CAFE Postdoctoral/Graduate Student Mentoring Seminar

Pulling your proposal together – the conceptual framework to filling in the forms.

By: **Joseph Chappell, Professor and Chair, Department of Pharmaceutical Sciences, University of Kentucky**.

Date:
Friday, September 20th, 2013

Time: 4:00-5:00 Seminar and discussion

Location: Room N-320, Agriculture Science Center - North, University of Kentucky Lexington, KY.

Ideas count

1. Understanding the wizardry of terpene metabolism in plants

2. Biofuels – the next generation of biofuels from agriculture

3. Techniques, methodologies, breakthroughs

Important question: If you are able to perform the experiment(s) you are thinking about, would the results be considered an incremental step or leap in our conceptual understanding or abilities

Funding Agencies

Federal

- NSF, USDA, DOE
- NIH
- EPA, FDA

State/local

- UK
- KSTC

Private/industrial

- Gates Foundation, HHMI
- Oil companies, chemical industry, biotech firms, agricultural firms

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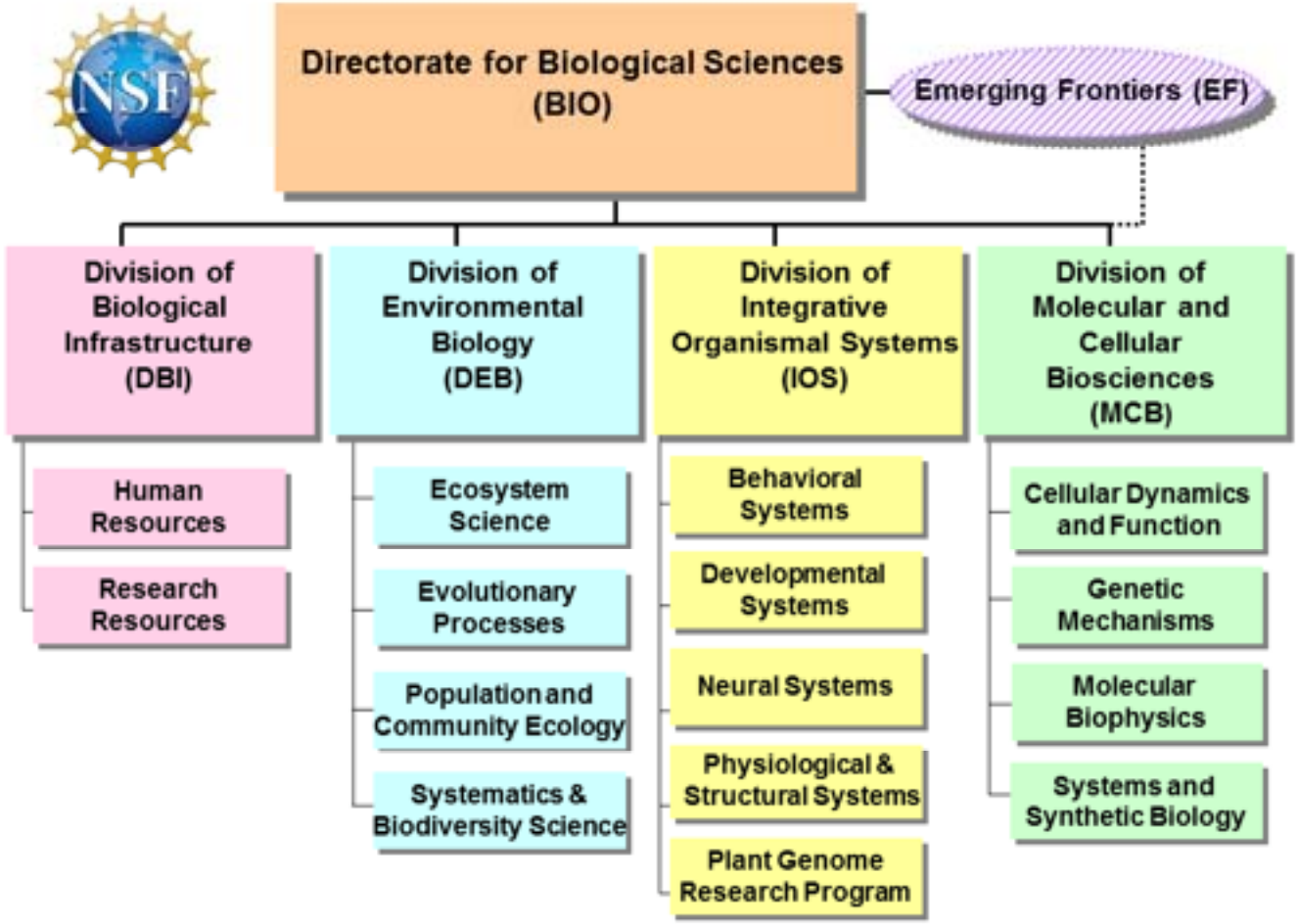
September 2013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Postdoctoral Research Fellowships in Biology (PRFB)

