PSNA 2005 Annual Meeting

Integrative Plant Biochemistry as We Approach 2010

July 30 - August 3, 2005
La Jolla, California

SALK INSTITUTE FOR BIOLOGICAL STUDIES

Where Cures Begin.

Phytochemical Society of North America

Welcome to this year's annual meeting of the Phytochemical Society of North America and to the Salk Institute for Biological Studies. The theme of this year's meeting, integrative plant biochemistry, is meant to celebrate the past accomplishments of the organization and its scientific focus, the growing importance of phytochemistry and plant biochemistry to the public, and to set a course for the future, linking the past with the present and attracting a wider breadth of scientists and disciplines to the society as we look to 2010 and beyond.

This is a critical time in plant biochemistry as the tools available to us all provide unprecedented opportunities for discovery. However, we are increasingly faced with shrinking funds available for discovery-based research. Moreover, as a society, we share a collective responsibility for mentoring future plant biochemists and phytochemists who will lead us beyond 2010. We also must increase our participation in the political process to ensure that the next generation of scientists have available the funds to make the seminal discoveries that increase our fundamental understanding of how plants have evolved to survive and prosper in challenging ecosystems. These discoveries provide the necessary foundation for translating basic research into technology that will play critical roles in the health and well being of mankind.

So while you all participate in the scientific discussions throughout the meeting, jot down ideas for future meetings, thoughts on how the society can evolve and play an increasingly critical role in advocating the economic importance of plant biochemistry for future generations and how the society can lead efforts to systemize and make available the exponentially expanding amount of data we are all accumulating. This is your society and we hope that each of you will come away from the meeting inspired to become active and vocal members of a society that must take a leadership role in the future of phytochemistry and plant biochemistry in North America and beyond.

Our scientific sessions have been chosen to highlight key areas of plant biochemistry that represent nearly limitless opportunities for our younger scientists to tap into novel biochemical pathways used so effectively by plants to survive and prosper in a myriad of diverse and challenging environments. I hope you all will agree that we have assembled a truly stellar class of scientists that represent the true diversity of a society such as ours.

I welcome feedback both positive and negative before, during and after this years meeting. Welcome to San Diego and the Salk Institute for Biological Studies. Now let the science begin!

Joseph Noel, Local Organizer

Program:

Saturday, July 30th

1:00 – 6:00 PM	Registration (Salk Foyer)
4:00 – 5:30 PM (Salk Parker Room)	PSNA Executive/Advisory Committee Meeting
6:30 – 7:30 PM	Buffet Dinner (Salk Foyer)
7:30 – 8:30 PM	After Dinner Speaker: Parag R. Chitnis (National Science Foundation) Obtaining National Science Foundation Grants for Phytochemistry Research: Opportunities and Challenges
8:30 – 9:30 PM	Beer/Wine Mixer

Sunday, July 31st

Session I – Metabolic Networks (Chair: Clint Chapple)

8:15 – 8:30 AM and Clint	Welcome and Introductory Remarks by Joe Noel
Symposium	Chapple – Scientific Sessions and the Neish
8:30 - 9:10 AM	Sue Rhee (TAIR, Carnegie, Stanford) – MetaCyc and AraCyc: Metabolic Pathway Databases and Beyond
9:10 - 9:30 AM	Christopher M. Fraser (Purdue University) – Dissecting the Metabolic Complexity of Sinapate Ester Production in Arabidopsis: A Serine Carboxypeptidase-like Protein that Synthesizes 1,2-Disinapoylglucose
9:30 - 10:10 AM	Bjoern Hamberger (University of British Columbia) - Plant Phenylpropanoid Metabolism – Neish Young Investigator Awardee
10:10 - 10:30 AM	Break
10:30 - 11:10 AM	Natalia Dudareva (Purdue University) – MEP Pathway and Rhythmic Terpene Emission

11:10 - 11:50 AM	Mary Wildermuth (University of California – Berkeley) – Metabolic Pathways and Networks involved in Plant-Pathogen Interactions – Neish Young Investigator Awardee
11:50 – 12:10 PM	Matthew Templeton (Horticulture and Food Research Institute of New Zealand) – Clustering of Genes Involved in Secondary Metabolism in Plants
12:15- 2:00 PM	Buffet Lunch (Salk Foyer and Courtyard)

Session II – Temporal and Spatial Regulation of Metabolism (Chair: Vladimir Shulaev)

2:00 - 2:40 PM	Peter Facchini (University of Calgary) – Cellular and Subcellular Compartmentalization of Alkaloid Biosynthesis
2:40 - 3:00 PM	Knut Meyer (Dupont) – Metabolic Engineering of Plants and Microbes for Production of Arbutin
3:00 - 3:20 PM	Break
3:20 - 4:00 PM	Jennifer Normanly (University of Massachusetts – Amherst) – Auxin Biology and Biosynthesis
4:00 - 4:20 PM	Joseph M. Jez (Donald Danforth Plant Science Center) – Evolving Phytochelatin-Based Heavy Metal Tolerance in Plants
4:20 - 5:00 PM	Jon Page (National Research Council – Saskatoon) – Tricks of the Trichome: Terpenophenolic Biosynthesis in Hops and Hemp
6:00 - 7:30 PM	Buffet Dinner (Salk Foyer)
7:30 - 10:00 PM	Poster Session / Wine-Beer-Cheese Mixer (Salk Foyer)

Monday, August 1

Session III – Biosynthesis and Regulation of Signaling Molecules (Chair: David Gang)

8:30 - 9:10 AM	Yunde Zhao (University of California – San Diego) – Auxin Signaling and Homeostasis – Neish Young Investigator Awardee
9:10 - 9:50 AM	Angus Murphy (Purdue University) – Flavonoid Roles in Auxin Transport and Other Targets of Flavonoid Activity
9:50 - 10:10 AM	Break
10:10 - 10:50 AM	W. David Nes (Texas Tech University) – Engineering Phytosterol Enzymes to Understand Function and Evolution of Activity
10:50 - 11:30 AM	Eran Pichersky (University of Michigan) – Methylation and Demethylation of Plant Signal Molecules
11:30 - 11:50 AM	John C. D'Auria (Max Planck Institute for Chemical Ecology) – What Does it Mean to Smell Green? The Molecular and Biochemical Characterization of a Plant Acyltransferase Responsible for Producing Greenl Volatile Esters
12:00 - 2:00 PM	Buffet Lunch (Salk Foyer and Courtyard)

Session IV – Translational Opportunities in Plant Biochemistry (Chair: Cecilia McIntosh)

2:00 - 2:30 PM	Troy Smillie (University of Mississippi) – The Natural Product Repository and Natural Products Quality Control
2:30 - 3:00 PM	Bryan Greenhagen (Allylix, Inc) – Heterologous Sesquiterpene Production Platforms
3:00 - 3:30 PM	Break
3:30 - 4:00 PM	Sekhar Boddupalli (Galileo Pharmaceuticals) – Inflammatory Pathway Modulators and Plant Natural Products

4:00 - 4:20 PM	Fabricio Medina-Bolivar (Arkansas State University) – Over-expression of Transcription Factors to Manipulate Specialized Metabolite Biosynthesis
4:20 - 5:00 PM	Agilent Technologies – Technologies for Profiling in Metabolomics Experiments
5:30 – 6:30 PM	PSNA Business Meeting (Salk Trustee's Room)
6:30 - 8:00 PM	Buffet Dinner (Salk Foyer)
8:00 - 10:00 PM	Poster Session / Wine-Beer-Cheese Mixer (Salk Foyer)

Tuesday, August 2 – PLEASE NOTE A CHANGE DUE TO A CANCELLATION

Session V – Lipids, Fatty Acids and Related Molecules (Chair: Rick Dixon)

8:30 - 9:10 AM	Basil Nikolau (Iowa State University) – Regulation of Plant Lipid Metabolism
9:10 - 9:50 AM	Eyal Fridman (University of Michigan) – Methylketones Biosynthesis in the Tomato Glandular Trichomes
9:50 - 10:10 AM	Break
10:10 - 10:30 AM	Daniel Cook (USDA-University of Mississippi) – Sorgoleone Biosynthesis and Plant Polyketide Synthases
10:30 – 11:10 AM	Ed Cahoon (Danforth) – Metabolic Engineering of Unusual Fatty Acid Biosynthetic Pathways for Enhanced Vegetable Oil Quality and Plant Pathogen Resistance – Neish Young Investigator Awardee
11:10 – 11:30 AM	Carlos L. Cespedes (UNAM) – Iridoids and phenylpropanoids from *Penstemon gentianoides* (Scrophulariaceae)

11:45 AM – 6:30 PM	Boxed Lunch To Go (Free Time) *San Diego Zoo, Quail Botanical Gardens, Beach, Surfing!*
2:00 – 5:00 PM	Agilent Technologies Metabolomics Workshop (Parker Room)
6:30 - 10:00 PM	Buffet Banquet and Presentation of Student Travel Awards and Best Poster Awards

Wednesday, August 3

Session VI – Conservation and Divergence in Enzyme Function (Chair: Joseph P. Noel)

8:30 - 9:10 AM	Joerg Degenhardt (Max Planck Institute for Chemical Ecology) – Diversification for Defense: the Role of the Maize Terpene Synthase Gene Family in Multitrophic Interactions
9:10 - 9:30 AM	Sarah O'Connor (MIT) – Chemistry and Mechanism of Alkaloid Biosynthesis
9:30 - 10:10 AM	Dan Panaccione (West Virginia University) – Origins and Significance of Ergot Alkaloid Diversity in Fungi
10:10 - 10:30 AM	Break
10:30 - 10:50 AM	Paul O'Maille (The Salk Institute) – Structural and Energetic Basis for Product Specificity Control by Sesquiterpene Cyclases
10:50 – 11:10 AM	Jean-Luc Ferrer (IBS CEA-CNRS-UJF) – The Structure of a Cyanobacterial Sucrose-Phosphatase Reveals the "Sugar Tongs" that Release Free Sucrose in the Cell
11:15 AM	Meeting Adjourns and Departure

Speaker Abstracts

Obtaining NSF Grants for Phytochemistry Research: Opportunities and Challenges

Parag R Chitnis
Division of Molecular and Cellular Biosciences
National Science Foundation, Arlington VA 22230

National Science Foundation supports research on biosynthesis, analysis and physiological relevance of phytochemicals through a variety of mechanisms. Some of them, such as research grants to disciplinary programs, are obvious to the community and are used routinely. However, there are several other opportunities that may be of interest to phytochemists.

This grantsmanship workshop will focus on:

- Information about NSF and its role in funding plant biology
- Current priorities in funding and the future directions
- Opportunities for funding phytochemistry research -
- The obvious and not so obvious programs
- Writing a compelling grant proposal
- Journey of a proposal at NSF
- Proposal content
- Tips for writing an effective proposal

At the end of the presentation, there will be sufficient time for general questions and discussion. Informal opportunities for discussion about specific projects will be available during the conference.

Session I
Metabolic Networks
Chair: Clint Chapple

MetaCyc and AraCyc: Metabolic Pathway Databases and Beyond

Peifen Zhang, Hartmut Foerster, Christophe Tissier, Ron Caspi*, Carol Fulcher*, Becky Hopkinson*, Pallavi Kaipa*, Markus Krummenacker*, Suzanne Paley*, John Pick*, Peter Karp*, and Seung Yon Rhee The Arabidopsis Information Resource, Carnegie Institution of Washington, 260 Panama Street, Stanford, CA 94305, USA; *Bioinformatics Research Group, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA

MetaCyc (http://metacyc.org/) is a metabolic pathway database that includes pathways, reactions, enzymes, and compounds from many organisms including microorganisms, plants, and human. Data in MetaCyc are curated using experimental evidence in the literature. Its goal is to represent experimentally verified pathway information from all organisms, which can then be used, among other applications, as a reference to predict the metabolic content of newly sequenced, annotated genomes, and to compare metabolic pathways from different organisms. AraCyc (http://www.arabidopsis.org/tools/aracyc/) is an Arabidopsis-specific pathway database, which was computationally predicted from the enzyme-coding genes annotated in the Arabidopsis genome using MetaCyc as the reference database. Since its initial build, AraCyc has been under continued curation to enhance its data quality and to increase the breadth of pathway coverage. The goal of AraCyc is to represent the complete metabolic content of a model plant, to be used as a base to predict the metabolic content of other plant genomes as well as to provide a metabolic context for the analysis of large-scale functional genomics data such as those arising from genome-wide expression profiling, and metabolomic profiling experiments. All of the predicted pathways are being verified and updated from the literature. Another major curation effort is devoted to expand the coverage of plant secondary metabolism in MetaCyc and AraCyc. Our immediate goal is to curate representative pathways for each of the main secondary metabolite classes, followed by more in-depth curation of secondary metabolism as well as incorporation of transporters to handle pathways that span multiple cellular compartments. Among other applications of MetaCyc and AraCyc, the rich and diverse information on metabolic pathways across species can assist comparative studies of pathways and facilitate metabolic engineering to improve traits in crops. Results from comparison of all plant pathways with pathway variants in other non-plant species will be presented. Both databases are freely accessible from the internet. In addition to querying and browsing the pathway data, the Omics Viewer (http://www.arabidopsis.org:1555/expression.html) - a data visualization and analysis tool - allows many different types of large-scale functional genomics data resulting from microarray expression, metabolite profiling, and proteomic profiling experiments to be overlaid onto the full metabolic pathway map of Arabidopsis.

Dissecting the metabolic complexity of sinapate ester production in Arabidopsis: a serine carboxypeptidase-like protein that synthesizes 1,2-disinapoylglucose.

Christopher M. Fraser, Michael G. Thompson[1], Amber M. Shirley[2] and Clint Chapple
Department of Biochemistry, Purdue University, West Lafayette, IN 47907; [1]current address, Department of Chemistry, Purdue University, West Lafayette, IN 47907; [2]current address, BASF Plant Science L.L.C., 26 Davis Drive, Research Triangle Park, NC 27707

The Arabidopsis genome contains fifty-one genes that encode serine carboxypeptidase-like (SCPL) proteins. A sequence analysis of these SCPL proteins has demonstrated that the majority of them fall clearly into two major groups, with one group being composed of nineteen SCPL proteins that are highly similar to one another. Two of these nineteen SCPL proteins have been characterized to date: sinapoylglucose: malate sinapoyltransferase (SMT) and sinapoylglucose:choline sinapoyltransferase (SCT). Hence, both of these SCPL proteins are acyltransferases that utilize sinapoylglucose as an activated acyl-donor molecule. Interestingly, the gene that encodes SMT (At2g22990) is one of five SCPL genes arranged in tandem to form a cluster on chromosome 2. An analysis of four different mutant lines harboring different combinations of mutations for this SCPL gene cluster has revealed that one gene (At2g23010) encodes sinapoylglucose:sinapoylglucose sinapoyltransferase (SST), an enzyme responsible for the production of 1,2-disinapoylglucose from two molecules of sinapoylglucose. Further, tissue-specific metabolic profiling of these mutants grown under different environmental conditions and examined at different developmental stages has begun to uncover a significant degree of metabolic complexity associated with the SCPL proteins encoded by these clustered genes. This work thus sheds further light on the relationship between sequence, regulation, and function found in the Arabidopsis SCPL gene family and provides another example of the genetic diversity that underlies plant secondary metabolism.

Bjoern Hamberger (University of British Columbia) - Plant Phenylpropanoid Metabolism

No abstract submitted.

Natalia Dudareva (Purdue University) – MEP Pathway and Rhythmic Terpene Emission

No abstract submitted.

Mary Wildermuth (University of California – Berkeley) – Metabolic Pathways and Networks involved in Plant-Pathogen Interactions

No abstract submitted.

Clustering of Genes Involved in Secondary Metabolism in Plants

Matthew D. Templeton[1], Ross N. Crowhurst, Richard D. Newcomb[1], Lesley L. Beuning[1], Edwige J. Souleyre[1], Ross G. Atkinson[1] and Richard A. Dixon[2]. [1]The Horticulture and Food Research Institute of New Zealand, Mt Albert Research Centre, Auckland, New Zealand and [2]Plant Biology Division, The Noble Foundation, Ardmore, Oklahoma, USA.

In many organisms the genes encoding enzymes involved in primary or secondary metabolism are clustered together within a genome. Classic examples are operons in bacteria, where the genes for a specific pathway are transcribed on a single polycistronic message. In fungi, many genes involved in secondary metabolism are clustered.

