NINTH ANNUAL

BIOTECHNOLOGY TRAINING RETREAT

Christian Brothers Retreat Center

Napa Valley

Saturday March 11, 2000

PROGRAM OVERVIEW

8:15 am	**Registration & Continental Breakfast**
8:45 am	**Introduction by John Yoder**, Chair of the Designated Emphasis in Biotechnology (DEB)
9:00 – 11:30 am	**Company presentations** may include brief overview of the company's mission; their research and development along project lines and/or a more in depth coverage of specific research projects. (Monsanto, Calgene campus, Chiron, DuPont, Genentech, Novo Nordisk Biotech, Roche Bioscience, Scios are our current affiliates). **Approximately 5 minutes for questions will be allotted at the end of each presentation**.
11:30 - 2:00 pm	**Poster Session & Lunch** Fellows, trainers, other students and invited companies will present Posters. There also will be opportunity for the fellows and other students to intermingle with the company trainers and gain a greater insight into research in the private domain and also, to develop ideas for suitable internship projects.
2:00 - 4:30 pm	**Faculty trainers & their present fellows presentations**. An overview of their research projects, beginning with a **5-minute overview** of the lab focus by the trainer, followed by a more detailed description by the fellow (**15-min**) of his/her research. **Approximately 5 minutes for questions will be allotted at the end of each presentation**.
4:45 pm	**Close**

PROGRAM OVERVIEW

8:15am Registration

8:45am Introduction

COMPANY PRESENTATIONS: Martina McGloughlin, Chair

9:00am	Monsanto research and product development	- Nordine Cheikh Site Director Monsanto, Calgene campus
9:20am	Chiron, a diversified biopharmaceutical company	- Steven Rosenberg, Research Director, Chemical Therapeutics Group Chiron Technologies
9:40am	An overview of biotechnology research at Dupont	- Julie Vogel, Senior Research Scientist Central Research & Development DuPont

10:00am BREAK

10:10am	Genentech, 2000: A view from the department of molecular oncology	- Ingrid Wertz Visiting Scientist Genentech, Inc.
10:30am	Cloning, expression, and industrial uses of fungal laccases	- Debbie Yaver Research Scientist Novo Nordisk Biotech
10:50am	Scios Research	- David Liu Research Scientist Scios Inc.

11:10am Presentation of Posters

12:00pm LUNCH, DISCUSSION & POSTERS

COMPANY FELLOW PRESENTATIONS: George Bruening, Chair

2:00pm	Molecular Virology/Vaccine Development	- Tilahun Yilma, VM-ILMB
	Enhancing the Immune Response To Recombinant Vaccinia Virus Vaccine	- Fatema Aziz Comparative Pathology
2:25pm	Statistical analysis of microarray data	- David Rocke Statistics/CPIPIC

	Variance component in DNA Microarray Data	- Blythe Durbin Statistics/CPIPIC
2:50pm	On-Going Research In The Hammock Lab	- Bruce Hammock, Entomology
	Epoxide hydrolase, juvenile hormone Esterase, plants and insects	- Tonya Severson, Entomology
3:15pm	Overview of Research	- John Yoder Vegetable Crops
	Gene Expression Profiles of an Induced Developmental Pathway in Parasitic Plant roots	-Manuel Torres Genetics

3:40pm BREAK

PRESENTATIONS CONTINUED: John Yoder, Chair

3:50pm	Plant – Plant Communication	- John Yoder, Vegetable Crops
	The Molecular and Genetic Basis of Haustorium Development in the Parasitic Plant, Triphysaria	- Denneal Jamison, Genetics
4:15pm	Overview of Research Nuclear Hormone Receptors	- Marty Privalsky, Microbiology
	Anti-Repression by thyroid hormone receptors	- Zhihong Yang Microbiology
4:45pm	Conclusion	
5:30pm	Bus departs	

POSTER TITLES

A. GENE CLONING AND MOLECULAR CHARACTERIZATION OF EXPANSINS IN GERMINATING TOMATO SEEDS

Feng Chen and Kent J. Bradford
Department of Vegetable Crops, University of California, Davis

B. REPAIR OF UV-INDUCED DNA DAMAGE IN ARABIDOPSIS

Fidantsef, A.L.*, Eads J. R., Estrada, A.K., Lei S., and Britt, A.B.
Section of Plant Biology, University of California, Davis

C. ANALYSIS OF DNA-MICROARRAY DATA USING PARTIAL LEAST SQUARES

Dahn Nguyen
Center for Image Processing and Integrated Computing, University of California, Davis

