating UGT80A2, seven enzymes showed a side-activity towards TCP. UGT72E2 and UGT75D1 were the most active, TCP-conjugating enzymes among the members tested. Their activities towards native and xenobiotic substrates were mutually inhibited. Thus, several isoenzymes may participate in detoxification of xenobiotics, which could interfere with endogenous reactions.

Flavonol glycosides constitute a prominent class of secondary metabolites in *A. thaliana*. However, the conjugating enzymes had been elusive. Based on sequence homologies to known flavonoid glycosyltransferases from other plant species, the UGT78D and UGT73C subgroups were selected as candidate flavonoid-UGTs from *Arabidopsis*. Metabolic profiles of two T-DNA knockout lines showed the reduction of flavonol-3-O-rhamnoside-7-O-rhamnosides (ugt78D1) and quercetin-3-O-rhamnoside-7-O-glucoside (ugt73C6 and ugt78D1). Recombinant UGT78D1 catalysed the rhamnosylation of the 3-OH group of quercetin and kaemp-

ferol, whilst UGT73C6 catalysed the glucosylation of the 7-OH group of kaempferol- and quercetin-3-O-rhamnosides. Thus, these analyses identified the first *A. thaliana* flavonol glycosyltransferases classifying UGT78D1 as UDP-rhamnose:flavonol-3-O-rhamnosyltransferase and UGT73C6 as UDP-glucose:flavonol-3-O-glycoside-7-O-glucosyltransferase. Furthermore, manipulation of flavonoid glycosylation will be a means to elucidate the role of these modifications in planta or to modify the bioavailability of flavonoid glycosides from crop plants.

#### **P6.14 Opportunities for plant isoprenoid engineering**

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Our knowledge of how isoprenoids are biosynthesised within plants has increased rapidly over the last few years, particularly since the discovery of the plastidic DXP pathway leading to geranyl diphosphate and geranylgeranyl diphosphate and the large number of metabolites derived from these key intermediates. Opportunities for the biotechnological manipulation of these pathways to produce high-value or bioactive terpenoids are numerous. The talk will highlight the central role of terpene synthases in the generation of the large diversity of isoprenoid structures present in the natural world. To illustrate the potential of these catalysts for bioengineering of useful products, the functional characterisation of a family of sesquiterpene synthase genes from goldenrod, *Solidago canadensis*, will be described. These genes encode enzymes that produce a number of volatile hydrocarbons that are active in plant-insect communications. These enzymes are highly homologous, yet are capable of exquisite stereocontrol over the cyclisation of the common substrate, farnesyl diphosphate. This results in isomeric products that show differences in structure ranging from ring size to, in one case, enantiomers of the same compound (germacrene D). Initial experiments in mutagenesis of these enzymes to investigate the reaction mechanism and to produce catalysts that produce new structures will also be discussed.

#### **P6.15 Dietary antioxidants outside and inside the body**

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Fruits and vegetables contain an array of flavonoids and phenolic compounds. Many of these compounds are antioxidants *in vitro* and have anti-atheroscleritic, anti-carcinogenic and anti-inflammatory properties. A

screening programme has identified major differences in the antioxidant capacity of different fruits and vegetables. A number of high antioxidant produce have been investigated using HPLC-MS<sup>n</sup> in conjunction with an on-line antioxidant detection system. This has enabled the compounds contributing to the antioxidant capacity to be identified. Subsequently, supplementation studies were carried out with raspberries which contain anthocyanins and ellagitannins, flavonol-rich onions, green tea which contains high levels of flavan-3-ols and coffee which is a rich source of antioxidant hydroxycinnamates. The investigation made use of subjects with an ileostomy as well as healthy human volunteers. Plasma and urine were collected and analysed by HPLC-MS<sup>n</sup>. The data obtained provide information on the absorption, metabolism, typically via glucuronidation, sulphation and methylation, and excretion of key dietary phenolics. Analysis of ileal fluid provides an insight of events occurring in the small intestine as well as on the components which pass to the large intestine where they may exert anti-carcinogenic effects.