Plants produce a vast array of secondary metabolites which are involved in many fundamental tasks such as pest and disease resistance, insect attraction and allopathy. While the catalogue of individual molecules is substantial, most are based on a relatively small number of carbon backbones, for example the phenyl propanoids and isoprenyl. These skeletons are then sequentially decorated in a modular fashion by specific enzymes from a several complex gene families including the heme-thiolate P450s, glycosyl transferases, O-methyl transferases and acyl transferases. The identification of all the enzymes in specific pathways for the synthesis of individual compounds is a significant area of interest in plant biochemistry. Genomics has provided a means for this to be achieved for all the secondary metabolic pathways for a single plant. While genomics has provided the information in terms of all the genes involved it has not facilitated the task of assigning function, this is particularly problematic for large gene families such as the heme-thiolate P450s.

Until recently, it was thought that the genes encoding these enzymes did not form clusters. Annotation the completed genomes of both the Arabidopsis and rice genomes has revealed that there incidences where genes encoding enzymes involved in secondary metabolism appear to be clustered. The significance of this observation for gene mining and assigning function to enzymes involved in secondary metabolism will be discussed.

Session II
Temporal and Spatial Regulation of Metabolism
Chair: Vladimir Shulaev

Cellular and Subcellular Compartmentalization of Alkaloid Biosynthesis

Peter J. Facchini, Nailish Samanani
Department of Biological Sciences, University of Calgary, Calgary, Alberta, T2N 1N4, Canada

Benzylisoquinoline alkaloids (BIAs) are a diverse group of secondary metabolites found in restricted plant taxa. Several BIAs including morphine, berberine, and sanguinarine are used as pharmaceuticals. Immunofluorescence labeling and *in situ* hybridization were used to identify the cell type-specific localization of seven BIA biosynthetic enzymes and their corresponding gene transcripts in opium poppy (*Papaver somniferum*). All seven enzymes were localized to the same cell type in the phloem. The presence of sieve plates and the co-localization of a sieve element-specific H^+-ATPase isoform suggested the identity of these cells. Moreover, corresponding gene transcripts were found in adjacent companion cells. Laticifers are adjacent to sieve elements in opium poppy and are the site of BIA accumulation, but not biosynthesis. The overall process from gene expression through product accumulation involves three cell types, and predicts the intercellular translocation of biosynthetic enzymes and alkaloids. In meadow rue (*Thalictrum flavum*), the accumulation of berberine occurs in the mature endodermis of roots and throughout the cortex of rhizomes. Despite extensive sequence identity between corresponding BIA biosynthetic genes in opium poppy and meadow rue, more than one cellular platform has evolved to support the biosynthesis and accumulation of alkaloids in related families. Gene transcripts for nine consecutive biosynthetic enzymes involved in berberine biosynthesis co-localized to the immature endodermis and pericycle in roots, and the protoderm of leaf primordia in rhizomes. Cell fractionation on a sucrose density gradient followed by western blot analysis, and immunogold labeling, showed the localization of BIA biosynthetic enzymes and sanguinarine to the endoplasmic reticulum in dedifferentiated opium poppy cell cultures. Individual cultured opium poppy cells appear to perform all of the steps that require three cell types *in planta*. The implications of the cell-type specific biosynthesis and accumulation of BIAs in opium poppy and meadow rue will be discussed.

Metabolic Engineering of Plants and Microbes for Production of Arbutin

Colleen M. McMichael, Dennis Flint, Paul V.Viitanen, Robert Kenvin, Drew E. Van Dyk and Knut Meyer

DuPont Central Research and Development, Rt. 141& Henry Clay Rd., P.O. Box 80328, Wilmington, DE 19880

Arbutin (hydroquinone β-D glucopyranoside) is a secondary metabolite present at high levels in certain plant species of the rosaceae, ericaceae, saxifragaceae families. Arbutin is an active ingredient in cosmetics for skin lightening. Arbutin can be isolated from plant tissues or it can be generated by biotransformation of hydroquinone using plant explants, suspension cultures or microbes expressing plant-derived hydroquinone-specific glucosyltransferase enzymes; moreover, routes for chemical synthesis of arbutin exist.

An opportunity for high volume production of arbutin at significantly lower cost may be created by engineering a pathway for arbutin biosynthesis into plants or microbes. The yeast *Candida parapsilosis* can grow on para-hydroxybenzoate (pHBA) as sole carbon source. The first step of pHBA breakdown uses a pHBA 1-hydroxylase (pHBA1H) enzyme, a soluble FAD-dependent monoxygenase that converts pHBA to hydroquinone. We purified this enzyme to homogeneity and cloned the corresponding gene. We demonstrate that DNA constructs expressing chorismate-pyruvate lyase (CPL) in the chloroplast and pHBA1H in the cytosol are sufficient to create a high flux pathway from chorismate to arbutin in transgenic plants. In addition, we demonstrate that co-expression of CPL and pHBA1H enzymes with a glucosyltransferase gene from arabidopsis (UGT72B1) in *E. coli* provides a biosynthetic route to arbutin from simple fermentable feed stocks.

Jennifer Normanly (University of Massachusetts – Amherst) –
Auxin Biology and Biosynthesis

No abstract submitted.

Evolving Phytochelatin-Based Heavy Metal Tolerance in Plants

Joseph M. Jez[1], Rebecca E. Cahoon[1], Philip A. Rea[2]
[1]*Donald Danforth Plant Science Center, St. Louis, MO*
[2]*Plant Science Institute, Dept. of Biology, University of Pennsylvania, Philadelphia, PA*

Phytochelatins play a key role in plant heavy metal tolerance by chelating metal ions for sequestration in vacuoles. Assembled by phytochelatin synthase (PCS), phytochelatin peptides consist of repeating units of γ-glutamylcysteine derived from glutathione. Since phytochelatins, along with other peptides including glutathione and metallothioneins, confer resistance to cadmium, arsenic, and lead, understanding and manipulating their biosynthesis is an important aspect of using plants for the remediation heavy metal contaminated soils. Here we describe the directed evolution of PCS to improve heavy metal tolerance in plants. While directed evolution is a powerful tool for engineering enzymes with tailored molecular function, it has not been widely applied to agricultural or environmental problems. We used directed evolution to generate a PCS mutant library. Screening of this library in yeast for cadmium tolerance identified a set of PCS genes that enhanced heavy metal protection. Expression of these evolved PCS mutants in yeast results in higher phytochelatin levels than in cells expressing wild-type PCS and improves tolerance to cadmium up to 10-fold. In addition, experiments using transgenic *Arabidopsis thaliana* expressing the most efficient first generation PCS variant displays enhanced protection against cadmium toxicity. These experiments offer proof-of-principle for this approach; however, these results are only the first round of mutant generation and selection. Ultimately, we envision an evolved PCS gene as a component in a molecular tool chest for developing a transgenic plant with multiple traits tailored for the remediation of heavy metal contaminated soils.

Tricks of the trichome: terpenophenolic biosynthesis in hops and hemp

Jonathan E. Page and Jana Nagel
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Members of the Cannabaceae synthesize a range of terpenophenolic secondary metabolites (prenylated polyketides) with diverse biological activities. Bitter acids and prenylflavonoids found in hops, *Humulus lupulus* L., contribute bitter flavour to beer and possess chemopreventative properties, respectively. *Cannabis sativa* L. (marijuana, hemp), a close relative of hops, is well known for its content of the psychoactive and medicinal cannabinoid, Δ^9-tetrahydrocannabinol (THC). Our research is attempting to understand the biosynthetic pathways by which these terpenophenolics are formed. Based on the fact that most of terpenophenolics found in hops and cannabis are synthesized in glandular trichomes, we are using a biochemical genomics approach that uses EST sequences derived from trichome-specific cDNA libraries. EST sequencing of a cannabis trichome cDNA library has yielded candidates for putative polyketide synthases involved in the cannabinoid pathway, but so far enzymatic activity has been elusive. Our hops trichome genomics project has uncovered the metabolic processes by which terpenophenolics are synthesized and has led to the discovery of several new genes potentially involved in bitter acid and prenylflavonoid biosynthesis. These include polyketide synthases and other enzymes, such as methyltransferases, that may function to decorate the polyketide core structure. Understanding how terpenophenolics are formed may allow the metabolic engineering of this important, and potentially valuable, branch of secondary metabolism in plants.

Session III
Biosynthesis and Regulation of Signaling Molecules
Chair: David Gang

Yunde Zhao (University of California – San Diego) – Auxin Signaling and Homeostasis

No abstract submitted.

Flavonoid Roles in Auxin Transport and Other Targets of Flavonoid Activity. Wendy Ann Peer and Angus S. Murphy, Dept. of Horticulture, Purdue University, West Lafayette, IN 47907, USA

Flavonols are thought to be natural regulators of auxin transport. Enhanced auxin transport and root elongation are seen in the flavonoid-deficient *tt4* mutant of Arabidopsis, and flavonols can displace auxin efflux inhibitors from membrane binding sites. Auxin transport is reduced in *tt7* and *tt3* seedlings that accumulate 5-10 times more of the flavonols kaempferol and quercetin than wild type. Flavonoids appear to primarily modulate movement of auxin into the transport stream at the root and shoot apices. However, flavonoids are not essential to auxin transport as auxin-mediated tropic growth responses occur in both flavonoid-deficient mutants and etiolated wild type seedlings which have no flavonoids.

Not all auxin-related growth phenotypes seen in *tt* mutants reflect direct flavonoid regulation. For instance, enhanced gravitropic bending of *tt4* mutant roots appears to reflect increased transport of shoot-derived auxin and consequent linear growth rather than altered graviresponsiveness. Other phenotypes, such as the inflorescence height and the number of secondary inflorescences in *tt4*, *tt7* and *tt3* may be influenced by altered auxin transport, but are light-dependent. Further, increased axillary branching reported in *tt4(2YY6)* segregates independently of the lesion in *CHS*. Some flavonol accumulations may be a response to auxin accumulation, as flavonols are effective scavengers of reactive oxygen species. Localized flavonol accumulations thought to induce altered auxin transport may instead be a response to free radical byproducts of IAA oxidation that provides initial protection from consequent oxidative stress.

Flavonoid binding and inhibition of plasma membrane PGP auxin transport proteins and the interacting trafficking protein APM1 suggest potential sites of flavonoid action. Flavonols may modulate the amount of auxin loaded into the polar auxin transport stream at the root and shoot apices by altering the activity of plasma membrane PGP auxin transporters. They also appear to modulate vesicular trafficking of auxin efflux complexes. Flavonoid modulation of the trafficking and enzyme activities of APM1 may indirectly affect auxin transport, as well as modulating the kinase and phosphatase activities of PID and RCN1, respectively. Flavonoids may also indirectly influence auxin transport by influencing membrane fluidity/rigidity. Finally, flavonoids may regulate auxin transport by altering auxin-responsive gene transcription as flavonols and flavonoid biosynthetic enzymes have recently been localized to the nucleus.

Engineering Phytosterol Enzymes to Understand Function and Evolution of Activity

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This review focuses on sterol methyltransferase (SMT) enzymes that use the corresponding Δ^{24}-sterol acceptor molecules as substrates to generate the diversity of sterol side chain structures characteristic of the phytosterol (24-alkyl sterols) family of natural products. Recently, several SMT genes have been cloned, sequenced and mutated for enzyme redesign, making it possible to address questions of SMT evolution and mechanistic enzymology. A comparison of the predicted amino acid sequences of SMTs from plants, fungi and protozoa is presented. Phylogenetic analyses based on protein sequence data indicated that SMTs were joined into a distinctive evolutionary branch within the AdoMet-dependent methyltransferase superfamily and revealed the existence of 5 SMT gene subfamilies, including SMT1 [accepts $\Delta^{24(25)}$-sterols) and SMT2 [accepts $\Delta^{24(28)}$-sterols]. These enzymes have different substrate and/or product specificities but share three regions of substantial amino acid identity, consistent with a divergent evolutionary relationship. Amino acid substitution of key residues in the active center of the yeast SMT alter molecular recognition and lead to a gain in enzyme function, making the mutant Erg6p plant-like. New methylated products synthesized by the mutant yeast SMTs were found to replace ergosterol as a membrane insert in *Saccharomyces cerevisiae* GL7. Thermodynamic activation analyses for SMT catalyzed reactions of diverse origin and phytosterol structure-occurrence data, based on chemical identification of phytosterols using MS and ^{1}HNMR, provide criteria by which C-methylation pathways can be distinguished primitive from advanced. The results of these studies imply that functional divergence influenced by sterol fitness to function in membranes regulated the appearance of different SMTs mutated to accept (1) $\Delta^{24(25)}$- sterols versus (2) $\Delta^{24(28)}$-sterols and produce (3) $\Delta^{24(28)}$-sterols versus (4) $\Delta^{25(27)}$-sterols; SMT1 is considered more primitive than the SMT2 whereas SMT isoform-1/3 is considered more primitive than the SMT isoform-1/4.

Methylation and Demethylation of Plant Signal Molecules

Yue Yang[1], Marina Varbanova[1], Jeannine Ross[2], Guodong Wang[1], Diego Cortes[3], Eyal Fridman[1], Vladimir Shulaev[3], Joseph P Noel[2], Eran Pichersky

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The Arabidopsis *thaliana* genome contains 24 genes encoding carboxyl methyltransferases belonging to the AtSABATH family. Our recent work has shown that enzymes in this MT family catalyze the formation of methylesters of salicylic acid (SA), indole-3-acetic acid (IAA), various gibberellins (GAs), farnesoic acid, nicotinic acid, as well as of jasmonic acid (JA), as previously described. JA, SA, IAA and GAs are known signal molecules in plants and play important roles in all facets of plant life. Some of these signal compounds, notably JA and IAA, have also been shown to be conjugated at their carboxyl moiety to other compounds such as sugars and amino acids, and such conjugations may affect the mobility and activity of these hormones. Signal molecules that are methylated at their carboxyl group cannot be further conjugated at this moiety; thus, carboxyl methylation of these signal molecules is antagonistic to sugar and amino acid conjugation and therefore may affect the ability of these hormones to perform their roles in the plant in yet unknown ways. We discuss the implication of this new mode of regulation on the activity of signal molecules. We also show evidence that *A. thaliana* has a family of genes encoding enzymes that hydrolyze methylesters of these signal molecules to give back the free acids.

This work was supported by the National Science Foundation as part of the Arabidopsis 2010 Initiative.

What does it mean to smell green? The molecular and biochemical characterization of a plant acyltransferase responsible for producing greenl volatile esters.

John C. D'Auria[1], Eran Pichersky[2], Jonathan Gershenzon[1] [1]Max Planck Institute for Chemical Ecology, Jena, Germany, [2]Department of MCDB, University of Michigan, Ann Arbor, MI, USA

Green leaf volatiles (GLV) are common products of mechanically and herbivore-damaged plants. These products derived from the lipoxygenase pathway are thought to be important for the prevention of the spread of bacteria and fungi in addition to serving as cues for herbivores and their predators and parasitoids. More recently, GLVs have been shown to act as plant signaling compounds that can induce several important plant defense pathways. We have identified a member of the BAHD acyltransferase gene family in *Arabidopsis thaliana* that specifies the ability to catalyze the formation of the green leaf volatile ester (*Z*)-3-hexen-1-yl acetate. The enzyme it encodes, acetyl-CoA:cis-3-hexen-1-ol acetyl transferase (CHAT), has been purified from transgenic plants expressing the CHAT gene under the 35S promoter as well as from *E. coli* heterologously expressing the protein. Kinetic data show that both the plant-purified and *E. coli*-produced CHAT enzymes have a high affinity for acetyl Coenzyme A as well as the ability to accept several medium-length chain aliphatic and benzyl-derived alcohols. Upon damage by mechanical wounding, CHAT gene transcript levels increase in a time-dependent manner with the highest levels detected between 3 and 6 hours after wounding. CHAT enzyme activity levels correlate with transcript levels, with CHAT enzyme activity levels peaking between 10 and 12 hours after mechanical wounding. Plants expressing an RNAi cassette for the *CHAT* gene show that the CHAT enzyme is responsible for the *in planta* production of Z-3-hexen-1-yl acetate.

Session IV
Translational Opportunities in Plant Biochemistry
Chair: Cecilia McIntosh

Troy Smillie (University of Mississippi) – The Natural Product Repository and Natural Products Quality Control

No abstract submitted.

Bryan Greenhagen (Allylix, Inc) – Heterologous Sesquiterpene Production Platforms

No abstract submitted.

Sekhar Boddupalli (Galileo Pharmaceuticals) – Inflammatory Pathway Modulators and Plant Natural Products

No abstract submitted.