D. DNA MICROARRAY ANALYSIS OF THE RESPONSE OF CYANOBACTERIA TO UV-B IRRADIATION

Lixuan Huang, Mike McCluskey, Melissa Jia, Zhixiong Xue, Bob LaRossa
Biochemical Science and Engineering, Dupont CR&D, Wilmington, Delaware

Oral Presentation Abstracts

MONSANTO, Calgene Campus – Research and Product Development

Nordine Cheikh
Site Director
Monsanto, Calgene campus
Davis, CA

As a life sciences company, Monsanto is committed to finding solutions to the growing global needs for food and health by sharing common forms of science and technology among agriculture, nutrition and health. Our family of 30,000 employees worldwide seeks to make and market high-value agricultural products, pharmaceuticals and food ingredients in a manner that achieves environmental sustainability.

Agriculture
Monsanto's Agriculture sector broadens and redefines agricultural niches through a wide range of ongoing initiatives that are transforming the way food and fiber are produced everywhere in the world. By integrating established technologies such as herbicide use and breeding techniques with advanced biotechnology, we strive to produce a sustainable level of nutrition for the world's rapidly growing population. Many of our products are aimed at helping farmers produce improved crops — crops that yield more and better food — while at the same time limiting the resource consumption and strains on the environment that accompany traditional agricultural production methods.

Nutrition & Consumer
The Nutrition & Consumer sector serves as a critical interface and bridge between Monsanto's agriculture and pharmaceutical divisions. The business is closely integrating its core capabilities in human and animal nutrition, food ingredient technology and consumer marketing with the company's strengths in agriculture, plant biotechnology, and healthcare.
These strengths will help Monsanto bring tomorrow's consumers tailored nutrition-based solutions from a new generation of food ingredients and food products systems, as well as innovative approaches to the enhancement of health. Our unique capabilities are opening up a new world of possibilities and changing the relationship between nutrition and health. Today, our global presence in this arena is marked by seven production facilities, three research and development centers, eighteen sales offices, and more than 100 distributors on five continents. The food ingredient and consumer food business of the Monsanto Company is headquartered in Chicago, Illinois. In Europe, NutraSweet AG, our joint venture partner, is the exclusive marketer of NutraSweet brand sweetener. Monsanto Calgene Campus is a research and development site within our Nutrition and Consumer Sector. The research at Calgene focuses primarily on improving quality traits for food and feed as well as nutritional approaches for the enhancement of health. The Calgene Campus is also the primary site within Monsanto for the genetic improvement of canola.

Pharmaceutical
Searle, the pharmaceutical sector of the Monsanto Company, is a research-based company principally located in Skokie, Illinois, that develops, manufactures and markets prescription pharmaceuticals and other healthcare solutions worldwide. Under the direction of co-presidents Al Heller and Philip Needleman, Searle's mission is to bring to the market innovative, value-added healthcare products that satisfy unmet medical needs.The company focuses its expertise on five therapeutic areas: arthritis; insomnia; cancer; cardiovascular disease; and women's reproductive health.

On December 19, 1999, **Monsanto Company and Pharmacia & Upjohn** announced a definitive agreement to merge. The new company, to be called Pharmacia Corporation. The merger is expected to close the second quarter of 2000.

CHIRON: A DIVERSIFIED BIOPHARMACEUTICAL COMPANY

Steven Rosenberg
V. P. Research
Chiron Technologies
Emeryville, CA

Chiron is a diversified biopharmaceutical company with businesses in vaccines, diagnostics and therapeutics, with a focus on infectious diseases, oncology, critical care and cardiovascular disease. Chiron's central research effort, Chiron Technologies, uses three methodologies: recombinant protein production; gene therapy and combinatorial chemistry to discover and develop new products for unmet medical needs. The targets for these efforts are discovered using high throughput functional gene discovery. In addition, this group has the mandate to develop new technologies, which can be used in the diagnosis, prevention and treatment of human disease. Examples of both present products and future directions will be presented.

An Overview of Biotechnology Research at Dupont

Julie Vogel
Senior Research Scientist
DuPont
Wilmington, DE

At DuPont, excellence in research and development has been a long standing tradition. The successful coordination of several scientific and engineering disciplines can enable safe and cost effective production of commodity chemicals and other products by biological agents. A general overview of development efforts in biotechnology will be presented.