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PROGRAM GUIDELINES

Solicitation 12-497

Important Notice to Proposers

A revised version of the NSF Proposal & Award Policies & Procedures Guide (PAPPG), NSF 13-1, was issued on October 4, 2012 and is effective for proposals submitted, or due, on or after January 14, 2013. Please be advised that, depending on the specified due date, the guidelines contained in NSF 13-1 may apply to proposals submitted in response to this funding opportunity.

Please be aware that significant changes have been made to the PAPPG to implement revised merit review criteria based on the National Science Board (NSB) report, National Science Foundation's Merit Review Criteria: Review and Revisions. While the two merit review criteria remain unchanged (Intellectual Merit and Broader Impacts), guidance has been provided to clarify and improve the function of the criteria. Changes will affect the project summary and project description sections of proposals. Annual and final reports also will be affected.

A by-chapter summary of this and other significant changes is provided at the beginning of both the *Grant Proposal Guide* and the *Award & Administration Guide*.

DUE DATES

Full Proposal Deadline Date: October 8, 2013

SYNOPSIS

The Directorate for Biological Sciences (BIO) awards Postdoctoral Research Fellowships in Biology to recent recipients of the doctoral degree for research and training in *selected* areas supported by BIO and with special goals for human resource development in biology. The fellowships encourage independence at an early stage of the research career to permit Fellows to pursue their research and training goals in the most appropriate research locations regardless of the availability of funding for the Fellows at that site. For FY 2013, these BIO programs are ***(1) Broadening Participation in Biology; (2) Intersections of Biology and Mathematical and Physical Sciences and Engineering; (3) National Plant Genome Initiative Postdoctoral Research Fellowships; and (4) International Postdoctoral Research Fellowships in Biology.*** These areas change periodically as new scientific and infrastructure opportunities present themselves. For this reason, this solicitation will be changed as necessary to reflect the areas being funded.

The fellowships are also designed to provide active mentoring of the Fellows by the sponsoring scientists who will benefit from having these talented young scientists in their research groups. The research and training plan of each fellowship must address important scientific questions within the scope of the BIO Directorate and the specific guidelines in this fellowship program solicitation. International and teaching options are also offered. Because the fellowships are offered only to postdoctoral scientists early in their careers, NSF encourages doctoral advisors to discuss the availability of these postdoctoral fellowships in biology with their graduate students early in their doctoral programs. Fellowships are awards to individuals, not institutions, and are administered by the Fellows.

Structure to Grant Applications

Specific aims
Project Summary/abstract (1/2 to 1 page)
Proposal (10 to 15 pages)
References cited
Biosketch
Major equipment available
Resources/Facilities (lab and office space, computer, etc)
Postdoctoral/Graduate student mentoring plan
Broadening participation plan
Support letters

Budget

Cover letter
Specific aims

Specific Aims

Natural products have served as important resources for drugs and drug development since the beginning of civilization and the new "omics" technologies are promising new avenues for improving the discovery pipeline from this rich and vast reservoir. Plant-derived natural products have had a profound impact on human health and include compounds that have been used successfully for decades, including drugs like digoxin, Taxol, vincristine, and morphine. The structural diversity and biological activities evident from the relatively limited number of all extant plants surveyed suggests many more medicinally relevant compounds remain to be discovered in plants. The diversity of plant natural products results from intertwining networks of secondary or specialized biosynthetic pathways. And while conventional screens for plant natural products have shown great promise for medical applications, uncovering the fully chemical diversity and complexity means understanding the genetic and molecular genetic underpinnings coding for these pathways. However, the functional identification of plant genes contributing to these networks remains a bottleneck. Here we propose to develop unprecedented resources to accelerate the identification and functional characterization of genes involved in plant natural product biosynthesis.