Over-expression of Transcription Factors to Manipulate Specialized Metabolite Biosynthesis. Argelia Lorence[1,3], Bonnie J. Woffenden[1], Matthew Smith[2], Craig L. Nessler[1] and Fabricio Medina-Bolivar[1,3]. [1]Department of Plant Pathology, Physiology and Weed Science, and [2]Department of Biochemistry, Virginia Tech, Blacksburg, VA. [3]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR.

Plant specialized (secondary) metabolites have been used as phytomedicines for hundreds of years. It is well known that many biosynthetic pathways leading to the formation of these molecules are highly inducible and hypothesized that key regulatory proteins act as major orchestrators of the specialized metabolism. The transcription factor ORCA3 (octadecanoic-derivative responsive *Catharanthus* APETALA2-domain protein) identified from *Catharanthus roseus*, is a master regulator of genes involved in both primary and secondary metabolism. ORCA3 enhances the expression of genes downstream of the stress-and wound-inducible plant hormone methyl jasmonate (MeJA), and is itself up-regulated by jasmonates. We cloned ORCA3 homologs from *Nicotiana tabacum* and *Hyoscyamus muticus* (named ORNA and ORHA, respectively) and are studying their expression in hairy root cultures treated with MeJA and cellulase. In addition, we constructed expression cassettes for either constitutive or inducible expression of ORNA and ORHA and generated transgenic tobacco plants containing these constructs. Molecular characterization of the plant lines and hairy roots generated from them is been conducted. Secondary metabolites produced in hairy roots of wild type and ORNA- and ORHA-over-expressing *N. tabacum* and *H. muticus* will be characterized. We anticipate the potential production of novel specialized metabolites in the transgenic hairy root cultures where specialized metabolism is expected to be enhanced. Ultimately, linking these newly developed products with bioassays may lead to the discovery of pharmaceutically important therapeutic drugs.

Agilent Technologies – Technologies for Profiling in Metabolomics Experiments

No abstract submitted.

Session V
Lipids, Fatty Acids and Related Molecules
Chair: Rick Dixon

Basil Nikolau (Iowa State University) – Regulation of Plant Lipid Metabolism

No abstract submitted.

Methylketones Biosynthesis in the Tomato Glandular Trichomes

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The glandular trichomes are prominent features of the foliage and stems in the genus *Solanum* and serve as the first physical and chemical line of defense against pests. One class of compounds that is known to be highly effective in protecting plants from a plethora of insects consists of medium-length methylketones. We have applied a biochemical genomics approach to study the hitherto unknown biosynthesis pathway of these compounds in the glandular trichomes of the tomato wild species *Solanum habrochaites f. glabaratum*. Based on comparative *in silico* analysis of gland EST databases we concluded that these compounds are not only stored in the glands, but are also synthesized *de novo* as a derivate pathway of the fatty acid biosynthesis. We have hypothesized that the substrate for the production of methylketones in the glands are the β-ketoacyl-ACPs, intermediates in fatty acid biosynthesis. Furthermore, we have developed an enzymatic assay process that enabled us to isolate and functionally test a gene, designated Methylketone Synthase1 (MKS1), that catalyzes the de-esterification and possibly the decarboxylation of β-ketoacyl-ACPs to the resultant methylketones in the glands.

We are applying several approaches to elucidate the biochemical mechanisms and the genetic basis that underline the biosynthesis of these compounds in the glands. Structural modeling of MKS1 suggests that, although it shares common features with the α/β-hydrolase protein family, a different mode of activity has evolved throughout evolution. Dissection of the wild species genome through analysis of interspecific F2 population between the wild species and the cultivated tomato indicates that the methylketones level in the glands is inherited as a quantitative trait and that more factors other than MKS1 regulate this trait. This study highlights the usefulness of the tomato glandular trichomes for the basic study of new biochemical pathways that can be used for crop protection.

Anacardic Acid Biosynthesis and Bioactivity

David J. Schultz[1,3,4], Nalinie S. Wickramasinghe[2,5], and Carolyn M. Klinge[2,3,5]. [1]Biology Department, [2]Biochemistry & Molecular Biology Department, [3]Center for Genetics and Molecular Medicine, [4]University of Louisville, [5]University of Louisville School of Medicine

Anacardic acid (2-hydroxy-6-alkylbenzoic acid) is a group of related molecules that differ with respect to alkyl chain length, double bond placement and/or the number of double bonds in the alkyl group. Although anacardic acid (AnAc) is known to occur in a number of plant families (most commonly found in *Anacardiaceae*), only in *Pelargonium* ×*hortorum* has a physiological function for AnAc been determined. In *P.* ×*hortorum*, AnAc is synthesized and secreted from glandular trichomes, thereby providing resistance to small pests such as spider mites, whitefly and aphid. In most other plants, AnAc is one component in a complex mixture of phytochemicals including related phenolic lipids (e.g. alkyl-resorcinols). The *P.* ×*hortorum* trichome system thus provides a less complex system for studying the bioactivity and biosynthesis of AnAc. Recently, we investigated the bioactivity of AnAc against insects as well as against tumor cell growth. We have demonstrated that purified $24{:}1^{\omega 5}$ AnAc inhibits the development of Colorado potato beetle larvae and thus may have potential activity against a wider range of pests than was previously known. We have also worked to identify the molecular mechanism(s) by which AnAc inhibits tumor cell growth. We found AnAc inhibits cell proliferation of breast and lung cancer cell lines. Using transient transfection assays, we determined AnAc alters estrogen receptor (ER) mediated gene transcription but does not act as a competitive inhibitor to estradiol. We are now investing the potential of AnAc for inhibition of prostaglandin synthesis and/or induction of apoptosis. To better understand the biosynthesis of AnAc, we have isolated metabolically active glandular trichome head cells. We have tested various substrates and found oleoyl-CoA and malonyl-CoA were the only substrates incorporated into AnAc at detectable levels. We are currently using this system to further explore the biosynthesis of AnAc in the glandular trichomes of *P.* ×*hortorum*.

Sorgoleone biosynthesis and plant polyketide synthases.

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Sorgoleone, an oily exudate secreted from the root hairs of sorghum (*Sorghum bicolor* (L.) Moench), acts as a potent allelochemical. Its phytotoxic properties make the elucidation of the biosynthetic enzymes participating in this pathway desirable. Previous studies suggest that the biosynthetic pathway of sorgoleone involves a polyketide synthase as well as a fatty acid desaturase, an O-methyl transferase, and a cytochrome P450 monooxygenase. This polyketide synthase is proposed to use a novel long chain fatty acyl-CoA (C16:3) as a starter unit followed by three iterative condensation reactions using malonyl-CoA to form a transient linear tetraketide that cyclizes to form a pentadecatriene resorcinol. To identify the polyketide synthase gene(s) involved in the biosynthesis of these alkylresorcinols, a root hair specific EST (expressed sequence tag) collection was mined for potential candidates. A total of nine polyketide synthase-like EST's were identified representing five unique contigs, three of which were preferentially expressed in root hairs. The molecular and biochemical characterization of these three candidate polyketide synthases will be presented of which two represent a novel type of type III plant polyketide synthase.

Metabolic Engineering of Unusual Fatty Acid Biosynthetic Pathways for Enhanced Vegetable Oil Quality and Plant Pathogen Resistance

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Hundreds of unusual fatty acids occur in plants, and many of these fatty acids have chemical, physical, or nutritional properties that make them attractive targets for the biotechnological enhancement of vegetable oils. My lab has maintained a long research interest in the identification of genes associated with the biosynthesis of unusual fatty acids, with the eventual goal of transferring these genes to existing crop species to generate oils with improved food, feed, or industrial quality. We have recently identified a class of enzymes that catalyzes the conversion of an existing double bond in linoleic acid ($18:2\Delta^{9,12}$) into two conjugated double bonds. These enzymes, which we have termed "fatty acid conjugases", are divergent forms of the ubiquitous Δ12 oleic acid desaturase. One type of fatty acid conjugase converts the Δ9 double bond of linoleic acid into Δ8 and Δ10 double bonds to form calendic acid ($18:3\Delta^{8,10,12}$), and a second type converts the Δ12 double bond into Δ11 and Δ13 double bonds to form eleosteraric acid ($18:3\Delta^{9,11,13}$). Fatty acid conjugases appear to have evolved numerous, independent times in the plant kingdom, as these enzymes occur in a limited number of species from diverse families including Asteraceae, Cucurbitaceae, and Euphorbiaceae. Oils enriched in the products of fatty acid conjugases are prone to oxidation and therefore have significant value as drying oils for the coatings industry. Our attempts to engineer pathways for conjugated fatty acid synthesis in seeds of Arabidopsis and soybean have met with only moderate success. We have been able to produce transgenic seed oils that contain 15 to 20% of either calendic acid or eleostearic acid. However, these levels are far below the 60+% that are typically found in seed oils that naturally accumulate these fatty acids. A bottleneck in the accumulation of conjugated fatty acids in seeds of transgenic plants appears to be the inefficient flux of these fatty acids from their synthesis on phospholipids to their storage as components of triacylglycerols, which is a major focus of our current research.

We have also recently reported the wide occurrence in the Asteraceae, Apiaceae, and Araliaceae families of a divergent form of the Δ12 desaturase that catalyzes the synthesis of a triple bond in linoleic acid. This enzyme, which is known as a fatty acid acetylenase, appears to catalyze an early step in the biosynthesis of biologically active polyacetylenic compounds that are produced by members of these families. These compounds include panaxynol, which has demonstrated pest resistance properties. The discovery of this widely occurring fatty acid acetylenase suggests that some unusual fatty acid biosynthetic pathways have arisen in plants for defense against pathogens and herbivores.

Iridoids and phenylpropanoids from *Penstemon gentianoides* (Scrophulariaceae).

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Penstemon gentianoides (HBK) Poiret, Lindl. Don. Scrophulariaceae a small shrub (common names: beardtongue, "jarritos", "jarra") grows on hilly areas above 3,000 m. in forest communities dominated by *Abies religiosa, Alnus jorullensis, Pinus pseudostrobus* and *Pinus hartwegii*, this plant is a popular medicinal species employed by different indigenous people from Mexico, they has profusely been used this plant for the treatments of diverse ailments. However, its main ethnomedicinal focus is in the treatment of inflammatory diseases. Previously we reports the anti-inflammatory and antioxidant effects of polar extracts from this species (Dominguez *et al*., 2004 PSNA Meeting; Dominguez *et al., J. Agric. Food Chem*., 2005, in press). Previous works in other Schrophulariaceae species reports the presence of iridoids, terpenes and flavonoids; however, there are not report about anti-inflammatory and antioxidant activities. Polar extracts and fractions were chromatographed; then natural compounds were isolated and purified by TLC and CC, afforded differents compounds: two iridoids (stansioside, 5-acetylstansioside), two iridoids similar to catalpol (one new, and globularicisine), two phenylpropanoids (martinoside and verbascoside), and two flavonoids. This is the first phytochemical study of this species.

This work was supported in part by DGAPA-UNAM grants # IN243802, IN211105 and USAID-TIES-ENLACES-PROGRAM.
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Session VI
Conservation and Divergence in Enzyme Function
Chair: Joseph P. Noel

Diversification for Defense: the Role of the Maize Terpene Synthase Gene Family in Multitrophic Interactions

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Despite the remarkable abundance and diversity of terpenoid secondary metabolites in plants, there are still large gaps in our knowledge of their biological and evolutionary origin. However, the availability of genetic and genomic resources for the model plant species maize and Arabidopsis provides an exciting array of new tools for exploring the ecological and evolutionary significance of this enormous class of natural products. The key step of terpene biosynthesis is catalyzed by the enzyme class of terpene synthases which employ an electrophilic reaction mechanism to form multiple products from single prenyl diphosphate substrates. In maize, terpene synthases are encoded by a large family of genes that share a common evolutionary origin. To identify the structures conveying the the functional diversity of terpene synthases, we subjected the closely related enzymes TPS4 and TPS5 to site directed mutagenesis. Two pockets in the active site were shown to carry out sequential parts of the reaction scheme resulting in multiple products and the stereospecific differences in product formation is determined by only four amno acids. Another factor increasing the diversity of maize terpene biosynthesis is the distribution of active and inactive alleles of TPS4 and TPS5 in maize varieties. To identify the function of terpene blends generated by the terpene synthases in the tritrophic interactions between maize, lepidopteran larvae and parasitoids, we overexpressed the corresponding genes in Arabidopsis and measured the attraction of the transgenic plants to the parasitoid *Cotesia marginiventris* using an olfactometer. A second tritrophic interaction was studied in the roots of maize which emit sesquiterpene hydrocarbons after insect damage. Feeding by larvae of the Western corn rootworm caused release of (-)-(E)-β-caryophyllene which was shown to attract enemies of the corn rootworm.

Chemistry and Mechanism of Alkaloid Biosynthesis

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Understanding the enzymes that catalyze natural product biosynthesis may enable production in more tractable host organisms, and may also allow reprogramming of biosynthetic pathways to produce "unnatural" natural products with potentially improved pharmacological activities. Many biosynthetic pathways of natural products found in higher plants remain largely uncharacterized. Our current efforts focus on determining whether enzymes from the terpene indole alkaloid pathway can utilize alternate substrates that result in modified alkaloid structures. Understanding the mechanism of these enzymes may also provide clues as to how to modulate substrate specificity.

Origins and Significance of Ergot Alkaloid Diversity in Fungi

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The ergot alkaloids are a complex family of mycotoxins derived from prenylated tryptophan in several species of fungi including the ergot fungi (*Claviceps* spp.), symbiotic grass endophytes in the genus *Neotyphodium*, and the opportunistic human pathogen *Aspergillus fumigatus*. Ergot alkaloids are well known from their historical role in human toxicoses and from the notoriety of the illicit drug LSD, but they also affect other organisms including bacteria, nematodes, and insects. Whereas the pharmacological effects of ergot alkaloids have been subjects of considerable research, the ecological significance of the alkaloids, which probably transcends their effects on mammals, is poorly understood. Interestingly, ergot alkaloid-producing fungi typically accumulate a characteristic profile of several ergot alkaloids rather than a single pathway end product. The diverse profile of ergot alkaloids observed in some fungi results in part from the apparent inefficiency of the pathway leading to accumulation of pathway intermediates to relatively high concentrations. Shunts off the main pathway and multiple peptide synthetases of varying substrate specificity also contribute to the diversity of ergot alkaloids observed in certain fungi. Different ergot alkaloids clearly have different biological activities and, thus, could play different roles in the biology and ecology of the producing fungus. The accumulation of intermediates and alternate products suggests that these alkaloids provide some benefits to the producing fungus that differs from those conferred by the pathway end product. This hypothesis is being tested by pathway truncation via gene knockout and subsequent analyses of the mutants.

Structural and Energetic Basis for Product Specificity Control by Sesquiterpene Cyclases

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Tobacco 5-epi-aristolochene synthase (TEAS) and henbane premnaspirodiene synthase (HPS) are evolutionarily related sesquiterpene cyclases which share a common reaction mechanism, but diverge at the penultimate step of catalysis where either a methyl migration or an alkyl shift occurs to produce 5-epi-aristolochene or premnaspirodiene, respectively. A contact map approach was developed to systematically identify differences in residues between TEAS and HPS that are in contact with active site residues shown to be in van der Waals contact with the farnesyl chain of the FPP analog (FHP) bound to TEAS. Mutating this identified collection of 9 residues in TEAS to the corresponding residues found in HPS led to the successful conversion of TEAS to HPS and represents a seminal discovery towards understanding the structural basis for product specificity control. The reciprocal conversion from HPS to TEAS was accomplished and demonstrates that these residues define an evolutionary pathway connecting their respective biosynthetic properties. Structure-based Combinatorial Protein Engineering (SCOPE) was used to create libraries encompassing all possible combinations of these mutations, in the context of TEAS and HPS, to further define a minimal collection of residues that are necessary and sufficient for this property change. Initial characterization of several mutants has identified a reduced set of mutations which interconvert the dominant product specificity as well as the recognition of minor products absent from the product distribution of the parent enzymes. Quantum mechanical treatment has been applied to the carbocation intermediate branch point (shown above) as well as the transition states leading to 5-epi-aristolochene or premnaspirodiene, and defines a theoretical basis for understanding the energetic control of the cyclization process exerted by TEAS and HPS. Ultimately, the full characterization of the biochemical properties of these mutants integrated with a theoretical chemistry framework will provide the first high-resolution pictures of the energetic landscape between subtle mechanistic differences that give rise to such diverse hydrocarbon skeletons in Nature.