Genentech, 2000:
A View from the Department of Molecular Oncology

Ingrid Wertz
Visiting Scientist
Genentech, Inc.
S. San Francisco, CA

Genentech, Inc. is a biotechnology company dedicated to the discovery, development, manufacturing, and marketing of human pharmaceuticals for significant unmet medical needs. Discovery research at Genentech focuses on three major defined therapeutic areas: Cardiovascular Medicines, Endocrinology, and Oncology. Specifically, the product pipeline from the Department of Molecular Oncology will be discussed, along with the rationale for the development of specific products.

CLONING, EXPRESSION, AND INDUSTRIAL USES OF FUNGAL LACCASES

Debbie Yaver
Research Scientist
Novo Nordisk Biotech
Davis, CA

Debbie Yaver*, Maria Del Carmen Overjero, Feng Xu, Stephen Brown, and Sakari Kauppinen, Novo Nordisk Biotech

Enzymes are used today in a wide variety of industries including paper, baking, brewing, textiles, animal feed, detergents and starch. Laccases are multi-copper enzymes (EC 1.10.3.2) that catalyze the oxidation of a variety of phenolic compounds and are widely distributed among plants, bacteria and fungi, In plants, laccase is involved in lignification. In fungi, laccases may be involved in many cellular processes including delignification, sporulation, pigment production, fruiting body formation, and plant pathogenesis. Many industrial applications for laccases have been proposed including paper processing, prevention of wine decoloration, detoxification of environmental pollutants, oxidation of dye and dye precursors, enzymatic conversion of chemical intermediates and production of chemicals from lignin. Before laccase can be used commercially for any of these applications, an inexpensive source of the enzyme must be available. A laccase from *Coprinus cinereus* had recently been shown to have activity at alkaline pH, and some of the potential applications for laccase will require an enzyme that functions at high pH. We cloned and sequenced three laccase genes (*lcc1*, *lcc2* and *lcc3*) from the ink cap basidiomycete *C. cinereus*. The *lcc1* gene contained seven introns while both *lcc2* and *lcc3* contained 13 introns. The predicted mature proteins (Lcc1-3) are 58% to 80% identical at the amino acid level. The predicted Lcc1 contained a 23 amino acid C-terminal extension that is rich in arginine and lysine, that suggests C-terminal processing may occur during its biosynthesis. We expressed the Lcc1 protein in *Aspergillus oryzae* and purified it. The Lcc1 protein as expressed in *A. oryzae* had an apparent molecular weight of 66 kDa on SDS-PAGE and two absorption maxima at 278 and 614 nm. Based on the N-terminal protein sequence of the laccase, a four-residue propeptide was processed during the maturation of the enzyme. The dioxygen specificity of the laccase showed an apparent K_m of 21 ± 2 μM and a k_{cat} of 200 ± 10 min^{-1} for O_2 with 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) as the reducing substrate at pH 5.5. The yields obtained of Lcc1 from *A. oryzae* will allow further characterization and testing of the enzyme in industrial applications. This is the first report of a basidiomycete laccase whose biosynthesis involves both N-terminal and C-terminal processing.

Scios Research

David Liu
Research Scientist
Scios, Inc.
Sunnyvale, CA

Overview of Scios and its research focus areas with a particular emphasis on the use of functional genomics to identify drug targets that are involved in pathways of intracellular signal transduction. Scios' research efforts will be focused in the coming years on the discovery and development of new chemical entities for the treatment of cardiovascular, renal, inflammatory, and fibrotic diseases.

UCDAVIS Biotechnology Program

Faculty and Fellow Abstracts

OVERVIEW OF RESEARCH:
Molecular Virology/Vaccine Development

Tilahun D. Yilma
Veterinary Medicine-PMI and International Laboratory of Molecular Biology for Tropical Disease Agents (ILMB), University of California, Davis

The mission of the International Laboratory of Molecular Biology (ILMB) is to conduct and coordinate a research program that brings together experts in molecular biology, in order to facilitate work on the pathogenetic mechanisms of human and animal tropical diseases. In addition to study of the molecular biology of diseases agents, the ILMB has specific goals of developing vaccines and rapid diagnostic kits to aid in tropical disease control. The ILMB has been named as a Collaborative Laboratory of the Food and Agriculture Organization of the United Nations, and has taken on additional responsibilities in this regard that are consonant with its major mission. Working within the guidelines of the FAO program to combat hunger in developing nations, the ILMB is broadly concerned with development of biotechnology programs for animal production and animal health, training of scientists from developing countries, transfer of biotechnology information and techniques to these countries, and formation of a network of laboratories dealing with specific biotechnology issues. The ILMB focus on vaccine development is not limited to animal health. The search for the efficacious and safe vaccine for human immunodeficiency virus (HIV) infection is a top priority. Simian immunodeficiency virus (SIV) in macaques is the vest available model for HIV in humans and is being used as a prototype for finding a vaccine.