Our Specific Objectives and Aims are:

1. The **GENE DISCOVERY** objective is *to establish an expandable, user-friendly computational platform to predict genes involved in NP biosynthesis.* This will involve four aims: **1.1 Assembling large and diverse transcriptomic and metabolomics data sets; 1.2 Providing a computational portal to annotate genes via structure predictions and annotate unidentified metabolites via classifications into related groups; 1.3 Develop and optimize computational approaches to enhance Natural Product discovery;** and **1.4 Creating a user-friendly big-data-enabled web platform.**

2. The **DESIGN AND CONSTRUCTION OF MODULAR EXPRESSION VECTORS AND DEVICES FOR HIGH-THROUGHPUT IDENTIFICATION OF FUNCTIONAL CAPACITY OF GENES TO PRODUCE NPS** *objective is to construct vectors and devices for rapid and efficient high-throughput functional analysis of genes in plant hosts undertaken in objective 3.* This will entail two specific aims: **2.1 Design and construct vectors for high-throughput cloning of single and multiple genes for transient, stable and inducible expression in heterologous plant-based platforms;** and **2.2 Explore, develop and evaluate the potential of protein-based and RNA-based metabolite sensors for natural product gene discovery.**

3. The **DEVELOPMENT OF HETEROLOGOUS PLANT-BASED EXPRESSION PLATFORMS FOR IDENTIFYING FUNCTIONAL CAPACITY OF GENES TO PRODUCE NPS** *objective is to experimentally validate the predicted functional capacity of genes assigned by the computational and bioinformatics analyses in objective 1.* This will be achieved by establishing robust and high-throughput plant-based heterologous expression platforms for functional characterization of gene products. These platforms will integrate expression devices developed in objective 2. This strategy will develop parallel expression systems to enable the identification of gene functions and uncover the unsuspected capacity of genes to produce novel metabolites. The specific aims include establishing: **3.1 Tomato fruits as a heterologous expression platform; 3.2 Transient expression in *N. benthamiana* leaves; 3.3 Hairy root cultures as heterologous expression platforms;** and **3.4 Carrot somatic embryogenesis system as a heterologous expression platform.**

4. The **CHEMICAL PROFILING** *objective is to provide comprehensive support to solve key problems in chemical characterization and metabolome data mining encountered in the entire GPNP project.* These efforts will be integrated in the form of metabolome data mining with the Gene Discovery working group, and deep non-targeted and metabolite profiling of plant extracts generated by the Expression Platforms working group in which target genes have been expressed. The primary focus of objective 4 is to generate precise measurements of metabolite levels that reflect the function(s) of each expressed gene based on alteration of metabolite profiles relative to control tissues. The specific aims include: **4.1 Advanced mining of existing and new metabolome data sets; 4.2 Surveying metabolite profiles in untransformed heterologous expression hosts; 4.3 Performing non-targeted high-throughput profiling of metabolites in transformed expression systems;** and **4.5 Providing problem-specific support of metabolite chemistry needs of the program.**

Abstract/Summary

Natural products have served as important resources for drugs and drug development since the beginning of civilization and the new “omics” technologies are promising new avenues for improving the discovery pipeline from this rich and vast reservoir. Plant-derived Natural Products have had a profound impact on human health and include compounds that have been used successfully for decades, including drugs like digoxin, Taxol, vincristine, and morphine. The structural diversity and biological activities evident from the relatively limited number of all extant plants surveyed suggests many more medicinally relevant compounds remain to be discovered in plants. The diversity of plant Natural Products results from intertwining networks of secondary or specialized biosynthetic pathways. And while conventional screens for plant Natural Products have shown great promise for medical applications, uncovering the fully chemical diversity and complexity means understanding the genetic and molecular genetic underpinnings coding for these pathways. However, the functional identification of plant genes contributing to these networks remains a bottleneck. Here we propose to develop unprecedented resources to accelerate the identification and functional characterization of genes involved in plant Natural Product biosynthesis. We are proposing to develop an expandable, user-friendly computational platform to predict genes involved in plant Natural Product biosynthesis, to provide an array of stable and transient expression vectors for introducing and monitoring expression of the targeted genes *in planta*, and to functionally characterize these genes of interest by identifying metabolic changes associated with their expression in a variety of host plant species. All these resources and resulting information will be disseminated to the scientific community via public databases and publications, as well as in an unrestricted, public resource operated and maintained by the Genomes to Plant Natural Products Consortium promising to advance our knowledge base of species-specific plant metabolism and accelerate the identification and functional analysis of genes involved in Natural Product biosynthesis associated with medicinal important plant species. These resources will provide the research community with user-friendly access to an unparalleled resource correlating functionally annotated genes with the biosynthesis of plant Natural Products, which we anticipate will have a translational effect on drug development.

Research Plan – Research Area

Natural products (NPs) have served as important resources for drugs and drug development since the beginning of civilization. Plant-derived NPs have had a profound impact on human health and include compounds that have been used successfully for decades, *e.g.*, drugs like digoxin, taxol, vincristine, and morphine. The chemical diversity and biological activities evident from the relatively limited number of all extant plants surveyed suggests many more medicinally relevant compounds remain to be discovered. Modern "omics" technologies are promising novel avenues for improving the discovery pipeline from this rich and vast reservoir (1, 2).