The Structure of a Cyanobacterial Sucrose-Phosphatase
Reveals the "Sugar Tongs" that Release Free Sucrose in the Cell

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Sucrose-phosphatase (SPP) catalyzes the final step in the pathway of sucrose biosynthesis in both plants and cyanobacteria, and the SPPs from these two groups of organisms are closely related. We have crystallized the enzyme from the cyanobacterium *Synechocystis* sp. PCC 6803 and determined its crystal structure, alone and in complex with various ligands. The protein consists of a core domain containing the catalytic site and a smaller cap domain that contains a glucose-binding site. Two flexible hinge loops link the two domains, forming a structure that resembles a pair of sugar tongs. The glucose-binding site plays a major role in determining the enzyme's remarkable substrate specificity, and is also important for its inhibition by sucrose and glucose. It is proposed that the catalytic reaction is initiated by nucleophilic attack on the substrate by Asp9, and involves formation of a covalent phospho-Asp9-enzyme intermediate. From modelling based on the SPP structure, we predict that the non-catalytic SPP-like domain of the *Synechocystis* sucrose-phosphate synthase could bind sucrose-6^F-phosphate, and propose that this domain might be involved in metabolite channelling between the last two enzymes in the pathway of sucrose synthesis.

Poster Abstracts

Enhancement of Alkaloid Production in *Papaver somniferum* and *Eschscholtzia californica* by Transactivation Using Heterologous Regulatory Factors

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Regulatory factors isolated from Arabidopsis, soybean, and corn have been identified to modulate expression of genes that encode for enzymes involved in the biosynthetic pathways leading to the production of morphinan alkaloids in Opium poppy (*Papaver somniferum*) and benzophenanthridine alkaloids in California poppy (*Eszcholtzia californica)*. Selected regulatory factors were evaluated by transforming poppy callus cultures of both species. Expression of metabolic pathway genes was monitored by quantitative RT-PCR and alkaloid yields were analyzed using LC-MS.

In Opium poppy, overexpression of selected regulatory factors increased transcription of *Codeinone reductase (CR)*, *S-adenosyl-L-methionine:3'-hydroxy-N-methylcoclaurine 4'-O-methyltransferase (HMCOMT)*, and *(R,S)-norcoclaurine 6-O-methyltransferase (NOMT)* genes 10- to >100-fold. These transcriptional activations translate to enhancement of alkaloid production in Opium poppy. Specifically, production of thebaine was enhanced 5-fold.

In California poppy, analysis of the transactivation effect of a regulatory factor from Arabidopsis indicates that transcription of *Norcoclaurine-N-methyl-hydroxylase (NMCH)* and *Berberine bridge enzyme* (*BBE)* genes were increased up to 60-fold. At the same time, the accumulations of alkaloid intermediates were enhanced up to 30-fold in the same transgenic lines. The transactivation effect of other regulatory factors lead to the accumulation of the same intermediates and has produced new alkaloids in California poppy callus culture.

Crystal Structure of the tomato Methylketone Synthase 1 reveals a novel α/β hydrolase active site.

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Medium length methylketones like 2-tridecanone and 2-undecanone are potent toxins to a number of herbivorous insects. The presence of methylketones within the glandular trichomes of certain wild species of tomato provide the tomato plant with a built-in defense mechanism. The production of both 2-tridecanone and 2-undecanone was recently linked to the expression of Methylketone Synthase 1 (MKS1), a member of the α/β hydrolase fold family of proteins. MKS1 is a monomeric plastid-localized protein shown to catalyze the production of 2-tridecanone from β-ketomyristoyl-ACP, a process requiring both de-esterification and decarboxylation.

Although proteins possessing the α/β hydrolase fold have little sequence identity, their active sites do conserve a catalytic triad consisting of a nucleophile activated by a histidine/acid pair. A homology model of MKS1 based on the structure of Hydroxynitrile Lyase (HNL), predicted that MKS1 contained only the central histidine of the canonical catalytic triad. We crystallized MKS1 and determined its three dimensional structure to 2.3 Å resolution by molecular replacement using this homology model. Conformational active site differences in the two monomers that make up the MKS1 crystal's asymmetric unit suggest a dynamic flexibility in solution that my be important for catalysis. The MKS1 structure also reveals a threonine-stabilized water molecule, poised next to the active site histidine, that is likely to serve as the MKS1 nucleophile. We are currently pursuing co-crystallization with substrate, intermediate and product analogues to explore a number of MKS1 mechanistic hypotheses.

Complete Saponin and Isoflavone Analysis of Whole Soybean, Germ, Hulls, and Cotyledons. Mark A. Berhow and Sandra M. Duval, USDA, ARS, NCAUR, 1815 N. University St., Peoria, IL 61604

Reproducible methodology for the recovery and analysis of all the isoforms of isoflavones and saponins in whole soybeans continues to be a problem. A standardized sample preparation and extraction method has been developed to prepare extracts for analysis of both total saponins and total isoflavones that has been shown to be reproducible in other labs as well as our own. The extracts can be analyzed with standard HPLC and LC-MS methods to identify and quantitate the malonyl-, acetyl-, glucosyl- and aglycone forms of the soy isoflavones, as well as the A-group and B-group forms (including the DMPP forms) of the soy saponins. Extractions were prepared from hand-separated soy seed components—germ, hulls and cotyledons. As has been previously reported the germ is rich in the daidzein and glycitein isoflavone isoforms, while the hypocots are rich in the genistein isoflavone isoforms. The cotyledons contain roughly two-thirds the total isoflavones found in the seed. Saponin analysis shows a significant portion of the saponins found in the soybean is concentrated in the germ. The germ contains nearly all the A-group saponins, while the B-group saponins are nearly equally distributed between the germ and cotyledons. The hulls contain little in the way of either isoflavones or saponins.

Selective Metabolism of Ginsenosides by *Pythium irregulare*

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Our earlier work supports the existence of an allelopathic relationship between ginseng saponins (ginsenosides) and soilborne microbes, in which ginsenosides promote the growth of the oomycete pathogen *Pythium irregulare* and inhibit that of the non-pathogenic fungus *Trichoderma hamatum*. Consequently, ginsenosides, like other saponins, are thought to play a role as pre-formed plant defense compounds. Two hypotheses have been presented to explain the apparent resistance of oomycetes to plant saponins: (1) innate resistance due to the lack of sterols in oomycete membranes, and (2) "detoxification" via deglycosylation. Herein we report that when *P. irregulare* was cultured in the presence of ginsenosides, nearly all of the 20(*S*)-protopanaxadiol ginsenosides (Rb_1, Rb_2, Rc, Rd, and to a limited extent G-XVII) were converted into a single product ginsenoside F_2, at least half of which was recovered from the isolated mycelia of the organism. No metabolism of the 20(*S*)-protopanaxatriol ginsenosides (Rg_1 and Re) was evident. By contrast, none of the ginsenosides added to the culture medium of *T. hamatum* were metabolized.

The selective metabolism of 20(*S*)-protopanaxadiol ginsenosides by *P. irregulare* occurs through the hydrolysis of terminal monosaccharide units from disaccharides present at C-3 and/or C-20 of ginsenosides Rb_1, Rc, Rb_2, Rd and G-XVII to yield a common product, ginsenoside F_2 and a minor product (possibly G-III). Interestingly, while remaining selective for 20(*S*)-protopanaxadiols, the extracellular proteins isolated from naïve *P. irregulare* cultures had different specific activities toward ginsenosides and the colourimetric substrate 4-nitrophenyl-β-D-glucopyranoside, than those isolated from ginsenoside-treated cultures. Taken together, these data support the hypothesis that *P. irregulare* is using 20(*S*)-protopanaxadiol ginsenosides as a chemical cue to find host roots.

Functional Characterization of Two *Medicago truncatula* Natural Product Glycosyltransferases

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Glycosyltransferases (GTs) transfer nucleotide-diphosphate-activated sugars to low molecular weight compounds, aiding in the stabilization, compartmentation and activation of a variety of secondary metabolites. Medicago truncatula, a model legume, contains many glycosylated secondary metabolites such as isoflavonoids, flavonoids, anthocyanins, and triterpene saponins. Using our extensive M. truncatula EST database, we have identified 63 full length putative GTs which may be involved in the glycosylation of secondary metabolites. An efficient HPLC method was developed to analyze the GT candidates against 31 potential substrates by dividing the substrates "equally" into four groups. The compounds in each group are readily separated by the HPLC method, and each compound within a particular group has a distinct UV spectrum. The enzyme assay mixed substrate conditions and HPLC method were optimized using two GTs (GTa and GTb). These results were reconfirmed for both enzymes by assaying them with individual substrates. GTa and GTb have some similarities in their glycosylation patterns of several different substrates, glycosylating at the same position(s). Yet they also have some strong differences in their substrate preferences as well as their apparent glycosylation site preferences. This poster will present the functional analysis of these two M. truncatula GTs in relation to one another.

Isolation and Identification of Antifungal and Antialgal Alkaloids from *Haplophyllum sieversii*

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Bioassay-guided fractionation of the hexane:ethyl acetate:water extract of the aerial parts of *Haplophyllum sieversii* was performed due to preliminary screening data that indicated the presence of growth inhibitory components against *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*. Fractionation was directed using bioautographical methods resulting in the isolation of the bioactive alkaloids flindersine, anhydroevoxine, haplamine, and an inactive lignan eudesmin. These four compounds were evaluated for activity against *C. fragariae*, *C. gloeosporioides*, *C. acutatum*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Phomopsis obscurans* in a dose-response growth-inhibitory bioassay at 50.0, 100.0, and 150.0 μM. Of the four compounds tested, flindersine demonstrated the highest level of antifungal activity. Additionally, flindersine, eudesmin, and haplamine were screened against the freshwater phytoplankton *Oscillatoria perornata*, *Oscillatoria agardhii*, *Selenastrum capricornutum*, and *Pseudanabaena* sp. (strain LW397). Haplamine demonstrated selective inhibition against the odor-producing cyanobacterium *O. perornata* compared to activity against the green alga *S. capricornutum*, with lowest-observed-effect concentration values of 1.0 μM and 10.0 μM, respectively.

Antioxidant , insecticidal and insect growth regulatory activities of lignans from Araucaria araucana against *Spodoptera frugiperda*.

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The methanol/H_2O extract from bark and wood of *Araucaria araucana* afforded secoisolariciresinol 1, lariciresinol 2, pinoresinol 3, methylpinoresinol 4, and eudesmin 5. These natural products showed insecticidal and insect growth regulatory activity against FAW *Spodoptera frugiperda,* an insect pest of corn. The most active compounds were pinoresinol and eudesmin, which had significant effects between 0.1 and 5.0 ppm in diets. These compounds were insecticidal at concentrations greater than 5.0 ppm. However, 1, 2 and 4 only showed an IGR effect at concentrations ranged between 15.0 to 50.0 ppm. In addition to crocin and β-carotene, some of these compounds also demonstrated scavenging properties toward DPPH, as well as, in TLC autographic and spectrophotometric assays. Our results indicate that these compounds appear to have selective effects on the pre-emergence metabolism of the insect. The results were fully comparable to known natural insect growth inhibitors such as toosendanin and *Yucca* extracts and have had a possible role as natural insecticidal agents.

This work was partially supported by FONDECYT, grant 1990444.

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Characterization of the biosynthesis of 5-alkyl resorcinols in rice

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Several rice cultivars have allelopathic potential against weeds common in paddy fields. Quantitative Trait Loci (QTL) analysis has associated the allelopathic trait with several rice chromosomes, suggesting that these weed-repressing varieties may produce several phytotoxins. Allelopathic rice cultivars produce glycosides of alkyl resorcinols, flavones, benzoxazinoids, as well as several momilactones and cyclohexenones. All of these compounds are known to be biologically active. Alkyl resorcinols are of particular interest because these secondary metabolites have been associated with pathogen resistance and allelochemical traits of other monocotyledonous species (eg. wheat, rye and barley) and have been identified as markers to evaluate the allelopathic potential of rice varieties. The biosynthesis of alkyl resorcinols is catalyzed by a novel type III polyketide synthase that accepts long chain fatty acid-CoA substrates instead of the usual coumaroyl-CoA substrate used by the more common polyketide synthases such as chalcone and stilbene synthases. The rice genome possesses at least 33 polyketide synthases. Of these, 8 polyketide synthase genes have similarity to the genes from sorghum responsible for the synthesis of a 5-pentadecatriene resorcinol intermediate in the formation of the allelochemical sorgoleone. These genes were cloned, overexpressed in *E. coli,* and the resulting his-tagged proteins were purified by affinity chromatography. The substrate specificity and biochemical characterization of these rice enzymes involved in the ring formation of these unusual resorcinols possessing long fatty acid-like side chains will be presented.

Analysis of Basil Mutants Gland Proteome Reveals EUGENOL SYNTHASE

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The phenylpropene class compounds (eugenol, isoeugenol, chavicol and their derivates) play an important role in the ecology of some plant species, either for defense or for attraction of insects for pollination. These compounds are recognized as the main contributors to the characteristic aroma of some of the most important spices that have shaped human history (e.g. cloves and allspice). Sweet basil (*Ocimum basilicum*) was shown previously to synthesize and accumulate these beneficial compounds in the peltate glands on the surface of its leaves. Although this pathway shares intermediates with the relatively known lignin/phenylpropanoid pathway, the last steps that lead to the phenylpropenes (e.g. modification of the allyl/propenyl side chain) have remained elusive.

We adopted a genetic approach that included ethyl methane sulfonate (EMS) mutagenesis of a eugenol producing basil line combined with high-throughput screening for mutants, followed by proteomic analysis of the glands. Proteomics analysis of two independent mutants, with significantly reduced levels of eugenol in the leaves, identified a single protein that was underrepresented in the mutants glands. Comparison of the peptide sequence to the GenBank database revealed that it shares app. 40% sequence identity with isoflavone reducatase homologs (IFRHs) proteins from several plants. Expression analysis of the identified basil IFRH (namely EUGENOL SYNTHASE 1; EGS1) showed specific expression in the glands and, interestingly, the expression of the gene in the leaves of the two mutants is not significantly reduced compare to the wild type plant. Feeding coniferyl alcohol to *E. coli* cells that expressed EGS1 led to detection of eugenol in the spent medium. This study highlights the power of combining genetics and proteomics for the study of specialized metabolism in glands.

Metabolic Profiling of In Vitro Micropropagated and Greenhouse Grown Ginger and Turmeric

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Ginger (*Zingiber officinale* Rosc.) and turmeric (*Curcuma longa* L.) are well known for their culinary and medicinal value, which are due to pharmacologically active compounds, such as the anti-inflammatory gingerols and curcuminoids, that these plants produce and store in their rhizomes. In our efforts to elucidate the biochemical pathways to these compounds, we found it necessary to develop a method to rapidly produce large numbers of ginger and turmeric plants from small amounts of starting material. As a result, we developed in vitro micropropagation methods for ginger and turmeric plants. One concern with such methods is that the resulting plants may not possess the same properties (such as presence or concentration of specific metabolites) as the parents. We used a metabolic profiling approach to address this question, by comparing ginger and turmeric plants that were propagated in vitro to plants propagated by traditional agronomic means. The targets of these investigations were the medicinally important curcuminoids, gingerols, mono- and diacetylgingerols, gingerdiols, 1-dehydrogingerdiones, paradols, shogaols, 3-dihydroshagaols, other diarylheptanoids, and methyl ether derivatives of these compounds, as well as major mono- and sesquiterpenoids. The in vitro propagated plants showed no qualitative differences in major volatile or non-volatile compound composition when compared to greenhouse grown plants. Media conditions used for micropropagation, including differences in concentrations of specific growth regulators, led to quantitative differences in accumulation of compounds such as [6]-, [8]-, and [10]-gingerols in ginger, and the curcuminoids in turmeric. For example, cytokinins stimulated or enhanced the production of gingerols and curcuminoids. When specific conditions were applied during in vitro micropropagation, ginger and turmeric plants with essentially the same metabolic profiles as the parental lines were produced.