ENHANCING THE IMMUNE RESPONSE TO RECOMBINANT VACCINIA VIRUS VACCINE

Fatema H. Aziz*, Paulo H. Verardi, Leslie A. Jones, and Tilahun D. Yilma.
International Laboratory of Molecular Biology for Tropical Disease Agents,
University of California, Davis

The long-term objective of this project is to develop safe and efficacious live recombinant vaccines. Using vaccinia virus as a vector, we want to evaluate the effects of expressing cytokine genes in addition to deleting viral immune-modulating genes on vaccine safety and immunogenicity. These optimized recombinant vaccinia virus vaccines (rVVs) are believed to elicit augmented protective immune responses. To investigate this hypothesis, we have constructed rVVs which lack the VV B13R and B22R immune modulating genes and co-express IFN-g or IL-18 as well as an immunogenic heterologous gene, vesicular stomatitis virus glycoprotein G. The VV B13R and B22R genes have sequence similarity to serine protease inhibitors (SERPINs). The B13R gene product inhibits the processing and release of IL-1b and blocks apoptosis; the function of the B22R gene product is still unclear. From our virulence studies, we have determined that both IFN-g expression as well as the insertional inactivation of the VV immune-modulating proteins B13R and B22R attenuate rVVs in both euthymic and athymic mice. Studies into the role of IL-18 as a viral attenuating agent are ongoing. Finally, we are currently testing the immunogenicity of these vaccines in a murine model for systemic disease, vesicular stomatitis virus by conducting humoral response and cell-mediated immune response studies.

STATISTICAL ANALYSIS OF MICROARRAY DATA

David M. Rocke*
Center for Image Processing and Integrated Computing (CPIPIC), University of California, Davis

Microarray technology, including DNA arrays, has the potential to revolutionize the biological sciences. These technologies allow the simultaneous determination of gene expression in thousands of genes from a single sample and related methods allow the determination of thousands of protein affinities or antibody bindings for immunoassays. In spite of the importance of the technology, they are sufficiently new that there has been little work on the statistical aspects of the problem. First, we need to know some fundamental things about measurement error on a gene-by-gene basis. There is little known about this now, even about such basic issues as when an expression measurement is statistically different from noise in an assay. Furthermore, to fully realize the potential of massive microarrays, it will be necessary to analyze the data in a multivariate fashion, using techniques of classification, clustering, and pattern recognition. We will need to use techniques from chemometrics to deal with the high dimensional data. Our group is attacking all of these problems in collaboration with investigators in many areas of the biological sciences.

VARIANCE COMPONENTS IN DNA MICROARRAY DATA

Blythe Durbin* and David M. Rocke
Center for Image Processing and Integrated Computing (CPIPIC), University of California, Davis

DNA microarray data contains a number of different sources of error due to variation in animal test subjects, construction of the slides, and measurement error. In this analysis, we attempt to quantify several of these sources of variation. A mixed model was fit to data from a number of different genes in an experiment involving biomarkers of exposure to a toxic substance (in mice). The model contains a fixed (non-random) component due to changing doses of a toxin and a random component due to variation between animals, between slides, between draws from the sample by the spotting pen, and between spots on the slide. We found differences between slides and between animals to be the most significant source of variation in these data, and variation between draws from the sample to be the least significant. Tests of significance of these components and a new method of identifying outlying observations will also be discussed.

WHAT'S GOING ON IN THE LAB

Bruce D. Hammock
Department of Entomology, University of California, Davis, CA 95616

Research in the lab is comprised of three areas: 1)detection of toxic substances and metabolites through immunoassay, 2)investigation into mechanisms of toxicity and biological function of compounds produced or degraded through activity of epoxide hydrolases and carboxylesterases, and 3) investigation of mechanisms of insect enzymes involved in developmental regulation, and of the utility of these enzymes and insect-selective toxins for control of pest insect populations with the baculovirus expression system. These areas dovetail within the realm of agriculture by taking research through detection of substances in the environment, understanding thresholds at which these substances are harmful and how they exert their toxicity, and finally, in developing biological alternatives for chemical insecticides that may reduce exposure and work effectively with a variety of agricultural pest control measures.