While plants continue to be important in direct screens for drug development, there is clear evidence that their full capacity for NP biosynthesis lies within their genomes and the genes coding for specialized metabolism (3). However, accessing and taking advantage of this potential poses serious challenges. Plant genomes are significantly larger than microbial genomes and it is difficult to glean particular biosynthetic pathways from such information due to multifold reasons. Plant biosynthetic pathway genes are often not clustered, thus the genome itself masks pathway discovery. Moreover, a simple, straightforward strategy for searching for genes contributing to yet undiscovered biosynthetic steps is not obvious (4). There is an urgent need to develop generalizable approaches for identifying plant genes contributing to specialized metabolism and tools for functional characterization. Coupling new advances in gene discovery with metabolic engineering of plants and microbes will catapult identification of new biologically active plant NPs for clinical screening of widespread medical uses.

The diversity of plant NPs results from intertwining networks of secondary or specialized biosynthetic pathways. While conventional screens for plant NPs have shown great promise for medical applications, uncovering the full chemical diversity and complexity means understanding the genetic elements and molecular mechanisms underpinnings complex metabolic networks. However, the functional identification of plant genes contributing to these networks remains a bottleneck. Here we propose to develop unprecedented resources to accelerate the identification and functional characterization of genes involved in plant NP biosynthesis.

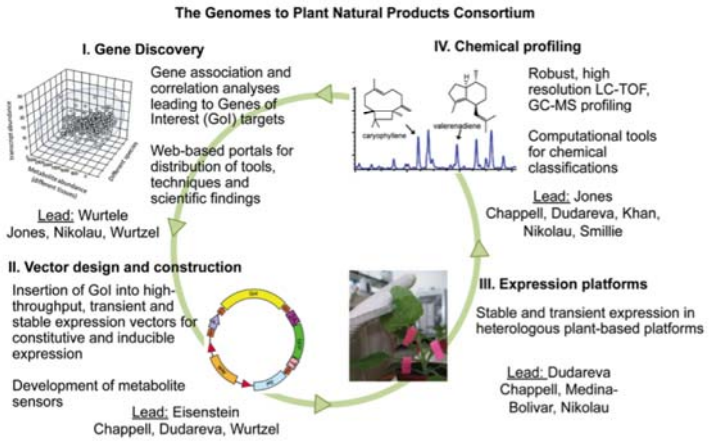

Fig. 1.0. Overview of the Genomes to Plant Natural Products Consortium Pipeline.

The Genomes to Plant Natural Products Consortium (GPNPC) will operate in four synergistic working groups (Fig. 1.0) to deliver tools, techniques and scientific findings to the worldwide scientific community. The *Gene Discovery* working group provides bioinformatic and computational tools to predict gene membership in NP pathways and select genes for functional analysis; the *Vector Design and Construction* working group incorporates these Genes of Interest (GoIs) into plant expression vectors and develops advanced molecular sensors for monitoring specialized metabolism manipulations in the expression hosts; the *Expression Platforms* working group performs heterologous expression of the GoIs in multiple hosts, taking advantage of diverse substrates and metabolic intermediates present in native environments; and the *Chemical Profiling* working group documents the functional contribution of GoIs to chemical changes in the NP profiles of the host species, and returns the combined data sets to the *Gene Discovery* working group for web portal distribution and iterative

BIOGRAPHICAL SKETCH

Joseph Chappell
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Professional Preparation
University of California, San Diego: B.A. in Biology, 1973-1977
University of California, Santa Cruz: Ph. D. in Biology, 1977-1981
Universität Freiburg and MPI, Cologne: Postdoctoral studies, 1981-1983
University of California, San Diego: Postdoctoral studies, 1984

Appointments
University of Kentucky: Assistant, Associate, Full Professor -1985, 1991, 1997
Salk Institute, Plant Biology Lab: Sabbatical leave, 1994-1995
University of Strasbourg, Dept. of Isoprenoids, Sabbatical leave, 2002

Research Publications (5 most relevant)