Purification, Crystallization and Mutational Analysis of the Plant Natural Product Glycosyltransferase UGT71G1

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Saponins are common plant secondary metabolites consisting of triterpenoid or steroid aglycones with various sugar side chains. Their antimicrobial activity, toxicity to insects and potentially useful anti-cancer, anti-cholesterol and hemolytic activities have attracted considerable attention. The uptake, metabolism, and biological effects of these compounds rely on the presence and nature of the glycosidic structures. Understanding the glycosylation of the triterpenes and other secondary metabolites is important for future metabolic engineering of their pathways. Glycosylation reactions are catalyzed by glycosyltransferases (GTs) which comprise a large and divergent mutigene super-family. GTs are currently divided into 69 different families on the basis of biochemical and sequence similarities. UGT71G1 has recently been characterized from the model legume *Medicargo truncatula*, and shown to glycosylate the triterpene aglycones medicargenic acid and hederagenin, as well as the flavonoid quercetin, using UDP-glucose as sugar donor. Here we report the purification, crystallization, crystal structure and mutational analysis of UGT71G1.

A new flavonoid from the fern *Dryopteris villarii.*

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A large number of flavonoids have been found[1] in ferns belonging to the genus *Dryopteris* but no flavonoid data have previously been reported for *Dryopteris villarii*.

From an ethanolic extract of aerial parts of the fern *Dryopteris villarii,* apigenin (**1**), kaempferol (**2**), quercetin (**3**), kaempferol 3-*O*-glucoside (astragalin) (**4**), kaempferol 3-*O*-rutinoside (nicotiflorin) (**5**), kaempferol 3-*O*-acetylrutinoside (**6**), quercetin 3-*O*-glucoside (isoquercitrin) (**7**), quercetin 3-*O*-rutinoside (rutin) (**8**) and quercetin 3-*O*-acetylrutinoside (**9**) have been isolated by preparative paper chromatography followed by Sephadex LH-20 column chromatography. These compounds have been identified by chemical and spectral methods.Flavonoid (**6**) is a new natural products whereas flavonoid (**9**) is a new constituent of ferns. Acylated flavonoids are reported for the first time from the genus *Dryopteris*.

The author thanks CNR/ISA (Avellino, Italy) for some electrospray mass spectra.

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A Comparative Systems Biology Approach to Investigate Specialized Metabolism in Sweet Basil Peltate Glandular Trichomes

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The peltate glandular trichomes (glands) of sweet basil (*Ocimum basilicum* L.) are an ideal system to investigate plant biochemical processes and their regulation in a single cell type. From gland cDNA libraries of 4 phytochemically distinct basil lines, we have constructed a database of 24,248 ESTs, which assembled into 4891 contigs plus 3516 singletons, for a total of 8407 non-redundant sequences. After BLASTing these sequences against the UniProt database, 69 % of contigs (accounting for 81 % of ESTs) were assigned a MatchID, and the transcriptomes of each line were functionally categorized. Comprehensive mining of this database has identified over 100 genes representing several classes of transcription factors, as well as several previously undescribed genes, such as a cinnamic acid carboxylmethyltransferase (CACMT) responsible for the biosynthesis of methylcinnamate, and candidate genes for yet uncharacterized steps of the biosynthesis of compounds such as eugenol and methoxylated flavonoids. Genes for a number of non-metabolism proteins, some with unknown functions, are highly expressed in all basil lines and appear to be important to peltate trichome biology. Expression levels for genes in major metabolic pathways were compared between lines using digital gene expression analysis, resulting in identification of, as on example, many genes across primary, shikimate, phenylpropanoid and 1-carbon pathways that are upregulated in conjunction with CACMT expression. Digital expression levels for several genes were confirmed using qRT-PCR. We further used this approach to evaluate transcript levels in other tissues, resulting in the identification of gland-specific gene expression patterns for several genes and confirming the exclusive biosynthetic capacity of peltate glandular trichomes for several important metabolites in basil, which corresponds well to metabolic profiling results. We have also characterized the soluble proteome of the peltate glandular trichomes of one basil line, using a combination of 2D-LC-MS/MS analysis and 1-D gel separation followed by in-gel digestion and MS/MS protein identification. A majority of the 412 soluble proteins that were thus identified corresponded to genes that are highly expressed according to the EST and qRT-PCR data.

Defining the 4-coumarate CoA ligase metabolic network in *Arabidopsis*: physiological roles and substrate specificities

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As part of a NSF 2010 project directed towards identifying the metabolic networks associated with phenylpropanoid (acetate) metabolism, a recent *in silico* analysis revealed that the *Arabidopsis* genome has fourteen genes annotated as putative 4-coumarate CoA ligase isoforms or homologues. Eleven were selected for detailed functional analysis *in vitro*, using all known possible phenylpropanoid pathway intermediates (*p*-coumaric, caffeic, ferulic, 5-hydroxyferulic and sinapic acids). Four were catalytically active *in vitro*, with fairly broad substrate specificities. Of these, At4CL1 best utilized *p*-coumaric, caffeic, ferulic and 5-hydroxyferulic acids, whereas At4CL2 readily transformed *p*-coumaric and caffeic acids into the corresponding CoA esters. At4CL3 displayed broad substrate specificity efficiently converting *p*-coumaric, caffeic and ferulic acids into their CoA esters. While At4CL5 was the only isoform capable of ligating sinapic acid, the preferred substrates were 5-hydroxyferulic and caffeic acids. Both At4CL1 and At4CL5 most efficiently utilized 5-hydroxyferulic acid with k_{enz} ~10 fold greater than At4CL2 and 3.

Although At4CL5 is able to convert both 5-hydroxyferulic and sinapic acids into the corresponding CoA esters, the physiological significance of the latter observation *in vitro* was in question, i.e. particularly since other At4CL isoforms can effectively convert 5-hydroxyferulic acid into 5-hydroxyferuloyl CoA. An homozygous line containing an enhancer trap insert (knockout) for *4cl5* was selected by screening. Then *Arabidopsis* stem and leaves tissues from the mutant line and wild type were individually subjected to detailed analyses for both lignin monomeric compositions and contents, and sinapate/sinapyl alcohol derivative formation, at different stages of growth and development until maturation. Relative to wild type, this "knockout" had no significant effect on either lignin content or monomeric composition, or accumulation of sinapate/sinapyl alcohol derivatives, i.e. no specific physiological role for At4CL5 in direct sinapic acid ligation was identified. The overall organization of the four-membered 4CL network *in planta* is the subject of ongoing inquiry for all stages of *Arabidopsis* growth and development.

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EST Mining of a Putative Flavonoid Glucosyltransferase Clone from *Citrus paradisi* Leaves: Cloning, Expression, and Functional Analysis. Starla Kiser, Lee M. Pike, and Cecilia A. McIntosh, Dept. Biological Sciences, East Tennessee State University, Johnson City, TN 37614, USA

Flavonoids contribute in diverse ways to plant biochemistry. The phenolic structure of flavonoids also make them potent antioxidants; they are thus of particular interest in human health. Flavonoids are divided into various classes based on molecular structure and are synthesized by a common pathway. Modifications of parent molecules result in over 5000 different flavonoid metabolites found in plants. Although much research has focused on the core pathway, less is known of the regulation of modification reactions involved in synthesis of the flavonoid derivatives.

Glucosylation is one of the most common modifications that occur after the core pathway of flavonoid synthesis. *Citrus paradisi* (grapefruit) is known for characteristic production and accumulation of flavanone and flavone glycosides and presents an ideal model system for the study of uncommon flavonoid GTs. For example, a flavanone-specific 7-O-GT (7GT) has been characterized from young leaf tissue that has a primary role in the "capture" of naringenin in the hyper-accumulation of the bitter flavanone diglycoside, naringin, in young leaves and fruit. Understanding the molecular mechanisms of the production of flavonoid glycosides in grapefruit would significantly contribute to the knowledge of flavonoid metabolism and could ultimately provide the possibility to develop transgenic plant lines with altered flavonoid contents.

A putative full-length GT clone, CP5D12, has been obtained from EST mining of a directionally cloned cDNA library. Characterization of the clone is focusing on 3 main objectives: independently verifying the sequence, expressing the protein coded by the full-length clone, and assaying the expressed protein for flavonoid GT activity. Sequencing confirmed the identity of a full-length putative GT clone. Expression of the protein was done using the pET system. Primers were designed to incorporate restriction sites at the ends of the CP5D12 insert via PCR. The PCR product was ligated into TOPO vector for amplification; isolated plasmids were subsequently digested, ligated into pCD1 expression vector, and transformed into BL21(DE3)RIL cells. Transformed colonies were induced with IPTG at various temperatures and time points in order to determine optimal conditions for protein expression. Analysis of the expressed protein for flavonoid GT activity is underway.

Dissecting the Regulation of Biochemical Pathways Using Oligonulceotide Microarrays, Metabolite Profiling and Pathway Map Visualization Tools

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Recently, we have introduced `BioPathAt`, a bioinformatic tool that integrates and displays complex genomic data sets directly in the context of biochemical pathways (Lange B.M. & Ghassemian M. (2005) *Phytochemistry* 66: 413-451) and we are using a photomorphogenesis experiment to demonstrate its utility. One of the key developmental processes during photomorphogenesis encompasses the differentiation of prolamellar bodies of proplastids into thylakoid membranes containing the photosynthetic pigment-protein complexes of chloroplasts. When dark-grown wild-type were exposed to continuous red light (Rc) a rapid greening was observed, whereas treatment with continuous far-red light (FRc) did not result in a visible phenotypic change. In Rc-treated seedlings gene expression and metabolite patterns were tightly correlated across multiple metabolic pathways (biogenesis of thylakoid membranes, assembly of photosystems, activation of chlorophyll, carotenoid, tocopherol, phylloquinone, carbohydrate and transitory starch biosynthesis, decrease in soluble amino acid pools), and appeared to be in accordance with the phenotypic changes (greening). The gene expression patterns in FRc-grown seedlings were very similar to those observed in Rc despite drastic differences in the experimental outcome (block of greening in FRc). However, the metabolite patterns in FRc clearly differed from those in Rc (lack of chlorophylls and reduced carotenoid, phylloquinone, ubiquinone and tocopherol levels), suggesting an uncoupling of gene expression patterns and phenotypic differentiation. The relevance of integrating different genomic data sets for the development of models describing the regulation of biochemical networks will be discussed.

Evidence for the Monophyletic Evolution of Benzylisoquinoline Alkaloid Biosynthesis in Angiosperms

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Benzylisoquinoline alkaloids (BIAs) are a diverse group of secondary metabolites consisting of more than 2,500 known structures, and include several compounds of medicinal and economic importance. While major producers of complex BIAs are concentrated within the order Ranunculales, these compounds also occur somewhat sporadically in several taxa throughout the angiospermae. Many BIAs are believed to function in plant defense, combatting herbivory and pathogenic attack. As such, the capacity to produce these compounds is expected to influence the reproductive fitness of certain plants. All BIAs are formed by the elaboration of (*S*)-norcoclaurine, the product of the first-committed step in BIA biosynthesis, (*S*)-norcoclaurine synthase (NCS; EC 4.2.1.78). Biochemical and molecular phylogenetic approaches were used to investigate the evolution of BIA biosynthesis in basal angiosperms. The occurrence of NCS activity in 90 diverse plant species was compared to the distribution of BIAs superimposed onto a molecular phylogeny. These results support the monophyletic origin of BIA biosynthesis prior to the emergence of the eudicots. Phylogenetic analyses of NCS, berberine bridge enzyme (BBE) and several *O*-methyltransferases suggest a latent molecular fingerprint for BIA biosynthesis in angiosperms not known to accumulate such alkaloids. The limited occurrence of the pathway outside the Ranunculales suggests that a highly specialized, yet evolutionarily unstable cellular platform might be required to accommodate, or potentially reactivate the pathway in divergent taxa. The molecular cloning and functional characterization of NCS from opium poppy (*Papaver somniferum L.*) is also reported. Pathogenesis-related (PR)10 and Bet v 1 major allergen proteins share homology with NCS, but recombinant polypeptides were devoid of NCS activity.

Structural bases for stereo-selectivity, regio-specificity and substrate promiscuity of isoflavonoid *O*-methyltransferases

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Isoflavonoids are natural products primarily limited to the Legume species, where they act as anti-microbial phytoalexins in plant defense responses, and as signaling molecules mediating bacterial or fungal symbioses. Moreover, isoflavonoids constitute the most potent group of phytoestrogens with potential dietary utility in humans for the prevention of cancer and cardiovascular disorder. Isoflavonoid *O*-methyltransferases catalyze the several critical reactions in the biosynthetic pathway leading to the formation a variety of pterocarpan phytoalexins in different legume species. Two families of isoflavonoid *O*-methyltransferase members were identified from *Medicago* species. Interestingly hydroxyisoflavanone 4'-*O*-methyltransferase (HI4'OMT) exhibited dual functionality for both the 4'-*O*-methylation of 2,7,4'-trihydroxyisoflavanone and the 3-*O*-methylation of (+)-6a-hydroxymaackiain, while isoflavone 7-O-methyltransferses (I7OMT) show promiscuity in substrate recognition. Protein X-ray crystallography of HI4'OMT-substrate complexes revealed the close conformational similarity of the bound phenolic 6aR,11aR-pterocarpans with 2S,3R-hydroxyisoflavanones thus structurally explaining the dual activities of MtHI4'OMT/HM3OMT toward two seemingly distinct isoflavonoid substrates. The structures of I7OMT-substrate complexes implicated conformational flexibility of the enzyme's architecture at the active site for properly binding the structurally distinct isoflavone substrates and for efficient methyl-transfer.

Heterologous expression of *Fusarium* trichothecene P450 genes

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Fusarium graminearum and *F. sporotrichioides* produce the trichothecene mycotoxins 15-acetyldeoxynivalenol and T-2 toxin, respectively. In both species, disruption of the P450 monooxygenase-encoding gene *Tri4* blocks production of the mycotoxins and leads to the accumulation of the trichothecene precursor trichodiene. *F. graminearum Tri4* (*FgTri4*) was heterologously expressed in the trichothecene-nonproducing species *F. verticillioides*. Transgenic *F. verticillioides* carrying the *FgTri4* converted exogenous trichodiene to the trichothecene biosynthetic intermediate isotrichodermin. Conversion of trichodiene to isotrichodermin requires seven steps, two of which can occur non-enzymatically. The results of this study suggest that the *FgTri4* protein catalyzes the first four oxygenation steps. We used heterologous expression to look at oxygenation steps near the end of the biosynthetic pathway controlled by *Tri1*. Experiments confirmed that *FsTri1* controls C-8 hydroxylation but showed that *FgTri1*controls both C-7 and C-8 hydroxylation. We also found that although wild-type *F. verticillioides* produces no trichothecenes it is capable of trichothecene C-3 acetylation, C-4 or C-15 deacetylation and C-8 oxidation.

Characterization of a bifunctional terpene synthase from *Antirrhinum majus* catalyzing the formation of a sesquiterpene, nerolidol and, a monoterpene, linalool

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Plants emit an array of volatiles that play an important role in pollinator attraction, defense against herbivores/pathogens and communication. *Antirrhinum* flowers emit a sesquiterpene, nerolidol derived from farnesyl diphosphate (FPP) in addition to three geranyl diphosphate (GPP) derived monoterpenes, myrcene, ocimene and linalool. A detailed search of the *Antirrhinum* petal specific EST library resulted in isolation of a cDNA (*ama1i06*) that has a high similarity to known sesquiterpene synthases. Sequence analysis revealed an open reading frame of 1,698 nucleotides that encodes a protein of 566 amino acids. The nucleotide and amino acid sequences share over 50% identity to three *Antirrhinum* monoterpene synthases (ama1e20, amaOc15 and amaOe23) and about 45% amino acid identity to *Fragaria ananassa* linalool/nerolidol synthase.

RT-PCR analyses of RNA from different floral tissues revealed that *AmNES* is highly expressed in upper and lower petal lobes with negligible expression in green tissues. A full length AmNES and a truncated AmNES from the second methionine lacking a signal peptide were expressed in *Escherichia coli* as C-terminal $(His)_6$-tagged proteins. Subsequent characterization using purified enzyme showed that the enzyme requires Mg^{2+} as a divalent metal cofactor for its activity. Full length AmNES catalyzed nerolidol and linalool production from FPP and GPP, respectively, whereas truncated AmNES catalyzed only nerolidol formation from FPP, suggesting the possibility of an alternate transcription start sites or alternative translation initiation codons to generate either the cytosolic or the plastidial isoforms. Purified enzymes were used to determine the substrate specificity, pH optimum, metal ion requirement and kinetic parameters.