EPOXIDE HYDROLASE, JUVENILE HORMONE ESTERASE, PLANTS AND INSECTS

Tonya Severson,* Virginia Ursin,[#] Jean Kridl,[#] and Bruce Hammock*
*Department of Entomology, University of California, Davis, CA 95616
[#]Calgene, Davis, CA 95616

We are interested in mechanisms of action of two α/β-fold enzymes, epoxide hydrolase (EH) and juvenile hormone esterase (JHE), at biochemical and physiological levels in plants and insects. Both enzymes effect the hydrolysis of their respective substrates. EH action results in hydrolysis of epoxides and formation of a vicinal diol. JHE action results in hydrolysis of the methyl ester moiety of JH to the carboxylic acid which has altered activity.

Epoxide hydrolases have been isolated from multiple organisms including plants, bacteria, fungi, insects, and mammals and are implicated in detoxification of toxic substances and in metabolism of arachidonate and linoleate fatty acid epoxides. We engineered *Arabidopsis thaliana* plants to over and under express the native epoxide hydrolase. These plants will facilitate testing the hypothesis that EH levels affect cuticular hydrocarbon profiles that may in turn be of utility in altering insect feeding behavior, influencing drought tolerance, and influencing fungal infection. Plants have been selected for further analysis based upon elevated and upon low levels of hydrolysis of [^{3}H]-*trans*-1,3-diphenyl propene oxide.

In a separate project, we genetically modified tomato plants (*Lycopersicon esculentum*) to express juvenile hormone esterase and explore the plausibility of targeting development and reproduction of phloem feeders such as whiteflies (*Bemisia tabaci*: Homoptera) for control of pest populations. JH esterase expression in these plants has been confirmed by Northern and Western blotting, and by partition assay of enzymatic activity using [^{3}H]-JH III as substrate.

PLANT – PLANT COMMUNICATION

John Yoder
Department of Vegetable Crops, University of California, Davis

The recognition of host plants by parasitic plants offers a unique opportunity to investigate plant-plant signaling. Phenolic molecules released by host roots trigger the development of haustoria in parasitic plants. Haustoria are globular outgrowths on parasite roots whose functions are to attach the parasite to the host, invade the host root cortex, and establish a vascular continuum through which the parasite robs host resources. The impact to the host plant can be debilitating, and agriculturally parasitic plants are devastating. In Africa, over two thirds of the 73 million hectares cultivated in cereals and legumes are infested with Striga. The FAO estimates that the lives of over 100 million Africans in 25 countries are threatened by crop losses by Striga. In the United States, dwarf mistletoe (Arcethobium) is estimated to destroy up to 3.2 billion board feet of lumber per year in western forests. Understanding the genetic mechanisms governing host recognition and haustorium development should enable us to develop rational strategies for engineering host resistance against parasitic weeds. We are using the parasitic plant Triphysaria to define the genetics of host recognition and haustorium development. Triphysaria is useful for these studies because it is a facultative parasite whose host range includes Arabidopsis, it is a simple diploid amenable to genetic analyses, and, haustorium development can be monitored in vitro. Using geographically defined ecotypes of Triphysaria, we have identified heritable variation in three parasite specific phenotypes; spontaneous hasutorium formation, sibling recognition, and, responsiveness to exogenous phenolic signals. These may represent recognition races for different signal molecules. We are also using suppressive subtraction to identify cDNAs differentially abundant in Triphysaria roots after treatment with haustorial inducing factors. Sequence comparisons are being used to assign tentative functions. Gene expression is being characterized in Triphysaria and the closely related, non-parasite Antirrhinum by in vitro and in situ hybridizations. Multi-array technology is being used to examine the global expression pattern of HIF induced genes in both parasites and non-parasites. A long-term objective is to assign biological functions to cloned genes by combining our genetics and molecular approaches.

GENE EXPRESSION PROFILES OF AN INDUCED DEVELOPMENTAL PATHWAY IN PARASITIC PLANT ROOTS

Manuel J. Torres* and John I. Yoder
Department of Vegetable Crops, University of California, Davis, CA, 95616