1. PJ Facchini and J Chappell. 1992. A gene family for an elicitor-induced sesquiterpene cyclase in tobacco. *Proc. Natl. Acad. Sci.* 89: 11088-11092.
2. S Wu, M Schalk, A Clark, RB Miles, RM Coates and J Chappell. 2006. Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. *Nature Biotech.* 24:1441-1447.
3. TD Niehaus, S Okada, TP Devarenne, DS Watt, V Sviripa, and J Chappell. 2011. Identification of unique mechanisms for triterpene biosynthesis in *Botryococcus braunii. Proc. Natl. Acad. Sci.*108:12260-12265.
4. TD Niehaus, S Kinison, S Okada, Y-S Yeo, SA Bell, P Cui, TP Devarenne, J Chappell. 2012. Functional identification of triterpene methyltransferases from *Botryococcus braunii* race B. *J. Biol. Chem*. 287:8163-8173.
5. YS Yeo, SE Nybo, AG Chittiboyina, AD Weerasooriya, YH Wang, E Góngora-Castillo, B Vaillancourt, CR Buell, D DellaPenn, MD Celiz, AD Jones, ES Wurtele, N Ranson, N Dudareva, KA Shabaan, N Tibrewal, S Chandra, T Smillie, IK Khan, RM Coates, DS Watt, J Chappell. 2013. Functional identification of valerena-1,10-diene synthase, a terpene synthase catalyzing a unique chemical cascade in the biosynthesis of biologically active sesquiterpenes in Valeriana officinalis. *J. Biol. Chem.* (in press)

(5 related)
1. CM Starks, K Back, J Chappell, and J Noel. 1997. Structural basis for cyclic terpene biosynthesis by 5-epi-aristolochene synthase from tobacco. *Science* 277: 1815-1820.
2. BT Greenhagen, PE O'Maille, JP Noel and J Chappell. 2006. Identifying and manipulating structural determinates linking catalytic specificities in terpene synthases. *Proc. Natl. Acad. Sci.* 103:9826-9831
3. S Takahashi, Y Yeo, BT Greenhagen, L Song, J Maurina-Brunker, R Rosson, JP Noel and J Chappell. 2007. Metabolic engineering of sesquiterpene metabolism in yeast. *Biotech. Bioeng.* 97:170-181.
4. S Lee and J Chappell. 2008. Biochemical and Genomic Characterization of Terpene Synthases in *Magnolia grandiflora. Plant Physiol.* 147:1017-1033.
5. S Wu, Z Jiang, C Kempinski, SE Nybo, S Husodo, R Williams and J Chappell. 2012. Engineering triterpene metabolism in tobacco. *Planta* **236**, 867-877

Synergistic Activities

Agricultural Biotechnology Degree Program – I helped initiate this degree program in 1988, which emphasizes genetics, biochemistry and molecular biology. The program graduates 40 to 45 students/year with ~75% of the students going on to graduate or professional programs. I am currently an academic advisor for the program and routinely direct 1 to 2 undergraduate research projects (a requirement of this program) per year. For example, Madison Wallace completed his ABT 395 project in Summer 2012 and gave his oral presentation of the work to the ABT Coordinating faculty. His project was to assess the transcriptome of *Digitalis purpurea* for putative lanosterol synthase genes, then to functionally characterized these by heterologous expression in bacteria and yeast. Madison is continuing to work in the lab.

ABT 120 – Genetics and Society, This is a new course I develop for the new University of Kentucky general education curriculum. This course welcomes freshman students from across the entire campus to learn and discuss the science of genetics, and to appreciate how genetics is pervading society at the personal level in terms of medical practices and the foods we eat, and at a national level with regards to the establishment of many new many public polices like DNA fingerprinting of anyone convicted of a crime.

DNA Science – An outreach effort for Middle to High Schools programs, Community College science instructors, Extension Specialists and 4H programs, since 1992. Our DNA Science operates as a resource center during the academic year, offering materials, supplies and experimental protocols for 2-3 area schools per semester, contact with 100-300 HS students per semester. Extended to international outreach when we provided training at 2 institutions in Indonesia in 2010.

Professional involvement (recent) – member of the Journal for Biological Chemistry Editorial Board (2006-2011), Co-Chair/Chair for the 2007/2009 Plant Metabolic Engineering Gordon Research Conference series, service to NSF and NIH grant review panels, and member of the Plant Physiology Editorial Board (2009-2014).

Collaborators and Co-Authors (last 5 years):

Robert Coates, University of Illinois
Dale Poulter, University of Utah
Anthony Clark, Firmenich, Switzerland
Michel Schalk, Firmenich, Switzerland
Joe Noel, Salk Institute
Pete Spielmann, University of KY
CJ Waechter, University of KY
David Watt, University of KY

Graduate and Postdoctoral Advisors:
Harry Beevers, UCSC, graduate advisor (deceased)
Maarten Chrispeels, UCSD, undergraduate and Postdoctoral advisor
Klaus Halhbrock, MPI, Germany, Postdoctoral advisor

Thesis advisor and Postgraduate-Scholar Training:
Current post-docs: Santosh Kumar, Eric Nybo, Sheba Goklany,
Current PhD students: Xun Zhang, Chase Kempenski, Zuodong Jiang, Stephen Bell, Kristin Linscott