Molecular and Genetic Analysis of *ETHYLENE INSENSITIVE 6* and the *ENHANCER OF ETHYLENE INSENSITIVITY*

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The plant hormone ethylene regulates a variety of developmental and stress responses, including the triple response displayed by etiolated seedlings. We have previously identified *ETHYLENE INSENSITIVE6* (*EIN6*) in a genetic screen for mutants deficient in the triple response. Mutations in *EIN6* are epistatic to *CONSTITUTIVE TRIPLE RESPONSE* (*CTR1*) and therefore *EIN6* acts downstream of *CTR1* in the ethylene signaling pathway. Further characterization of this mutant revealed that it contained a second recessive mutation, *ENHANCER OF ETHYLENE INSENSITIVITY* (*EEN*), which dramatically enhanced the *ein6* ethylene phenotype. In the absence of *een*, *ein6* plants display an ethylene insensitive root phenotype. Mutations in the *EEN* gene alone show no ethylene phenotype, but dramatically enhance the *ein6* ethylene insensitive root phenotype; the ein6een double mutants show a near complete lack of the triple response. *EIN6* was mapped to the bottom of chromosome 3. Positional cloning of *EIN6* revealed that it encodes a DNA binding protein, consistent with its downstream position late in the ethylene signaling pathway. Protein levels of the transcription factor EIN3 and its related family members are regulated by the *EIN6*. In the *ein6een* double mutant EIN3 protein levels are nearly abolished. This indicates that the ethylene signaling pathway works through either the *EIN6* gene or the *EEN* gene. Further work from genetic crosses shows that *EEN* is controlling EIN3 protein levels and is possibly working in parallel with the F-box proteins EBF1 and EBF2.

Evidence for non-random alkali-labile aryl-O-ether linkages in *Arabidopsis fah* 1-2, C4H::F5H and *irx4.*

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Lignins from various transgenic/mutant lines have recently been established to have non-random alkali-labile aryl-*O*-ether linkages. That is, the amounts of monolignol-derived moieties released by thioacidolytic cleavage of (aryl-*O*-4-linkages) were determined to be directly proportional to their corresponding total lignin contents and independent of monomeric composition; together, they account for approximately 35-40% of overall lignin content. These *Arabidopsis* lines included: *fah* 1-2 (a ferulate 5-hydroxylase [F5H] mutant), C4H::F5H (a F5H up-regulated transgenic), and the CCR1-*irx4* mutant (cinnamoyl-CoA reductase 1). The invariant nature of this predominant inter-unit linkage is indicative of explicit, regular (non-random), first order, primary lignin structures in the native macromolecules, which are independent of degree of methoxylation at positions 3 and 5. Each of these lines was also analyzed by histochemical methods and the results obtained revealed a conserved lignin assembly process. Futhermore, we examined the lignin-enriched isolate from the CCR1-*irx4* mutant using nuclear magnetic resonance (NMR) techniques and confirmed that the apparent assembly of its lignin was both normal and specific with no evidence for abnormal lignin deposition. Taken together, these results provide further evidence that lignin assembly occurs in a highly regular, elegant and predictable manner.

Liquid suspension culture as a model for studying defensive terpene metabolism in Norway spruce (*Picea abies*)

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Conifer species produce copious amounts of terpenoid products as a defensive secretion to deter predatory insects such as bark beetles as well as their pathogenic fungal symbionts. The activation of terpene biosynthesis is suspected to involve upregulation of both the MEP pathway and terpene synthases, the former supplying substrates for the latter. However, difficulties inherent in working with intact spruce (long generation time, RNA extraction, large genome, etc.) have slowed the development of molecular tools to dissect the coordination of isoprenoid pathways during a defensive response.

Here we report the utility of embryogenic spruce liquid suspension cultures to emulate this defensive response in a system more amenable to gene discovery, gene regulation studies, and transformation. We have determined that suspension cultures and intact spruce trees are similar in many aspects of induced terpenoid metabolism and its regulation. For example, we found that suspension cultures biosynthesize small amounts of monoterpenes *de novo* with a product profile similar to that of adult trees and that this production could be induced by methyl jasmonate (MJ) or chitosan treatment, as in intact trees. The enhanced production of *de novo* biosynthesized monoterpenes in culture was paralleled by an increase in monoterpene synthase activity as measured by *in vitro* enzyme assays (nearly a 10-fold increase was observed in 24 h post-MJ treatment). Using quantitative Real-Time PCR, we have also determined that MJ induces upregulation of a specific isoform of *dxs* in culture, a highly regulated gene in the MEP pathway.

Using liquid culture as a guide, these tools will help to determine how regulation of terpene production in spruce is controlled as well as understand how the multi-gene families in the MEP pathway are differentially activated to satisfy cellular needs for isoprenoid intermediates for both primary and defensive metabolism. Despite the enormous differences between suspension cultures and intact spruce trees in tissue organization, the similarities in their induced terpenoid responses suggest that tissue culture can be a useful system for the detailed dissection of the regulation of terpenoid production in spruce.

Protein-protein interactions in the MEP pathway.

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Isoprenoids (= terpenoids) are the most structurally diverse family of compound found in nature. More than 23 000 isoprenoid molecules are known to date. Many isoprenoids have biotechnological applications as drugs, flavours, pigments, perfumes or agrochemicals.

IPP and the isomeric compound, DMAPP are the fundamental building blocks of isoprenoids in all organisms. Until recently it was generally assumed that IPP was derived solely from mevalonate synthesized from the condensation of three molecules of acetyl-CoA. However, in the early 1990s two research groups independently demonstrated the existence of a novel, mevalonate-independent pathway for IPP synthesis known as the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. This latter mevalonate-independent pathway utilizes pyruvate and glyceraldehyde 3-phosphate as starting materials for production of IPP.

In the past few years, efforts have focused on the discovery of the enzymes involved in this MEP pathway. However, possible protein-protein interactions between proteins of the pathway have not been investigated. . In order to address this question, we are using yeast two-hybrid and tap-tagging approaches, to map interactions between all the members of the pathway.

Expression, Purification, and Characterization of a Recombinant *S*-adenosyl-L-methionine: Cinnamic Acid Carboxylmethyltransferase from Sweet Basil (*Ocimum basilicum*)

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Plants produce a wide variety of methyl ester compounds. These compounds are often responsible for attracting pollinators or acting as airborne chemical "warning signals" in response to physical injury. One such methyl ester, methyl cinnamate, is an important component of the floral scent of many plants. It is also an important component in the aroma and flavor of cinnamon basil (*Ocimum basilicum*). Using a biochemical genomics based approach, we have identified the enzyme responsible for formation of methyl cinnamate in sweet basil. This enzyme, cinnamic acid carboxymethyl transferase (CACMT), uses *S*-adenosyl-L-methionine (SAM) as methyl group donor. A cDNA encoding CACMT was found by identifying in an EST database a gene that had homology to known carboxyl methyltransferases such as benzoic acid methyltransferase (BAMT), salicylic acid methyltransferase (SAMT), and jasmonic acid methyltransferase (JMT). CACMT was only expressed in basil lines that produce methyl cinnamate. Sequence analysis of the resulting cDNA revealed an open reading frame of 1,122 nucleotides that encodes a protein of 374 amino acids. This sequence shares over 50% sequence similarity to 3 *Arabidopsis thaliana* SAMT-like proteins and about 35% identity (~50% similarity) to *Clarkia breweri* SAMT, *Petunia hybrida* BSMT, and *Antirrinum majus* SAMT and BAMT. For functional characterization of the protein encoded by the isolated cDNA, the open reading frame was subcloned into the expression vector, pET 28a, which contains an N-terminal polyhistidine (6×His) tag, and expressed in *E. coli*. Overexpression and subsequent nickel column-based purification allowed for characterization of the recombinant enzyme *in vitro*. Substrate specificity and kinetic characterization of the protein revealed high specificity for cinnamic acid as substrate, in contrast to related proteins (SAMT and BAMT) which did not utilize this compound as substrate.

Relaxed specificity in aromatic prenyltansferases

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Prenylated aromatic natural products possess bioactivities and therapeutic potential distinct from their non-prenylated aromatic precursors. These hybrid natural products serve in microbes and plants as anti-microbials, and in mammals they play a variety of roles as anti-oxidants, anti-inflammatories, anti-virals, anti-proliferatives, and anti-cancer compounds. Prenyltransferases (PTases) utilize isoprenoid diphosphates as substrates to catalyze the addition of the acyclic prenyl moiety onto isopentenyl diphosphate (IPP), longer prenyl diphosphates, aromatic molecules and proteins. Until recently, only a few "aromatic" prenyltransferases of secondary metabolism had been isolated and biochemically characterized, and none at the structural level. Here we report the gene identification, biochemical characterization and structural elucidation of a novel aromatic prenyltransferase, Orf2 from *Streptomyces* sp. strain CL190, involved in naphterpin biosynthesis [1]. This biosynthetically promiscuous PTase catalyses the formation of a C-C bond between a prenyl group and an aromatic acceptor, and also possesses a novel C-O bond forming activity. This first reported structure of an aromatic prenyltransferase of secondary metabolism obtained with substrates and substrate analogues bound, displays an un-expected and non-canonical barrel architecture.

Furthermore, these structures provide a mechanistic basis for understanding prenyl chain length determination and aromatic co-substrate recognition in this structurally unique family of aromatic prenyltransferases. The high resolution structure is a useful starting point for engineering the novel diversification of aromatic small molecules in a regio-specific manner using a biosynthetic approach.

1. **Kuzuyama T. Noel J-P & Richard SB.** *Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products*. **Nature**. 2005 (435)983-7.

Structural analysis of *Arabidopsis thaliana* methyltransferases involved in isoprenoid methylation

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We have previously reported the identification of a new family of plant methyltranseferases (MTs), the SABATH (for Salicylic Acid, Benzoic Acid, THeobromine synthase)family, that uses S-adenosyl-L-methionine (SAM) to methylate the carboxyl or nitrogen moiety of a diverse array of plant small molecules.

Here we describe the use of a combination of structural biology and biochemistry to elucidate the nature of the active sites of three *Arabidopsis* SABATH family isoprenoid MTs, with emphasis on one of the enzymes that shows preference for farnesoic acid (FAMT). We have used structural modeling to make predictions about the identity of active site residues and active site mutagenesis to test the validity of our models. The results of the mutagenesis and enzyme activity assays indicate that our active site models accurately approximate the location of the isoprenoid substrate binding site, as the mutations to predicted active site residues result in perturbation of the carbon chain selectivity of the FAMT enzyme.

Does the Chemical Composition of the Outermost Cuticle of Soybean Seed Coats Determine its Water Permeability?

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The seed coats of different cultivars of soybean (*Glycine max* L) show different degrees of water permeability. Some seeds readily imbibe water and are termed "soft-seeded", while others remain non-permeable even after several days in water and are termed "hard-seeded". Recent evidence has established that the permeability of soybean seed coats correlates with the degree of cracking in the thin outermost cuticle, and points to the importance of the surface chemistry to water permeability. In the present work, we have conducted a detailed chemical analysis of the outer soybean seed coat in an attempt to link the chemistry of the walls of the cells in soybean seed coats to their water permeability. As a result, we demonstrate that (1) very little surface wax is present, and does not contribute to resistance to water uptake, (2) there is no correlation between seed coat lignin and permeability, (3) the outermost cuticle has an unusual composition, particularly rich in 2-hydroxy- and ω-hydroxy fatty acids and (4) there is a close association between the aliphatic monomers of the surface cutin and the underlying carbohydrates of the walls of the cells to which they are attached. Of these results, the most significant appears to be the composition of the outermost cuticle, since the non-permeable cultivar studied herein contained a disproportionately high amount of hydroxylated fatty acids relative to that of the permeable ones. The association between aliphatic monomers and the carbohydrates of the underlying cell walls, which appears to be more integrated in the non-permeable cultivar, may influence the mechanical stability of the cutin layer. These results support the hypothesis that the unique chemical composition of the outermost cuticle of non-permeable soybean seed coats may be responsible for the structural integrity that prevents cracking, and thereby ready permeability.

Functional and Comparative Genomic Analysis of Plant acyl:CoA ligase-like Genes

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4-coumarate:CoA ligase (4CL), an enzyme encoded by a small gene family in *Arabidopsis*, plays a key role in phenylpropanoid metabolism. 4CL belongs to the adenylate-forming enzyme superfamily, and is related to ATP-dependent CoA ligases involved in lipid metabolism. Our *in silico* similarity search of the *Arabidopsis* genome using the amino-acid sequences of *4CL*s identified twelve putative genes of unknown function as members of the acyl-CoA ligase superfamily most closely related to true 4CLs. We call these genes acyl:CoA ligase likes (*ACLLs*). Most of the ACLLs have the peroxisome targetting signal PST1, indicating a possible function in this organelle, where pathways requiring CoA activation of fatty acids, like β-oxidation steps, occur. In order to shed a light on the evolution and potential functions of ACLLs in plants, we identified putative *ACLL* genes in the poplar and rice genomes. Most of these contain the PST1 target signal, and phylogenetic analyses suggest that the poplar and rice ACLLs are orthologous to those of *Arabidopsis*, but that these plants have a higher number of *ACLL*s. Recently, one ACLL (At5g63380) was biochemically characterized and shown to activate the jasmonic acid (JA) precursor OPDA, suggesting a role for this enzyme in the peroxisomal JA biosynthetic pathway. However, the biological functions of this and other ACLLs remain unclear. Our goal is to functionally characterize these genes *in vivo*. Promoter analysis revealed a strong wound response of some ACLLs, including At5g63380, in agreement with the role of jasmonates in defense responses. Metabolic profiling of At5g63380 knock-out seedlings, revealed accumulation of OPDA and raffinose sugars. Further experiments to establish the biological role of At5g63380 and other ACLLs are underway.

Flavonoid Glucosyltransferases in *Citrus paradisi*: Evaluation of Putative Clones Obtained from Grapefruit Leaves using SMART RACE RT-PCR and EST Mining.

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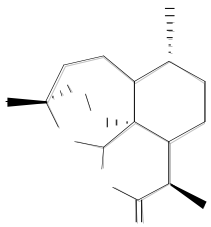
Flavonoids play roles in plant coloration, plant/animal interactions, and also possess antioxidant properties. They are chemically modified by glycosylation, hydroxylation, methylation, etc. During glycosylation, the sugar moiety from a UDP-sugar is transferred to aglycone flavonoid substrates by specific enzymes. Those catalyzing glucose transfer are known as glucosyltransferases GTs). Grapefruit contains up to5 different flavonoid GTs that demonstrate differences in substrate and position specificity. The five flavonoid GTs include: flavonol 3-O-GT, flavonol 7-O-GT, chalcone 4'-O-GT, flavone 7-O-GT, and flavanone 7-O-GT. Flavanone-specific 7-O-glucosyltransferase (7GT) catalyzes the first glycosylation reaction leading to production of bitter naringin in young leaves and fruit. This research was designed to obtain full-length secondary product GT cDNA clones, express them, and characterize them. Specific primers were designed from the Plant Secondary Product Glucosyltransferase (PSPG) box to amplify grapefruit GTs. RNA was extracted from young grapefruit leaves followed by SMART RACE cDNA synthesis and RT-PCR to obtain 5' clones. Clone specific primers were used to obtain the corresponding 3' ends. Compiled sequences were obtained by matching overlaps of partial clones. Primers were designed from compiled sequences to obtain full-length cDNA clones. To date, 2 full-length clones have been inserted into vector pCD1 and have been transformed into expression host E. coli BL21 (DE3) RIL. An additional compiled full-length clone has been obtained and is undergoing PCR reactions to obtain the full-length clone. Experiments to obtain optimal expression conditions are in progress. Expressed enzymes will be analyzed for GT activity towards chalcone, flavone, flavanone, and flavonol aglycones.

In another approach, a directionally cloned cDNA library is being EST mined to identify additional unique putative GT clones. A partial GT candidate, EST CP1-D10, is being used for clone-specific primer design for use in PCR reactions to obtain a full-length compiled sequence of this clone. Other putative GT candidates from the EST library are also being analyzed at this time.

A Molecular Genetics Approach to the Biosynthesis of Artemisinin in *Artemisia annua*. **K. H. Teoh, D.R. Polichuk, D.W. Reed** and **P.S. Covello**, National Research Council-Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK., Canada S7N 0W9

Artemisinin is an antimalarial drug that comes from a Chinese herb known as *Artemisia annua*. The drug has been shown to be far more effective in treating malaria than the traditional drugs of chlorquinine and sulfadoxine-pyrimethamine. It is a sesquiterpene lactone with an unusual endoperoxide bridge believed to be essential for its antimalarial activity. The superiority of artemisinin has the World Health Organization (WHO) elevating the drug to the preferred first therapy for all malaria infections caused by the parasite, *Plasmodium falciparum*. Artemisinin is found only in the aerial parts of the plant in the range of 0.01% to 0.5% dry weight, with the highest level in the leaves and inflorescences. Indirect evidence points to the glandular trichomes as possible sites of artemisinin biosynthesis. Here, we describe a molecular genetics approach to identify and characterize the genes involved in the biosynthesis of artemisinin with the ultimate aim of enhancing biological production of artemisinin.



