FIFTH ANNUAL
BIOTECHNOLOGY
TRAINING GRANT RETREAT
NAPA VALLEY
APRIL 13, 1996

PROGRAM OVERVIEW

8:45am	Introduction by Roy Doi, Director of the Training Grant
9:00 - 11:30am	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. (Calgene, Chiron, Genentech, Novo Nordisk Biotech, Roche Bioscience)
11:30 - 2:30pm	Poster Session & Lunch: Posters will be presented by new trainees, trainers and other students from trainer laboratories. There will also be opportunity for the trainees, first year 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.
2:30 - 5:30pm	Each of six faculty trainers and their present trainees will present an overview of their research projects, beginning with a short overview of the lab focus by the trainer (5 mins) and continuing with a more detailed description by the trainee (15 mins) of his/her research and ending with five minutes for questions.
5:30pm	Close by Roy Doi.

COMPLETE PROGRAM

8:30	Registration	
8:45	Introduction	- Roy H. Doi, Molecular and Cellular Biology, Training Grant Director

COMPANY PRESENTATIONS:

CHAIR: Roy Doi, Molecular and Cellular Biology

9:00	The future of CaIgene research and product development	- Rick Stonard, Chief Technical Officer, Caigene Inc.
9:30	Chiron, a diversified biotechnology company Chemical Therapeutics Group,	- Steven Rosenberg, Research Director, Chiron Technologies
10:00	BREAK	
10:10	Research and development at Genentech	- Steven Chamow Senior Scientist, Genentech
10:40	Overview of enzymes in commercial processes	- Alan V. Klotz, Department of Protein Chemistry, Novo Nordisk Biotech
11:10	Overview of research & development at Roche Biosciences	- Jim Barnett, Roche Bioscience
11:40	Posters	
12:00	LUNCH, DISCUSSION & POSTERS	

TRAINEE AND TRAINER LAB PRESENTATIONS:

Chair: George Bruening, Plant Pathology/CEPRAP

2:30	Cellular regulation of bovine leukemia virus (BLV) replication	- Kathryn Radke, Avian Sciences
	Host cell state and BLV expression	- Ed Fulton, Avian Sciences

2:55	Overview of research	- Alan Bennett Vegetable Crops
	Cell wall disassembly in ripening melon fruit	- Kristen A. Hadfield Vegetable Crops
3:20	Pursuing emerging protoza	- Pat Conrad Vet Med: Pathology, Microbiology and Immunology
	Role of cytokines in pathogenesis of human Babesia isolates in mice	- Ruth Hemmer Vet Med: Pathology, Microbioloby and Immunology
3:45	BREAK	

TRAINEE AND TRAINER LAB PRESENTATIONS:

Chair: Kathryn Radke, Avian Sciences

3:55	Overview of research	- Dewey Ryu, Chemical Engineering
	Development of a novel bioreactor for high value phospholipic synthesis	- Rajesh Krishnan, Chemical Engineering
4:20	Overview of research	- Marty Privalsky, Microbiology
	Dissection of tetramer formation by X receptors: a novel form of DNA recognition by nuclear hormone receptors	- Ben Lin, Microbiology
4:45	Extraction and controlled release technologies using micelles and microemulsions	- Stephanie Dungan, Chemical Engineering
	Micellar properties of quillaja saponin	- Shuman Mitra, Chemical Engineering
5:10	Conclusion	- Roy H. Doi, Training Grant Director
5:30	Bus departs	

POSTER TITLES

A. GENERATION OF A SOLUBLE RECEPTOR FROM AN IMMUNOADHESIN BY SPECIFIC CLEAVAGE: THE EXAMPLE OF INTERFERON γ RECEPTOR
Steven Chamow
Genentech

B. ORGANIZATION AND LINKAGE OF THE CHICKEN SARCOMERIC MYOSIN HEAVY CHAIN MULTIGENE FAMILY
I. Analysis of 5 fast and 4 slow cardiac genes expressed in skeletal muscles.
Q. Chen*, L.A.Moore, Q. Zhang, and E. Bandman
Food Science and Technology
University of California, Davis

C. CHARACTERIZATION OF MUTANTS IN MID TO LATE STAGE *ARABIDOPSIS THALIANA* OVULE INTEGUMENT DEVELOPMENT
Jacinto M. Villanueva and Charles Gasser.
Molecular and Cellular Biology
University of California, Davis

D. PHENOTYPIC CHARACTERIZATION OF AN ALLELIC SERIES OF *DRAGON* MUTANTS REVEALS THAT THIS LOCUS IS ESSENTIAL FOR EARLY STAGES OF OVULE DEVELOPMENT
Shawn C. Baker, Kay Robinson-Beers, and Charles S. Gasser
Molecular and Cellular Biology
University of California, Davis

E. CHARACTERIZATION OF PROTEINS SECRETED BY *TRICHOSANTHES KIRILOWII* PLANT CELL CULTURES
Remi Shih, Karen McDonald and Alan Jackman
Department of Chemical Engineering and Materials Science
University of California, Davis