Former co-workers (last 5 years):
Yunsoo Yeo, Sabbatical Scholar – Research Scientist, Nat. Inst. Ag. Biotech., Suwan, Korea
Shunji Takahashi, Postdoctoral Scholar – Associate Professor, Riken, Japan
Shuiqin Wu, Postdoctoral Scholar/Research Assistant – Research Scientist, Sapphire Energy
Sungbeom Lee, earned Ph.D. 2008, - Senior Scientist, ARTI, Deajeon, South Korea
Thomas Niehaus, Ph.D. 2011 – Postdoctoral Associate, University of Florida, Gainesville
Walter Suza, Postdoctoral Scholar – Lecturer, University of Nebraska
Jeanne Rasbery, Postdoctoral Scholar – currently working with another UK faculty member

Resources – Chappell - UK

The Chappell laboratory has workspace allocations in the Plant Science (PS) building and in the new Bio-Pharmaceutical Complex (BPC) building. The BPC laboratory of approximately 1,000 sq. ft. with 10 workstations is located contiguous with laboratories performing similar types of research using common experimental techniques and equipment. One immediate neighboring laboratory houses 2 high-speed Sorvall centrifuges, one maintained by the Chappell lab. The laboratory on the other side maintains a Beckman ultra-centrifuge with multiple fixed-angle and swing-bucket rotors. The Chappell laboratory is equipped for standard chemical, biochemical and molecular genetic work and includes multiple rotoevaporators and glassware for standard analytical work; electrophoresis equipment for both protein and nucleic acid work; a single beam, high resolution spectrophotometry; multiple PCR machines; and various small equipment items typical in biochemical laboratories (scales, pH meter, microfuges, vortexes, gel electrophoresis, gel documentation station, refrigerators, freezers, etc.). This laboratory also houses a chemical fume hood.

The Chappell BPC laboratory has space in the equipment corridor and is assigned several equipment rooms of approximately 600 sq. ft. each. A Packard liquid scintillation counter and 2 -80^{o}C freezers (one designated as a repository for our various Medicinal Plant projects) are located in the equipment corridor, along with 2 temperature controlled gyratory shakers and 2 standing temperature-controlled incubators. One equipment room is set-up for microbial and plant cell cultures including sterile transfer hoods, additional 2 gyratory shakers, a light bank, and a BioFlor 115 fermentation system. The second equipment room is set up for analytic work and includes a new Agilent 7890 GC-FID equipped with an autosampler, a new (2012) Agilent GC-MS with autosampler with an assortment of columns, and a Waters HPLC-PDA-autosampler system with C18 reverse phase and a variety of preparative and analytical silica columns.

In addition, the Chappell BPC laboratory shares space in a walk-in cold room and enjoys access to general services including 2 large autoclaves, liquid nitrogen, nitrogen gas and ice supplies.

The Chappell PS laboratory is shared space within a 1,000 sq. ft., fully equipped laboratory (scales, chemical stocks, spectrophotometry, gel electrophoresis equipment, Sorvall centrifuge, refrigerator, freezers). This space also contains 2 fume hoods and is adjacent to an equipment corridor that is organized with common use equipment housed in separate rooms. A tissue culture facility includes 6 large laminar flow hoods and a temperature controlled room for plant and microbial cultures. The general utility room houses 4 -80 ^{o}C freezers, an ultracentrifuge with various rotors, a freeze dryer and chemical cabinets for excess solvent storage. The cold room (4^{o}C) is organized for general laboratory use with spacious workbenches and a sonication system with various size probes. The autoclave room houses 1 large capacity autoclave and 1 small. The microscopy room contains several low-resolution dissection type microscopes as well as a high-resolution system equipped for fluorescence microscopy and digital image-capture.

A greenhouse section of ~800 sq. ft. within the UK greenhouse complex is also assigned to the Chappell laboratory. Full access to headhouse and pesticide storage facilities are also granted to the Chappell laboratory.

Support Staff

Scott Kinison is the primary research technician in the Chappell laboratory and supports day-to-day operations. Scott maintains supplies of chemicals, reagents, solutions and consumables, is responsible for the microbial and plant cell cultures, and is the resident radiation and hazardous materials officer. Many of Scott's duties are supported by undergraduate assistants.

Offices

All research personnel have separate desk space outside of the BPC laboratory with internet access, including that to extensive electronic library resources. These spaces are equipped with several PC and MacIntosh desktop computers. The PI's BPC office is approximately 400 sq. ft and is equipped with an iMAC computer. Dr. Chappell also has an office in the PS building and maintains separate office space for postdoctoral associates and graduate students in this building as well. All offices and laboratories have hardwire and wireless access to internet connectivity and IP phone service, including access to extensive electronic library resources. The Chappell postdoc office in the PS building houses the Chappell genomics workstation – a Mac Quadra system with a CLC workbench license.