Artemisinin

Regulation of (*E*)-β-ocimene and (*E*,*E*)-α-farnesene formation in leaves of different *Arabidopsis thaliana* ecotypes – an interesting example for natural variation of terpene volatile biosynthesis

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Arabidopsis thaliana is emerging as a valuable model system to investigate the biochemistry and function of terpene volatile biosynthesis. Previously, we have shown that Arabidopsis flowers constitutively emit monoterpene and sesquiterpene volatiles and identified several terpene synthase (*TPS*) genes out of a large Arabidopsis *TPS* gene family responsible for the biosynthesis of these compounds (Chen *et al*. 2003). It has also been shown that *A. thaliana* rosette leaves emit terpene volatiles in response to biotic stresses such as herbivory (Van Poecke *et al*. 2001). We are interested in the natural variation of stress induced terpene volatile biosynthesis and the molecular mechanisms controlling these differences. By employing volatile metabolite profiling of rosette leaves from several *A. thaliana* ecotypes treated with the fungal elicitor alamethicin or the coronatine analogon 6-ethyl indanoyl isoleucine, we identified significant qualitative and quantitative differences of induced terpene volatile blends. For example, a comparison of the two ecotypes Columbia (Col) and Wassilewskija (Ws) showed that small amounts of (*E,E*)-α-farnesene were released from foliage of the Col ecotype while (*E*)-β-ocimene was observed as the predominant terpene volatile emitted from leaves of the Ws ecotype. In addition, both ecotypes showed emission of the C_{16}-homoterpene (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Based on gene expression analysis and product identification of recombinant enzymes, we identified two closely related *TPS* genes that are most likely responsible for the observed induced monoterpene and sesquiterpene emissions. We also demonstrate that the ecotype related differences in emissions of (*E*)-β-ocimene and (*E,E*)-α-farnesene appear to be the result of differential mutation and expression of those genes as well as differences in subcellular compartmentation of their encoded enzymes, as shown by targeting of green fluorescent protein (GFP) in fusion to potential signal peptides of these enzymes.

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Altering the Physical Environment Affects Growth, Morphogenesis and Essential Oil Production in *Mentha spicata* L. Shoots in vitro

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Altering the physical environment profoundly alters the growth (fresh weight), morphogenesis (leave, root and shoot numbers) and secondary metabolism [i.e., production of the monoterpene (-)-carvone] of *Mentha spicata* L. (spearmint) shoots cultured on Murashige and Skoog medium. The type of physical support (e.g., agar, liquid, sponge, platform, or glass) employed within Magenta vessels were tested. Mint shoots grown on the liquid medium produced 3-x fold greater fresh weights than shoots grown on the agar medium. However, carvone concentrations in shoots grown on agar were higher than in shoots grown on liquid medium. Replacing culture media more frequently within Magenta vessels during an 8 week culture period significantly increased growth and morphogenesis without affecting carvone concentrations. The influence of culture vessel capacity on spearmint shoots was tested by culturing shoots on a variety of culture vessels including: culture tubes, Magenta vessels and 1.89 l jars. Positive correlations occurred between culture vessel capacities and shoot growth, morphogenesis and carvone concentrations. A comparative study was conducted testing growth, morphogenesis and secondary metabolism occurring with 3 different spearmint cultivars grown on either culture tubes containing 25 ml agar medium or in an automated plant culture system (APCS, a sterile hydroponics system) employing a one-l medium reservoir. The APCS allowed for the production of greater biomass (e.g., ~12 to 15-x fold increase in fresh weight), and morphogenesis to occur compared to that obtained within culture tubes. However, shoots grown in the APCS produced less carvone compared to shoots grown in agar medium. Increasing the number of media culture immersions (4, 8, 12, or 16 immersions day^{-1}) employing the APCS increased growth and morphogenesis responses while lowering carvone levels. Optimum growth rates is not necessarily associated with optimum secondary metabolism in vitro.

Gibberellin carboxyl methyltransferases – potential regulation of gibberellin action

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The *Arabidopsis* genome encodes a group of related carboxyl mehtyltransferases able to transfer a methyl group from S-adenosyl-methionine to various compounds, thus forming methylesters. Previously, three members of this methyltransferase family have been shown to methylate jasmonic acid, salicylic acid and benzoic acid, and indole-3-acetic acid, respectivly. Here we report that two other members of this methyltransferase family in *Arabidopsis thaliana,* designated as GAMT1 and GAMT2*,* are capable of converting gibberellins into their methyl esters *in vitro*. Purified GAMT1 most efficiently methylates GA9 with K_M value of 14 μM. Purified GAMT2 most efficiently methylates GA4 with K_M value of 6 μM. Both enzymes have no or little activity with gibberellins that do not contain a lactone ring, and no activity with any other substrate among more than 100 different plant-occurring acids tested. Transgenic *Arabidopsis* plants, overexpressing *GAMT1* and *GAMT2* under 35S promoter, exhibited a dwarf growth habit, a typical GA-deficient phenotype.

* This work was supported by the National Science Foundation as part of the Arabidopsis 2010 Initiative.

Biofumigation potential of glucosinolate-containing seedmeals

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Defatted seedmeals from fifteen glucosinolate-containing plant species were analyzed for biofumigation potential by determining inhibition of seedling emergence when added to a sandy loam soil containing wheat (*Triticum aestivum* L.) and sicklepod [*Senna obtusifolia* (L.) H. S. Irwin & Barneby] seeds at rates of 0.1, 0.5 and 1.0 % (w/w). In general, the seedmeals were more phytotoxic to wheat than sicklepod. For wheat, nine of the seedmeals completely inhibited seedling emergence at the 1% level, but at the 0.1% rate only brown mustard [*Brassica juncea* (L.) Czern.] was as inhibitory. For sicklepod seedling emergence, while none of the seedmeals were completely inhibitory at the 0.1% rate, six of the seedmeals were completely inhibitory at the 1% rate {brown mustard, arugula [*Eruca vesicaria* (L.) Cav. subsp. *sativa* (Mill.) Thell.], Siberian wallflower (*Erysimum x allionii*), English wallflower [*Erysimum cheiri* (L.) Crantz], sweet alyssum [*Lobularia maritima* (L.) Desv.] and evening-scented stock [*Matthiola longipetala* (Vent.) DC.]} and three were completely inhibitory at the 0.5% rate (brown mustard, arugula and sweet alyssum). Intact glucosinolates and their corresponding hydrolysis products varied among the seedmeals with the highest activity. The major hydrolysis products produced by the seedmeals, respectively, were allyl isothiocyanate by brown mustard seedmeal, erucin (4-methylthiobutyl isothiocyanate) by arugula seedmeal, cheirolin (3-methylsulfonylpropyl isothiocyanate) by Siberian wallflower and English seedmeals, 3-butenyl isothiocyanate and lesquerellin (6-methylthiohexyl isothiocyanate) by sweet alyssum seedmeal, and sulforaphene (4-methylsulfonyl-3-butenyl isothiocyanate) by evening-scented stock seedmeal.

The Role of *S*-Adenosyl-L-methionine: nicotinic acid methyltransferase (NAMT) in the salvage pathway of NAD^+ in Arabidopsis

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Methylation is one of the most common enzymatic modifications in plant secondary metabolism. We have identified a new group of carboxyl methyltransferase that have no significant sequence similarity to other known methyltransferases. Among the 24 members of this group in Arabidopsis, we identified one enzyme that showed high specific activity with nicotinic acid (NA), an important intermediate compound in the salvage biosynthetic pathway of NAD^+, where NA is found in the amide form. NAD^+ is an essential compound in all organisms, both as a coenzyme for oxidoreductase and as a source of ADP-ribosyl groups used in various reactions. The carboxyl methylation of NA, to give methyl nicotinate, precludes it from forming the amide. The *S*-Adenosyl-L-methionine: nicotinic acid methyltransferase (NAMT) of Arabidopsis has a K_m value of 4.0 μM for nicotinic acid and 16.2 μM for *S*-Adenosyl-L-methionine (SAM). GUS staining showed that 2-week-old Arabidopsis seedlings express NAMT in all tissues. Northern blot analysis showed that in mature plants, NAMT expression was detected in flowers, roots, and siliques and it was induced by NaCl, ABA, trigonelline, quinolinic acid and nicotinic acid. Currently, both a knockout mutant and overexpressing plants are being examined to determine how the activity of NAMT affects the salvage pathway of NAD^+ *in planta*.

This work was supported by the National Science Foundation as part of the Arabidopsis 2010 Initiative

Comparison of HPTLC, HPLC and HPCE on Fingerprinting of *Pueraria Radix*

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Abstract

For the purpose of evaluating the reliability and applicability of chromatographic methods chosen for fingerprinting, and gaining comprehensive information of chromatographic fingerprint, several chromatographic techniques including HPTLC, HPLC and HPCE are applied in development of fingerprint of *Pueraria Radix* for the first time. The results showed that RSD of R_f values, retention times and the percentages of peak areas among the samples from different sources of *Pueraria lobata* all perfectly satisfied the demands of the national standard, and chromatographic techniques of HPTLC, HPLC and HPCE have been successfully used in development of fingerprint of RP. Although the three methods applied in development of RP fingerprint are highly selective and reproducible, there are still significant differences of reliability and applicability among results obtained by those approaches, which was illustrated in this paper.

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Differential Detection of Metabolites using Mass Hunter and Mass Profiler.

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Introduction Differential detection of metabolites in biological matrices was accomplished via new algorithms using retention time, accurate mass, and intensity to identify "features" and statistical comparison software to differentiate experimental variations from real expression level changes. Metabolic samples typically exhibit significant complexity. 100-1000's of compounds co-eluting over wide concentration ranges make manual extraction of compounds of interest from liquid chromatography/mass spectrometry (LC/MS) time-of-flight data sets nearly prohibitive. A prototype "Mass Hunter" program automatically extracts and identifies individual molecular features from the data sets, in addition to reducing LC/MS data file sizes. Differential expression, spectral alignment, and normalization is possible using a prototype "Mass Profiler" software package.

Methods: An *Arabidopsis* extract (~800 μg) was used to test the functionality of the software tools for profiling of plant metabolites. Accurate mass LCMS data was obtained using the Agilent Series 1100 Capillary HPLC system interfaced to the Agilent LC/MSD TOF mass spectrometer. Separation was performed using a Zorbax SB C18, 5.0, 150 x 0.5 mm column, a solvent gradient: 0-5 min, 5% B, 5-25 min, 5%-95% B with a 20 minute hold; at a flow rate of 20 μL/min; a 1.0 μL injection volume; and water (A) and acetonitrile (B) with 0.1% formic acid as mobile phases. Mass spectrometer conditions: positive-ions, *m/z* 50 to 1,100, and 1.29 cycles/second. To ensure low-ppm mass accuracy, the internal reference mass correction was utilized to correct for scan-to-scan variations in mass.

Results: Profiling of complex metabolomics samples is dependent on a reproducible analytical system that can deliver repeatable measures of retention time, ion intensity, and accurate mass, and the appropriate bioinformatics software to process the data. Retention time variation was reduced using a flow controlled capillary HPLC and mass measurement variation was reduced using internal reference mass correction. The "Mass Hunter" software first finds peaks in all spectra, and removes chemical background by subtracting the baseline for each *m/z* bin (EIC). The peaks were then clustered in RT (in seconds) and *m/z* in 2D space to form 3-D peaks. These 3-D peaks were centroided and a peak-volume was assigned to each peak. Individual 3-D peaks that are related to each other via isotopes, adducts, dimers, trimers, and charge state were all combined together as a neutral mass and total volume value. For differential expression analysis the Mass Profilier software used 2-D and 3-D visualization and statistical tools to rapidly sort features into multiple categories: those present in all sets; unique to one set and present in all sets but with differential expression.

The lycophyte *Selaginella moellendorffii*: an emerging plant model for studying comparative genomics and the evolution of phenylpropanoid metabolism

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The lycophyte *Selaginella moellendorffii* is a member of one of the oldest lineages of vascular plants on Earth. Fossil records show that the lycophyte clade arose 400 million years ago, 150-200 million years earlier than angiosperms, a group of plants that includes the well-studied flowering plant *Arabidopsis thaliana*. We sequenced 2181 Expressed Sequence Tags (ESTs) from a *S. moellendorffii* cDNA library. These ESTs were assembled into 1301 putative unigenes, annotated using the BLASTX algorithm, surveyed for their abundance within the dataset, and classified into functional groups according to the Gene Ontology (GO) hierarchy. A comparative genomics approach was used for comparing *S. moellendorffii* ESTs with those of *A. thaliana* and *Physcomitrella patens* to look for genes that may be unique to lycophytes. These results give an initial insight into its transcriptome that will aid in the study of the *S. moellendorffii* genome in the near future.

Our interest in *Selaginella* was piqued by the observation that although syringyl (S) lignin is often regarded as being restricted to angiosperms, it is found in lycophytes as well. To gain insight into the evolution of lignin biosynthesis and phenylpropanoid in general, we cloned candidates for the *S. moellendorffii* homologs of the three phenylpropanoid P450s that play essential roles in determining the lignin composition in angiosperms. Their functions in *S. moellendorffii* are being assessed by cross-species complementation and heterologous expression of P450s for kinetic analysis. This project will provide important information for better understanding the evolution of phenylpropanoid pathway in vascular plants.

Methylation and demethylation—A novel mechanism in maintaining auxin homeostasis

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The plant hormone auxin is involved in many processes of plant growth and development. Indole-3-acetic acid (IAA), the predominant naturally occurring auxin, can be conjugated to amino acids and sugars as part of IAA homeostasis. Known endogenous IAA conjugates may function as storage forms of active IAA, since they could be hydrolyzed to free IAA, or may have roles in IAA degradation. Here we report the characterization of an *Arabidopsis* S-adenosyl-L-methionine (SAM): IAA carboxyl methyltransferase (*At*IAMT) which converts IAA to IAA methyl ester (MeIAA). Under steady-state kinetics, IAMT exhibits K_M values of 13 μM and 19 μM for IAA and SAM, respectively. Protein structure modeling using the experimentally determined *Clarkia breweri* SAMT crystal structure suggests a reasonable model for IAA recognition in the IAMT active site. Expression analysis of IAMT using RT-Real-time PCR and promoter-GUS fusion revealed higher expression levels in flower and silique. MeIAA was detected in wildtype siliques by GC-MS analysis but not in siliques of an IAMT knockout line. Plants overexpressing IAMT displayed loss of gravitropism in roots indicative of auxin deficiency, and upward-curling leaves suggesting disruption of auxin gradient in leaf. We hypothesize that through conversion of free IAA to MeIAA, IAMT may function to fine-tune levels of active IAA, and thus may have roles in the establishment of IAA gradient. We further hypothesize the existence of MeIAA esterase(s) which counteract the activity of IAMT in IAA-MeIAA homeostasis. Several *Arabidopsis* enzymes with MeIAA esterase activity have been identified.

MetaCyc and AraCyc: Metabolic Pathway Databases and Beyond

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MetaCyc (http://metacyc.org/) is a metabolic pathway database that includes pathways, reactions, enzymes, and compounds from many organisms including microorganisms, plants, and human. Data in MetaCyc are curated using experimental evidence in the literature. Its goal is to represent experimentally verified pathway information from all organisms, which can then be used, among other applications, as a reference to predict the metabolic content of newly sequenced, annotated genomes, and to compare metabolic pathways from different organisms. AraCyc (http://www.arabidopsis.org/tools/aracyc/) is an Arabidopsis-specific pathway database, which was computationally predicted from the enzyme-coding genes annotated in the Arabidopsis genome using MetaCyc as the reference database. Since its initial build, AraCyc has been under continued curation to enhance its data quality and to increase the breadth of pathway coverage. The goal of AraCyc is to represent the complete metabolic content of a model plant, to be used as a base to predict the metabolic content of other plant genomes as well as to provide a metabolic context for the analysis of large-scale functional genomics data such as those arising from genome-wide expression profiling, and metabolomic profiling experiments. All of the predicted pathways are being verified and updated from the literature. Another major curation effort is devoted to expand the coverage of plant secondary metabolism in MetaCyc and AraCyc. Our immediate goal is to curate representative pathways for each of the main secondary metabolite classes, followed by more in-depth curation of secondary metabolism as well as incorporation of transporters to handle pathways that span multiple cellular compartments. Among other applications of MetaCyc and AraCyc, the rich and diverse information on metabolic pathways across species can assist comparative studies of pathways and facilitate metabolic engineering to improve traits in crops. Results from comparison of all plant pathways with pathway variants in other non-plant species will be presented. Both databases are freely accessible from the internet. In addition to querying and browsing the pathway data, the Omics Viewer (http://www.arabidopsis.org:1555/expression.html) - a data visualization and analysis tool - allows many different types of large-scale functional genomics data resulting from microarray expression, metabolite profiling, and proteomic profiling experiments to be overlaid onto the full metabolic pathway map of Arabidopsis.