The focus of investigations being conducted by the Yoder Lab is aimed at clarifying mechanisms of plant-plant interactions in the rhizosphere. We believe that subterranean interactions between plants are a common adaptation in the natural environment and can be characterized as self-defense mechanisms, symbiotic associations and in our experimental model, parasitism. The defining feature of root parasitic plants is the ability to form haustoria in response to host-plant recognition signals secreted as root exudates in the rhizosphere. We have developed an in vitro method for inducing haustoria formation using DMBQ (2,6-p-dimethoxy-benzoquinone). We have constructed a cDNA library enriched for differentially abundant early-DMBQ-induced (EDI) transcripts from root-tissue mRNA subtracted against a water treated control tissue using PCR-based SSH, suppression subtractive hybridization. We intend to array this cDNA library onto glass slides using a robotic-arrayer and, where possible, by clusters of putative functional categories. The specific aims of this research project are: 1.) Interrogate an array containing transcript probes derived from an SSH cDNA library enriched for DMBQ induced transcripts with target elements prepared from DMBQ-induced root tissue mRNA within hours of exposure, 2.) Monitor transcriptional differences over time, between two closely related genera the parasite *Triphysaria,* and the non-parasite, *Lindenbergia*; and following treatment with a known haustorium inducing factor and structurally related non-inducing analogs, 3.) Analyze expression data using cluster analysis software and, 4.) Create a searchable electronic record or database with the EDI transcript collection and data generated in gene expression profile experiments. The main objective of this investigation is to identify clusters of coordinately expressed genes and combine this information with putative functional assignments of the transcript collection to construct a pathway or metabolic network of interacting genes expressed during early haustorium formation.

THE MOLECULAR AND GENETIC BASIS OF HAUSTORIUM DEVELOPMENT IN THE PARASITIC PLANT, *TRIPHYSARIA*

Denneal Jamison*, Angela Wojtowicz and John Yoder*
Department of Vegetable Crops, University of California, Davis

Root-parasitic plants in the Scrophulariaceae recognize common secondary metabolites in the rhizosphere in order to direct developmental events critical to the parasitic lifestyle. Exuded by the roots of host plants, these signal molecules trigger the formation of the haustorium. This parasitic organ attaches and penetrates the host root, forming a vascular continuum and facilitating the transfer of minerals, nutrients and water from the host to the parasite.

Triphysaria is an annual, root-parasitic angiosperm belonging to the Scrophulariaceae. Using an *in vitro* assay, haustorium development can be readily induced and monitored in *Triphysaria* root tips. Induction occurs in response to the presence of a host root and the direct application of host root exudates. Specific haustorium inducing factors (HIF's) have been identified in analyses of host root exudates including phenolic acids, quinones and anthocyanidins.

DMBQ, 2,6-dimethoxy-benzoquinone, is a commonly found HIF that has been used to induce haustorium development in root-parasitic species. Differential recognition of DMBQ has been observed among three species of *Triphysaria.* Genetic analyses of the DMBQ recognition trait in defined *T. pusilla* families and in F1 progeny generated between inducing and non-inducing species have shown that DMBQ recognition is a heritable phenotype.

Using a subtractive PCR method, the molecular basis of DMBQ recognition has been investigated in the lab and approximately 200 DMBQ-induced transcripts have been isolated, sequenced and assigned putative functions. Of these transcripts, there are two quinone NAD(P)H oxidoreductases, TvQR1 and TvQR2, encoding proteins that may interact directly with DMBQ. Northern analyses have been performed using TvQR1 and TvQR2 as probes on root tip RNA isolated from *Triphysaria* species that differentially recognize DMBQ. Differential induction of these transcripts appears to be correlated with the observed phenotypes. Further characterization of the transcription of these genes *Triphysaria* species will be carried out using genetically defined *T. pusilla* families.

OVERVIEW OF RESEARCH: Nuclear Hormone Receptors

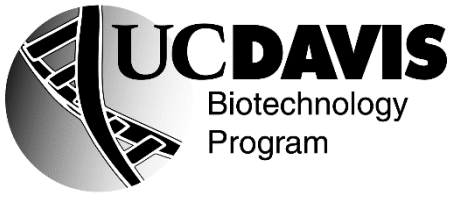
Martin Privalsky
Section of Microbiology, Division of Biological sciences, University of California, Davis

Our general interest is in the mechanisms by which proliferation and differentiation is regulated in normal cells, and in the abnormal processes that occur in neoplasia. Our specific goal is a better understanding of the actions of the nuclear hormone receptors in normal cells and in oncogenesis. Nuclear hormone receptors are a family of ligand-regulated transcription factors, and include the steroid, retinoid, and thyroid hormone receptors. These receptors directly modulate gene expression in response to hormones of extracellular origin, and play critical roles in metazoan homeostasis, differentiation, and reporduction. In addition, aberrant nuclear hormone receptors have been implicated as causal or contributory factors in a variety of human an dhon-human cancers. My research seeks to exploit these aberrant oncogenic receptors, both to better elucidate the actions of their normal counterparts, and to determine the molecular pathways that operate in neoplasia.