Major Equipment Available for this Project – Chappell - UK

Major equipment available in the Chappell laboratory spaces (lab 343 Plant Science Building, labs 29-32, 4th floor BioPharma Complex) include:

2 high-speed Sorvall centrifuges;
1 Beckman ultracentrifuge with multiple fixed-angle and swing-bucket rotors;
1 Perkin-Elmer liquid scintillation counter;
6 temperature controlled, gyratory shakers for microbial and plant cell cultures;
1 Bio-Rad protein purification system with a wide range of purification columns;
6 laminar flow hoods (for microbial and plant cell culture work);
3 chemical fume hoods;
1 Agilent 7890 GC-FID with autosampler;
1 Agilent GC-MS (quadrapole) with autosampler, wide range of columns (including chiral columns);
1 Waters HPLC-PDA-autosampler system with C18 reverse phase and silica columns;
8 PC and 2 Macintosh computers;
1 800 sq. ft. greenhouse section;
3 -80 ^{o}C freezers;
2 walk-in cold rooms (4^{o}C) organized for protein biochemistry; and
3 chemical fume hoods

Major equipment available within UK sponsored facilities:

AGTC – The UK Advanced Genetic and Technology Center, a high throughput DNA sequencing facility (re-charge basis);

UKMSF – The UK Mass Spectrometry Facility, home to a wide range of mass spectrometers, providing analysis using many different methods of sample introduction, ionization, and mass measurement such as MALDI and ESI (re-charge basis);

UK Chemistry NMR Facility - The NMR center provides two upgraded GEMINI 200 MHz instruments and two Varian INOVA 400 MHz instruments capable of z-pulsed field gradients (PFG), variable temperature capabilities, waveform generators, and can perform used for 2- and 3-dimensional NMR experiments (COSY, NOESY, ROESY, TOCSY, DEPT, INADEQUATE, and other advanced NMR pulse sequences) – walk-on use.

UK College of Pharmacy - Varian Vnmr 500 NMR spectrometer (re-charge basis)

Budget – Chappell - UK

3 Year Grant			**Year 1**					
	% Effort on Project	Institutional Base Salary	Salary Requested	Fringe Benefits	Total Requested	**Year 2**	**Year 3**	All Years
Faculty	15%	$100,000	$15,000	$3,182	$18,182	$20,453	$21,372	$60,007
project manager	50%	$40,000	$20,000	$8,911	$28,911	$30,369	$31,917	$91,197
Lab Technician (Staff)	20%	$40,000	$8,000	$3,564	$11,564	$12,148	$12,767	$36,479
Post Doc	100%	$40,000	$40,000	$12,742	$52,742	$55,455	$58,340	$166,537
Graduate Student	100%	$0	$0	$0	$0	$0	$0	$0
Undergraduate Students*	100%	$4,000	$4,000	$346	$4,346	$4,520	$4,701	$13,566
Total Personnel			$87,000	$28,745	$115,745	$122,945	$129,096	$367,786
Equipment					$0	$0	$0	$0
Supplies					$24,000	$24,000	$24,000	$72,000
Travel					$2,000	$2,080	$2,163	$6,243
Publication Charges					$0	$1,500	$1,560	$3,060
Other direct costs					$2,000	$2,080	$2,163	$6,243
Tuition					$0	$0	$0	$0
Total Direct Costs					$143,745	$152,605	$158,983	$455,333
Indirect Costs	49%				$70,435	$74,776	$77,901	$223,113
Total Costs					**$214,180**	**$227,382**	**$236,884**	**$678,446**
	Information purposes only -- modified direct costs for indirect calculations				$143,745	$152,605	$158,983	
	Modified Direct Costs are direct costs less tuition and equipment							

Faculty Fringe	21.25%	(7.65% FICA; 10% Retirement; 3.6% Miscellaneous)
Staff Fringe	21.35%	(7.65% FICA; 10% Retirement; 3.7% Miscellaneous)
Graduate Student, Post Doc, Temp Fringe	8.65%	(7.65% FICA; 1% Miscellaneous)
Yearly Salary & Supplies Increase	4.00%	
Faculty & Staff Family Health & Life Insurance	$9,282	
Graduate Student Health Insurance	$1,000	
Yearly Health Insurance Increase	10.50%	
2010-11 Tuition	$10,300	
2011-12 Tuition	$11,100	
2012-13 Tuition	$12,100	
2013-14 Tuition	$13,100	
2014-15 Tuition	$14,100	
Indirect Rates		
On Campus Ag Research	39.0%	
On Campus Public Service/Ag Extension	33.9%	
Off Campus Ag Research	19.2%	
Off Campus Public Service/Ag Extension	21.6%	
On Campus non Ag Research	48.50%	

*Undergraduate students will be part time employees paid hourly for this spreadsheet It's just easier to say 100% of the time is on the grant.
The salary is usually something like 10 hours a week times $8 or whatever you think is reasonable and then 40 hours a week in the summer.