Dissecting the metabolic complexity of sinapate ester production in Arabidopsis: a serine carboxypeptidase-like protein that synthesizes 1,2-disinapoylglucose.

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The Arabidopsis genome contains fifty-one genes that encode serine carboxypeptidase-like (SCPL) proteins. A sequence analysis of these SCPL proteins has demonstrated that the majority of them fall clearly into two major groups, with one group being composed of nineteen SCPL proteins that are highly similar to one another. Two of these nineteen SCPL proteins have been characterized to date: sinapoylglucose: malate sinapoyltransferase (SMT) and sinapoylglucose:choline sinapoyltransferase (SCT). Hence, both of these SCPL proteins are acyltransferases that utilize sinapoylglucose as an activated acyl-donor molecule. Interestingly, the gene that encodes SMT (At2g22990) is one of five SCPL genes arranged in tandem to form a cluster on chromosome 2. An analysis of four different mutant lines harboring different combinations of mutations for this SCPL gene cluster has revealed that one gene (At2g23010) encodes sinapoylglucose:sinapoylglucose sinapoyltransferase (SST), an enzyme responsible for the production of 1,2-disinapoylglucose from two molecules of sinapoylglucose. Further, tissue-specific metabolic profiling of these mutants grown under different environmental conditions and examined at different developmental stages has begun to uncover a significant degree of metabolic complexity associated with the SCPL proteins encoded by these clustered genes. This work thus sheds further light on the relationship between sequence, regulation, and function found in the Arabidopsis SCPL gene family and provides another example of the genetic diversity that underlies plant secondary metabolism.

Clustering of Genes Involved in Secondary Metabolism in Plants

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In many organisms the genes encoding enzymes involved in primary or secondary metabolism are clustered together within a genome. Classic examples are operons in bacteria, where the genes for a specific pathway are transcribed on a single polycistronic message. In fungi, many genes involved in secondary metabolism are clustered.

Plants produce a vast array of secondary metabolites which are involved in many fundamental tasks such as pest and disease resistance, insect attraction and allopathy. While the catalogue of individual molecules is substantial, most are based on a relatively small number of carbon backbones, for example the phenyl propanoids and isoprenyl. These skeletons are then sequentially decorated in a modular fashion by specific enzymes from a several complex gene families including the heme-thiolate P450s, glycosyl transferases, O-methyl transferases and acyl transferases. The identification of all the enzymes in specific pathways for the synthesis of individual compounds is a significant area of interest in plant biochemistry. Genomics has provided a means for this to be achieved for all the secondary metabolic pathways for a single plant. While genomics has provided the information in terms of all the genes involved it has not facilitated the task of assigning function, this is particularly problematic for large gene families such as the heme-thiolate P450s.

Until recently, it was thought that the genes encoding these enzymes did not form clusters. Annotation the completed genomes of both the Arabidopsis and rice genomes has revealed that there incidences where genes encoding enzymes involved in secondary metabolism appear to be clustered. The significance of this observation for gene mining and assigning function to enzymes involved in secondary metabolism will be discussed.

Metabolic Engineering of Plants and Microbes for Production of Arbutin

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Arbutin (hydroquinone β-D glucopyranoside) is a secondary metabolite present at high levels in certain plant species of the rosaceae, ericaceae, saxifragaceae families. Arbutin is an active ingredient in cosmetics for skin lightening. Arbutin can be isolated from plant tissues or it can be generated by biotransformation of hydroquinone using plant explants, suspension cultures or microbes expressing plant-derived hydroquinone-specific glucosyltransferase enzymes; moreover, routes for chemical synthesis of arbutin exist.

An opportunity for high volume production of arbutin at significantly lower cost may be created by engineering a pathway for arbutin biosynthesis into plants or microbes. The yeast *Candida parapsilosis* can grow on para-hydroxybenzoate (pHBA) as sole carbon source. The first step of pHBA breakdown uses a pHBA 1-hydroxylase (pHBA1H) enzyme, a soluble FAD-dependent monoxygenase that converts pHBA to hydroquinone. We purified this enzyme to homogeneity and cloned the corresponding gene. We demonstrate that DNA constructs expressing chorismate-pyruvate lyase (CPL) in the chloroplast and pHBA1H in the cytosol are sufficient to create a high flux pathway from chorismate to arbutin in transgenic plants. In addition, we demonstrate that co-expression of CPL and pHBA1H enzymes with a glucosyltransferase gene from arabidopsis (UGT72B1) in *E. coli* provides a biosynthetic route to arbutin from simple fermentable feed stocks.

Evolving Phytochelatin-Based Heavy Metal Tolerance in Plants

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Phytochelatins play a key role in plant heavy metal tolerance by chelating metal ions for sequestration in vacuoles. Assembled by phytochelatin synthase (PCS), phytochelatin peptides consist of repeating units of γ-glutamylcysteine derived from glutathione. Since phytochelatins, along with other peptides including glutathione and metallothioneins, confer resistance to cadmium, arsenic, and lead, understanding and manipulating their biosynthesis is an important aspect of using plants for the remediation heavy metal contaminated soils. Here we describe the directed evolution of PCS to improve heavy metal tolerance in plants. While directed evolution is a powerful tool for engineering enzymes with tailored molecular function, it has not been widely applied to agricultural or environmental problems. We used directed evolution to generate a PCS mutant library. Screening of this library in yeast for cadmium tolerance identified a set of PCS genes that enhanced heavy metal protection. Expression of these evolved PCS mutants in yeast results in higher phytochelatin levels than in cells expressing wild-type PCS and improves tolerance to cadmium up to 10-fold. In addition, experiments using transgenic *Arabidopsis thaliana* expressing the most efficient first generation PCS variant displays enhanced protection against cadmium toxicity. These experiments offer proof-of-principle for this approach; however, these results are only the first round of mutant generation and selection. Ultimately, we envision an evolved PCS gene as a component in a molecular tool chest for developing a transgenic plant with multiple traits tailored for the remediation of heavy metal contaminated soils.

What does it mean to smell green? The molecular and biochemical characterization of a plant acyltransferase responsible for producing greenl volatile esters.

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Green leaf volatiles (GLV) are common products of mechanically and herbivore-damaged plants. These products derived from the lipoxygenase pathway are thought to be important for the prevention of the spread of bacteria and fungi in addition to serving as cues for herbivores and their predators and parasitoids. More recently, GLVs have been shown to act as plant signaling compounds that can induce several important plant defense pathways. We have identified a member of the BAHD acyltransferase gene family in *Arabidopsis thaliana* that specifies the ability to catalyze the formation of the green leaf volatile ester (*Z*)-3-hexen-1-yl acetate. The enzyme it encodes, acetyl-CoA:cis-3-hexen-1-ol acetyl transferase (CHAT), has been purified from transgenic plants expressing the CHAT gene under the 35S promoter as well as from *E. coli* heterologously expressing the protein. Kinetic data show that both the plant-purified and *E. coli*-produced CHAT enzymes have a high affinity for acetyl Coenzyme A as well as the ability to accept several medium-length chain aliphatic and benzyl-derived alcohols. Upon damage by mechanical wounding, CHAT gene transcript levels increase in a time-dependent manner with the highest levels detected between 3 and 6 hours after wounding. CHAT enzyme activity levels correlate with transcript levels, with CHAT enzyme activity levels peaking between 10 and 12 hours after mechanical wounding. Plants expressing an RNAi cassette for the *CHAT* gene show that the CHAT enzyme is responsible for the *in planta* production of Z-3-hexen-1-yl acetate.

Over-expression of Transcription Factors to Manipulate Specialized Metabolite Biosynthesis. Argelia Lorence[1,3], Bonnie J. Woffenden[1], Matthew Smith[2], Craig L. Nessler[1] and Fabricio Medina-Bolivar[1,3]. [1]Department of Plant Pathology, Physiology and Weed Science, and [2]Department of Biochemistry, Virginia Tech, Blacksburg, VA. [3]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR.

Plant specialized (secondary) metabolites have been used as phytomedicines for hundreds of years. It is well known that many biosynthetic pathways leading to the formation of these molecules are highly inducible and hypothesized that key regulatory proteins act as major orchestrators of the specialized metabolism. The transcription factor ORCA3 (octadecanoic-derivative responsive *Catharanthus* APETALA2-domain protein) identified from *Catharanthus roseus*, is a master regulator of genes involved in both primary and secondary metabolism. ORCA3 enhances the expression of genes downstream of the stress-and wound-inducible plant hormone methyl jasmonate (MeJA), and is itself up-regulated by jasmonates. We cloned ORCA3 homologs from *Nicotiana tabacum* and *Hyoscyamus muticus* (named ORNA and ORHA, respectively) and are studying their expression in hairy root cultures treated with MeJA and cellulase. In addition, we constructed expression cassettes for either constitutive or inducible expression of ORNA and ORHA and generated transgenic tobacco plants containing these constructs. Molecular characterization of the plant lines and hairy roots generated from them is been conducted. Secondary metabolites produced in hairy roots of wild type and ORNA- and ORHA-over-expressing *N. tabacum* and *H. muticus* will be characterized. We anticipate the potential production of novel specialized metabolites in the transgenic hairy root cultures where specialized metabolism is expected to be enhanced. Ultimately, linking these newly developed products with bioassays may lead to the discovery of pharmaceutically important therapeutic drugs.

Iridoids and phenylpropanoids from *Penstemon gentianoides* (Scrophulariaceae).

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Penstemon gentianoides (HBK) Poiret, Lindl. Don. Scrophulariaceae a small shrub (common names: beardtongue, "jarritos", "jarra") grows on hilly areas above 3,000 m. in forest communities dominated by *Abies religiosa, Alnus jorullensis, Pinus pseudostrobus* and *Pinus hartwegii*, this plant is a popular medicinal species employed by different indigenous people from Mexico, they has profusely been used this plant for the treatments of diverse ailments. However, its main ethnomedicinal focus is in the treatment of inflammatory diseases. Previously we reports the anti-inflammatory and antioxidant effects of polar extracts from this species (Dominguez *et al*., 2004 PSNA Meeting; Dominguez *et al., J. Agric. Food Chem*., 2005, in press). Previous works in other Schrophulariaceae species reports the presence of iridoids, terpenes and flavonoids; however, there are not report about anti-inflammatory and antioxidant activities. Polar extracts and fractions were chromatographed; then natural compounds were isolated and purified by TLC and CC, afforded differents compounds: two iridoids (stansioside, 5-acetylstansioside), two iridoids similar to catalpol (one new, and globularicisine), two phenylpropanoids (martinoside and verbascoside), and two flavonoids. This is the first phytochemical study of this species.

This work was supported in part by DGAPA-UNAM grants # IN243802, IN211105 and USAID-TIES-ENLACES-PROGRAM.
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Structural and Energetic Basis for Product Specificity Control by Sesquiterpene Cyclases

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Tobacco 5-epi-aristolochene synthase (TEAS) and henbane premnaspirodiene synthase (HPS) are evolutionarily related sesquiterpene cyclases which share a common reaction mechanism, but diverge at the penultimate step of catalysis where either a methyl migration or an alkyl shift occurs to produce 5-epi-aristolochene or premnaspirodiene, respectively. A contact map approach was developed to systematically identify differences in residues between TEAS and HPS that are in contact with active site residues shown to be in van der Waals contact with the farnesyl chain of the FPP analog (FHP) bound to TEAS. Mutating this identified collection of 9 residues in TEAS to the corresponding residues found in HPS led to the successful conversion of TEAS to HPS and represents a seminal discovery towards understanding the structural basis for product specificity control. The reciprocal conversion from HPS to TEAS was accomplished and demonstrates that these residues define an evolutionary pathway connecting their respective biosynthetic properties. Structure-based Combinatorial Protein Engineering (SCOPE) was used to create libraries encompassing all possible combinations of these mutations, in the context of TEAS and HPS, to further define a minimal collection of residues that are necessary and sufficient for this property change. Initial characterization of several mutants has identified a reduced set of mutations which interconvert the dominant product specificity as well as the recognition of minor products absent from the product distribution of the parent enzymes. Quantum mechanical treatment has been applied to the carbocation intermediate branch point (shown above) as well as the transition states leading to 5-epi-aristolochene or premnaspirodiene, and defines a theoretical basis for understanding the energetic control of the cyclization process exerted by TEAS and HPS. Ultimately, the full characterization of the biochemical properties of these mutants integrated with a theoretical chemistry framework will provide the first high-resolution pictures of the energetic landscape between subtle mechanistic differences that give rise to such diverse hydrocarbon skeletons in Nature.

The Structure of a Cyanobacterial Sucrose-Phosphatase
Reveals the "Sugar Tongs" that Release Free Sucrose in the Cell

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Sucrose-phosphatase (SPP) catalyzes the final step in the pathway of sucrose biosynthesis in both plants and cyanobacteria, and the SPPs from these two groups of organisms are closely related. We have crystallized the enzyme from the cyanobacterium *Synechocystis* sp. PCC 6803 and determined its crystal structure, alone and in complex with various ligands. The protein consists of a core domain containing the catalytic site and a smaller cap domain that contains a glucose-binding site. Two flexible hinge loops link the two domains, forming a structure that resembles a pair of sugar tongs. The glucose-binding site plays a major role in determining the enzyme's remarkable substrate specificity, and is also important for its inhibition by sucrose and glucose. It is proposed that the catalytic reaction is initiated by nucleophilic attack on the substrate by Asp9, and involves formation of a covalent phospho-Asp9-enzyme intermediate. From modelling based on the SPP structure, we predict that the non-catalytic SPP-like domain of the *Synechocystis* sucrose-phosphate synthase could bind sucrose-6^F-phosphate, and propose that this domain might be involved in metabolite channelling between the last two enzymes in the pathway of sucrose synthesis.

How does Nature evolve new natural product scaffolds? Diversification of intramolecular carbon-carbon bond formation within the Type III Polyketide Synthase superfamily.

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Type III (chalcone synthase-like) polyketide synthases (PKSs) are structurally simple but multifunctional enzymes that utilize a conserved Cys-His-Asn catalytic triad within an internal active site cavity to iteratively condense acetyl units derived from malonyl-CoA to a CoA-linked substrate. Type III PKS-catalyzed polyketide chain extension typically terminates via one of three intramolecular cyclization mechanisms. Family members vary in their selection of starter molecule, number of extensions catalyzed, and mechanism of intramolecular polyketide cyclization. Homology-based analysis and experiments following the first chalcone synthase (CHS) crystal structure suggested that type III PKS superfamily substrate and product diversity stems from 'steric modulation' of chemically inert residues lining the active site cavity. To explore the existence of mechanistic design principles other than steric modulation, we focused our subsequent crystallographic attention upon two type III enzymes whose cyclization specificity determinants resisted illumination by sequence comparisons with the CHS structure. Both of these new crystal structures revealed unanticipated structural modifications of the type III PKS fold and generated novel mechanistic hypotheses for further experimentation. Protein engineering guided by our stilbene synthase (STS) crystal structure identified the 'aldol switch' emergent hydrogen bond network responsible for this enzyme's production of resveratrol via a C2->C7 intramolecular aldol condensation of its tetraketide intermediate (rather than the more typical C6->C1 Claisen cyclization leading to chalcones). We then solved the first bacterial type III PKS crystal structure, that of a *Streptomyces coelicolor* 1,3,6,8-tetrahydroxynaphthalene synthase (THNS), a PKS that synthesizes two fused hydroxylated rings from five molecules of malonyl-CoA. This latter structure's novel active site spawned additional hypotheses regarding enzymatic control of reactive intermediates during multi-step polyketide biosynthetic reaction mechanisms. Insights from the STS and THNS crystal structures have expanded and refined our understanding of the processes determining particular biosynthetic routes through the branching mechanistic pathways available to type III PKS active sites.

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