This investigation was part-funded by a flexible funding grant from the Scottish Executive Rural Affairs Department.

#### **P6.16 Plant stress signalling: understanding and exploiting insect-plant and plant-plant interactions**

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When plants are attacked by insects, volatile chemical signals can be released systemically from other parts of the plant, and after cessation of feeding. These plant-derived signals are perceived by olfactory sensory mechanisms in both herbivorous insects and their predators and parasitoids, and can be characterised chemically by means of electrophysiological assays using the insect sensory system. Evidence is mounting that such signals can also affect neighbouring intact plants which are caused to initiate defence, either through the generation of mechanical or antibiotic mechanisms, or by the induction of further signalling systems such as those increasing parasitoid foraging. Furthermore, insect electrophysiology can be used to identify plant compounds having effects on the plants themselves. It has recently been found that certain plants release stress signals even when undamaged, and that these can also cause defence responses in intact plants. Again, these signals, released constitutively by intact plants, can influence insect behaviour at second and third trophic levels, which provides opportunities for further identification of semiochemicals acting directly on plants.

### **P6.17 Serine hydrolases involved in xenobiotic metabolism in in *Arabidopsis thaliana***

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Classic inhibition studies with organophosphates have suggested that the plant esterases involved in the hydrolysis of xenobiotic esters in plants are serine hydrolases (Cummins et al., (2002) *Phys Plant.* **113**, 477–485). On the basis of searching the genome databases with catalytic motifs which are characteristic of serine hydrolases very large and disparate gene superfamilies can be identified in *Arabidopsis thaliana*, though the functions of the respective proteins is largely unknown. Using a directed biochemical and proteomic approach we are currently interested in identifying the major serine hydrolases involved in hydrolysing model xenobiotic esters, such as *p*-nitrophenyl acetate, which are expressed in plants and suspension cultures of *Arabidopsis*. To help identify these enzymes, we have synthesised a fluorophosphonobiotin (FP-biotin) inhibitor which covalently labels active site serines in the course of catalytic activation to label the respective hydrolase with biotin. Having optimised the use of this probe in proteomic studies, the resolution and further characterization of *Arabidopsis* serine hydrolases will be reported.

### **P6. 18 Functional characterisation of a glucosyltransferase from *Arabidopsis* involved in the detoxification of dichloroaniline**

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The conjugation of synthetic compounds with sugars in plants by glycosyltransferases is a major route of detoxification of both pollutants and pesticide metabolites. Using *Arabidopsis thaliana* as a model plant species, we have demonstrated that both plants and cell cultures rapidly detoxify the recalcitrant pollutant 3,4-dichloroaniline by *N*-glycosylation [1]. The enzyme responsible for the greater part of 3,4-dichloroaniline conjugation in *Arabidopsis* cell cultures has been purified and identified using proteomics as a family 1 type glucosyltransferase UGT72B1 [2]. The recombinant protein was highly active in conjugating 3,4-dichloroaniline, as well as the chlorinated phenol 2,4,5-trichlorophenol, demonstrating both *N*-glucosyltransferase and *O*-glucosyltransferase activity. The importance of UGT72B1 in the detoxifi-

cation of 3,4-dichloroaniline in *Arabidopsis* was examined using a T-DNA insertional mutant designated *ugt72B1*. *Ugt72B1* showed reduced activity toward 3,4-dichloroaniline when assayed *in vitro* and was found to be impaired in its ability to metabolise 3,4-dichloroaniline *in planta*. Ongoing studies with the plants are focused on defining the effects of modifying the expression of UGT72B1 on the ability of plants to tolerate toxic synthetic compounds as well as identifying endogenous substrates of the enzyme.