BUDGET JUSTIFICATION – Chappell, UK

For participating in the Expression Platforms and Chemical Profiling Working Groups and serving as the contact Program Director

Personnel:
Joe Chappell will serve as the contact Program Director for the overall proposed activities (i.e. will be responsible for communicating with NIH program officers and fulfilling all the reporting functions), as well as overseeing the Chappell Collaborator Unit responsibilities to both the Expression Platforms and Chemical Profiling groups. Given the expected dedication of time and effort to this project, funds to cover Dr. Chappell's salary for 2 months per year are request.

Funds to support a **Project Manager** are request. The Project Manager will be expected to develop and maintain a LIMS tracking system for all the proposed activities at the University of Kentucky and all the participating institutions. This initiates with selection of target genes by the Gene Discovery working group through to obtaining the LC-TOF and GC-MS profiles for each gene expressed in each host plant species by the Chemical Profiling working group. The Project Manager will also participate in all the bi-weekly, monthly and project review meetings, will assist in preparing written records for each of these, and will help to facilitate information/report sharing between the various functional units, the Executive and the Advisory committees and the NIH. The Project Manager is also expected to visit each of the cooperating institutions within 6-months of the grant beign initiated to provide administrative support for each. Funds to support a 50% time Project Manager are thus requested.

The Chappell Collaborator Unit is expected to receive substantial numbers of gene constructs for transient expression studies in Nicotiana species and stable expression studies in Valeriana hairy root cultures. The Chappell unit is expected to record receipt of materials, to deposit aliquots in repository facilities (-20 to -80^{o}C freezers), to introduce these expression constructs into the appropriate host species and to collect appropriate plant materials for chemical profiling. Aliquots of the collected plant material will be extracted for LC-TOF, while a second aliquot will be extracted for GC-MS analyses. The Chappell unit will also receive samples for GC-MS analyses from other Collaborator units. Hence, we expect to receive up 300-600 different expression cassettes within the first 3 years for expression analysis and chemical profiling, and to receive another 600 to 1,2000 samples for GC-MS analysis during the first 3 years of the proposed project. Funding for **1 technical assistant** and **1 Postdoctoral Scholar** are requested to meet to these expectations.

The technician would be assigned to introducing the expression vectors into the various host plant materials and propagating these materials until they are harvested. **Scott Kinison**, M.S. Engineering, is a Senior Research Technician in the Chappell laboratory and has been responsible for maintaining much of the biological materials used for research over the last 8 years. This has included assisting in generation of transgenic plant lines and maintenance of sterile tissue culture materials. Scott also has a great deal of experience running and maintaining our various GC instruments. Partial funding for Scott is requested.

The **Postdoctoral Scholar,** to be named, would provide oversight and coordination for all these activities, plus prepare the necessary tissue extracts for chemical profiling and running all the GC-MS samples.

Undergraduate students – We will recruit 2 undergraduate students from the Agricultural Biotechnology program operating within the UK College of Agriculture to assist in receiving and archiving materials, and to help the technical staff. We hope to recruit two undergraduates to work during the academic months and 2 students to work full-time during the summer. The students would work directly with the technician, but be responsible to the Post-doctoral Associate.

Fringe Benefits:
Annual salary adjustments have been calculated at 4%, assuming satisfactory progress. The current UK fringe benefit allowances are 8.55% for graduate students and Postdoctoral Scholars, and 21.35% for staff members.

Equipment:
We have all the necessary facilities to perform all the proposed work, but are requesting 2 -80^{o}C freezers ($20,000) to store and archive materials. Funds for the purchase of a platform shaker to be used in preparing metabolite extracts is also requested ($10,000).

Travel:
Funds to cover the costs for the program manager and Dr. Chappell to visit all the research sites to implement the coordination activities are requested in year 1. Funds to help cover the costs for attending the annual review meetings is also requested. The intention of the annual meetings will be to coordinate all the proposed activities, establish performance standards (including timelines), and to provide a means for all investigators to participate in discussions and analyses of data prior to public dissemination.

Supplies and Other direct costs:
Funds to cover routine expenses in propagating the various plant materials, preparing extracts and running GC-MS analyses are requested. Costs for shipping various materials between collaborating units are included as well.

Subcontracts:
As the institution serving to coordinate all the research activities, UK is requesting funds to distribute to the other participating institutions according to their sub-project budget requests.