ANTI-REPRESSION BY THYROID HORMONE RECEPTORS

Zhihong Yang* and Martin Privalsky
Section of Microbiology, Division of Biological Sciences
University of California Davis 95616

Thyroid hormone receptors (T3Rs) belong to the nuclear hormone receptor superfamily and function as hormone-regulated transcription factors. Different T3R isoforms are expressed in tissue-specific and developmentally-regulated manner. The T3Rα-1, β-0, and β-1 isoforms exhibit bimodal transcriptional regulation, typically repressing target gene expression in the absence of hormone and activating transcription in the presence of hormone. Intriguingly, however, the T3Rβ-2 isoform fails to repress, and instead is able to activate transcription in both the absence and presence of hormone. We wished to understand the molecular mechanism behind this absence of repression by T3Rβ-2. Repression by T3Rα-1, β-0, and β-1 is mediated by the ability of these isoforms to physically recruit a SMRT/N-CoR corepressor complex. We report here that the unliganded T3Rβ-2 also recruits the SMRT corepressor; in contrast to the α-1, β-0, and β-1 isoforms, however, the T3Rβ-2 protein interacts not only with the C-terminal "receptor-interaction domain" of SMRT, but also mediates additional contacts with the N-terminal "silencing domain" of the SMRT corepressor. These additional T3Rβ-2-specific contacts block the subsequent association of SMRT with mSin3, a crucial second subunit of the corepressor holo-complex. We propose that T3Rβ-2 regulates transcription through a novel anti-repression mechanism, recruiting SMRT in a fashion similar to that of the other T3R isoforms, but employing additional inhibitory contacts with SMRT to abort the subsequent formation of a functional corepressor complex.



Poster Abstracts

A. GENE CLONING AND MOLECUALR CHARACTERIZATION OF EXPANSINS IN GERMINATING TOMATO SEEDS

Feng Chen* and Kent J. Bradford
Department of Vegetable Crops, University of California,
Davis, CA, 95616

For seeds whose embryos are embedded in a rigid endosperm, germination is controlled by two critical factors: the weakening of the endosperm tissue enclosing the radicle tip (endosperm cap) and the elongation of radicle. These two events are related to cell wall modification. We are interested in identifying factors that might be essential for these two events. Expansins are novel cell wall proteins that are proposed to be involving in both cell wall elongation and cell wall disassembly, even though their biochemical mechanism is unknown. Based on the conserved regions of known expansin amino acid sequences, degenerate primers were designed and used to amplify expansin genes from a tomato seed cDNA library constructed from mRNA from GA-treated *gib-1* mutant tomato (*Lycopersicon esculentum* Mill.) seeds. The PCR products were used to screen the cDNA library and 6 full-length expansin cDNAs were obtained. These six cDNAs share 60-80% sequence identity. By using gene-specific probes, RNA gel blot analysis showed that during seed germination, strong signal can be detected for *LeEXP4*, *LeEXP8* and *LeEXP10*, but no signal or very weak signal can be detected for *LeEXP1*, *LeEXP2* and *LeEXP9*. RNA gel blot analysis showed that LeEXP4 was specifically localized to endosperm cap region. In gibberellin (GA)-deficient (*gib-1* mutant) seeds, which germinate only in the presence of exogenous GA, GA induces the expression of *LeEXP4* within 12 h of imbibition. When *gib-1* seeds were imbibed in GA solution combined with 100 μM abscisic acid (ABA), the expression of *LeEXP4* was not reduced although radicle emergence was inhibited. In wild-type seeds, *LeEXP4* mRNA accumulation was blocked by far-red light and decreased by low water potential but was not affected by ABA. The presence of *LeEXP4* mRNA during seed germination parallels endosperm cap weakening determined by puncture force analysis. We hypothesize that *LeEXP4* is involved in the regulation of seed germination by contributing to cell wall disassembly associated with endosperm cap weakening. Thus, expression of *LeExp4* is correlated with conditions conducive to tomato seed germination, specifically to endosperm cap weakening. By doing tissue prints and anatomical analysis, *LeEXP8* mRNA was localized to the cortex of the radicle, the tissue that is responsible for embryo elongation during seed germination. Further immunohistochemical and transgenic analyses will elucidate more about the physiological roles of expansins in seed germination. Supported by NSF grant IBN-9722978 to K.J.B.

References:

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2) Cosgrove D. J. Creeping walls, softening fruit, and penetrating pollen tubes: the growing roles of expansins. Proc. Natl. Acad Sci USA 1997, 94: 5504-5505.