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### **P6.19 Foliar uptake of polar compounds: transport across the plant cuticle**

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The plant cuticle covers the leaf surfaces of higher plants. The cuticle protects leaves from uncontrolled losses of water and ions, and prevents or diminishes the uptake of compounds from the environment into the leaf interior. Cuticular uptake is desired for foliar applied fertilizers or systemic agrochemicals. Organic compounds can be divided by their octanol/water partition coefficient in polar ( $\log K_{O/W} < 0$ ) or lipophilic ( $\log K_{O/W} > 0$ ) compounds. Two different pathways of penetration across the cuticle are postulated. The lipophilic pathway is created by the cutin matrix and the cuticular waxes. This pathway is accessible to lipophilic compounds. The polar pathway is limited to hydrophilic or charged compounds. While the lipophilic pathway is well understood, less is known about the polar pathway. In order to find further information on the mechanisms which govern cuticular uptake of polar compounds, penetration experiments under steady-state and non-steady-state conditions were conducted. In these experiments isolated leaf cuticles of ivy (*Hedera helix*) and grape vine (*Vitis vinifera* cv. Nelly) were used. New information on the mode of uptake of polar compounds will be substantial to improve cuticular penetration of foliar applied agrochemicals and fertilizers.

### **P6.20 Carotenoid accumulation during potato tuber development and storage**

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There is an increasing awareness of the health benefits associated with the availability of carotenoids in the human diet. Carotenoids are implicated in the reduced risk of several chronic health disorders including some forms of cancer, heart disease and eye degeneration. Human health could benefit if the carotenoid composition could be improved in a staple food such as potato. Our approach has been to carry out a detailed comparison of carotenogenesis in a range of potato germplasm. Tuber carotenoid content is particularly high in a *Solanum phureja* accession (DB375\1). These tubers accumulate high levels of zeaxanthin and also contain significant levels of antheraxanthin, lutein and violaxanthin. We have demonstrated that tuber carotenoids accumulate during tuber development; levels remain high during tuber maturation and are stable during 9 months of tuber storage at 4 °C. During storage however, there are changes in individual tuber carotenoids possibly indicating carotenoid inter-conversions. In parallel, the transcript levels of the genes encoding carotenogenic enzymes have been profiled in a range of germplasm during tuber development. Surprisingly, we have discovered an inverse relationship between the zeaxanthin epoxidase transcript level and tuber carotenoid content. We are currently developing transgenic strategies for manipulating tuber carotenoid content and balance. For example, we have over-expressed an algal gene (*crtO*) and also down regulated expression of the two lycopene cyclase genes. Preliminary results indicate significant alterations to tuber carotenoid content and balance in the transgenic lines.

### **P6.21 Transgenic manipulation of tuber carotenoid content in *Solanum phureja* and *Solanum tuberosum***

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Carotenoids are not synthesised by vertebrates but they play a range of functions in human. Perhaps the best known function of carotenoids is as a precursor of provitamin A. They also protect against a lot of diseases and serve as natural colorants in food or organisms that lack carotenoid synthesis. An enhancement of levels in staple foods such as potato would be highly significant. The aim of this work is to develop an understanding of carotenogenesis in potato tubers. A transgenic approach was chosen using germplasm available at SCRI. Several potato accessions with a high level of carotenoids content was identified. A tuber specific promoter (patatin) was used to confine expression of transgenes to the tuber:

\*Overexpression of bacterial genes that encode key-activities in the carotenoid biosynthesis pathway.

\*Down-regulation of other enzyme activities using an antisense strategy in order to accumulate carotenoids of interest and/or modify flux in the pathway.

\*Transformation in Desiree, a cream-fleshed *Solanum tuberosum* cultivar that contains a low level of carotenoid. Transformation of this cultivar is generally straightforward and is a model for *Solanum tuberosum* cultivars with good agronomic traits.

\*Transformation in *Solanum phureja* 337/37, a high tuber carotenoid content cultivar.

Comparison of those 2 systems, Desiree/ *Solanum phureja* 337/37, should enhance our understanding of potato tuber carotenogenesis. The effects produced by the transgene, like up- or down-regulation of other enzymes of the pathway, will help our comprehension of the mechanisms that regulate carotenoids accumulation in potato tuber.