3) Rose J. K.; Lee H. H.; and Bennett A. B. Expression of a divergent expansin gene is fruit specific and ripening-regulated. Proc Natl Acad Sci USA 1997, 94: 5955-5960

B. REPAIR OF UV-INDUCED DNA DAMAGE IN ARABIDOPSIS

Fidantsef, A. L.*, Eads J. R., Estrada, A. K., Lei S., and Britt, A. B.
Section of Plant Biology, University of California, Davis, CA, 95616

Our laboratory is interested in studying repair and tolerance mechanisms of UV-induced DNA damage using *Arabidopsis thaliana* as a model system. In plants, UV-induced pyrimidine dimers are repaired by two pathways, a light-dependent and a light-independent pathway. We have already isolated and characterized the genes required for photoreactivation. However, the molecular mechanism for the "dark repair" pathway remains to be determined. To accomplish this objective, we are currently screening T-DNA tagged lines for mutants defective in the genes involved in the dark repair of UV-induced DNA damage products using two different approaches. The first approach consists of a classical mutant screening, where T-DNA tagged line seedlings are irradiated with UV-B and putative mutants are selected based on a characteristic UV-sensitive phenotype. The second approach consists of a reverse "molecular" genetic approach. This approach uses the sequence information available for dark repair genes that have been cloned from yeast and humans. Homologous sequences can be found in the Arabidopsis genome database. We can then use PCR to screen for insertions in these homologs and check their function. Also, we have been using these homologs to complement our existing collection of dark repair mutants. Three NER sequences analyzed complemented three of our dark repair mutants, indicating that, indeed, NER occurs in plants.

C. ANALYSIS OF DNA-MICROARRAY DATA USING PARTIAL LEAST SQUARES

Danh Nguyen
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Data arising from DNA-microarrays present some interesting statistical analysis problems. We review some basic statistical methodologies for prediction and classification/discrimination using DNA-microarray data, particularly using the partial least squares (PLS) method. Examples of some applications using real data and will be provided.

D. DNA MICROARRAY ANALYSIS OF THE RESPONSE OF CYANOBACTERIA TO UV-B IRRADIATION

Lixuan Huang, Mike McCluskey, Melissa Jia, Zhixiong Xue, Bob LaRossa
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The UV-B (280-320 nm) component of sunlight has significant detrimental effects on biological systems ranging from bacteria to plants and humans. The main targets of UV-B irradiation are tRNA, proteins, lipids, and especially photosystems in photosynthetic organisms. We have used the cyanobacterium *Synechocystis* PCC sp.6803 as a model organism to study the response of cells to UV-B radiation. We constructed a DNA microarray consisting of a near complete collection of all 3168 ORFs of *Synechocystis* sp. PCC 6803. Synechocystis cells were exposed to a moderate level of UV-B light (20 mES^{-1} m^{-2}) for 20 minutes, and the resultant gene expression profile was analyzed. The microarray-derived data was quantified using Array Vision (Molecular Dynamics) and interpreted with Genespring (Silicon genetics). *psbA*, which encodes photosystem II D1 protein, is a major target of UV-B damage. Consistent with literature data, *psbA* was among the genes most induced by the UV-B treatment. There is a significant decrease in the biosynthesis of phycobilisome and chlorophyll associated mRNA, suggesting an overall lessening of light harvesting and photosynthesis. In conclusion, DNA microarray analysis has provided us with a global picture of how cyanobacteria copes with UV-B irradiation, a major environmental stress factor for photosynthetic organisms. The same technology can define the response of cyanobacteria to other stress factors and provide further insights into the global gene regulatory networks.

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Designated Emphasis in Biotechnology Program

Description

The Designated Emphasis in Biotechnology (DEB) is an inter-graduate group program that allows Ph.D. students to receive and be credited for training in the area of biotechnology. The purpose is to provide graduate students an opportunity to explore biotechnology through seminars and courses and examine the relationship of academia to industry through internships. This program supplements a student's Ph.D. curriculum and those completing the DEB program will obtain an official designation on their transcript indicating a qualification in biotechnology.

Students interested in participating in DEB must be enrolled in the Ph.D. program of one of the participating graduate groups. Participating graduate programs currently include Genetics, Nutrition, Plant Biology, Biochemistry and Molecular Biology, Physiology, Microbiology, and Chemical Engineering and Material Sciences. In addition to the specific graduate group requirements, students wishing to obtain DEB must enroll in MCB 263 - Biotechnology Fundamentals and Application, and MCB 294/ECH 294 - Current Progress in Biotechnology Seminar. In addition, students must complete a minimum of three months internship at a participating biotechnology company. This last requirement should be particularly appealing to students interested in working for such a company after graduation.

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