F. THE DEVELOPMENT OF A NOVEL BIOREACTOR SYSTEM FOR PRODUCTION OF HIGH VALUE PHOSPHOLIPIDS
Rajesh Krishnan
Department of Chemical Engineering and Materials Science
University of California, Davis

G. ENZYMATIC HYDROLYSIS OF PRETREATED RICE STRAW
Elena Yu. Vlasenko, Hanshu Ding, John M. Labavitch, and Sharon P. Shoemaker
Food Science and Technology
University of California, Davis

H. CALIFORNIA INSTITUTE OF FOOD AND AGRICULTURAL RESEARCH
Sharon Shoemaker, Director - CIFAR

ORAL
ABSTRACTS

Rick Stonard
Chief Technical Officer
Calgene Inc.

Plant biotechnology is entering into a new phase of its development. For the past 15 years this field has resided in the realm of science. In the mid-1990's it finally entered the realm of commerce. Products ranging from insect resistant cotton to modified canola oils to tomatoes which have better improved flavor are being sold to farmers and consumers. Calgene too is evolving rapidly with a new corporate partnership and a significant repositioning of its produce research program. This presentation will summarize briefly the industries progress in commercializing GE plants with focus on Calgene's activities. Secondly, a status report on Calgene's research programs in oils, fiber and produce will be provided.

CHIRON: A DIVERSIFIED BIOPHARMACEUTICAL COMPANY

Steve Rosenberg
Research Director,
Chemical Therapeutics Group
Chiron

Chiron is a diversified biopharmaceutical company with businesses in vaccines, diagnostics, and therapeutics, with a focus on infectious diseases, oncology, critical care, and ophthalmology. 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. 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.

RESEARCH AND DEVELOPMENT AT GENENTECH

Steven Chamow
Senior Scientist
Genentech

Genentech, Inc. is a pioneer biotechnology company using human genetic information to discover, develop, manufacture and market pharmaceuticals that address significant unmet medical needs. Genentech commits itself to high standards of behavior in contributing to the best interests of patients and the medical profession, and makes its products available to all patients who need them, regardless of their ability to pay. The company has headquarters in South San Francisco and is traded on the New York and Pacific Stock exchanges under the symbol GNE.

Genentech's most important asset is its science. The company's research has led to ten - almost half - of biotechnology-based products on the market today. Genentech is the only biotechnology company to have taken five of its own products from the laboratory to the marketplace

Science at Genentech focuses on six areas of medicine: Cardiovascular/pulmonary, cancer, immunology/allergy, endocrinology, neuroscience and blood cell formation. Nurturing quality research through a commitment to basic science, the company encourages scientists to pursue their own interests with time for discretionary research.

Genentech manufactures five products and markets them in the United States: Protropin® (somatrem for injection) human growth hormone for treating children with growth hormone inadequacy (GHI); Nutropin® (somatropin for injection) human growth hormone also for treating GHI in children and for treating growth failure in children due to chronic renal insufficiency (Nutropin also comes in a liquid version, Nutropin AQ™); Activase® (Alteplase, recombinant) tissue-plasmogen activator to dissolve blood clots in the arteries of patients with acute myocardial infarction (heart attack) and patients with acute massive pulmonary embolism (blood clots in the lungs); Actimmune® (interferon gamma-1b) for treating chronic granulomatous disease, a rare, inherited deficiency of the immune system; and Pulmozyme® (domase alfa) DNase, the first new therapeutic approach for cystic fibrosis in 30 years.

Some other potential products Genentech is investigating in clinical trials are the HER2 humanized monoclonal antibody, a potential treatment for severe cases of breast and ovarian cancer; and insulin-like growth factor (IGF-1) as a possible treatment for diabetes. Other indications targeted by the many new products in Genentech's pipeline include: other cancers, various side effects of cancer chemotherapy, asthma and a type of acute renal failure.

Genentech is also evaluating three of its marketed products for new indications: Activase for ischemic stroke, for which the company is awaiting regulatory approval; Nutropin for growth

hormone inadequacy in adults and for short stature associated with Turner's Syndrome; and Actimmune for metastatic renal cell carcinoma (kidney cancer)

Genentech and Roche are collaborating on several R&D projects to maximize complementary scientific strengths. These collaborations utilize advanced technology to develop potential new pharmaceuticals for untreated medical conditions.

Genentech also has R&D agreements with several biotechnology companies studying related technologies. An example of these is its relationship with IDEC Pharmaceuticals to develop a monoclonal antibody, IDEC-C2B8, currently in Phase III trials for non-Hodgkin's B-cell lymphoma. Genentech continues to seek out and develop strategic alliances with other companies when a synergistic fit exists.

OVERVIEW OF ENZYMES IN COMMERCIAL PROCESSES

Alan V. Klotz
Department of Protein Chemistry
Novo Nordisk Biotech

Enzymes are used as industrial catalysts to substitute for conventional chemical reagents in a growing number of commercially significant processes. This presentation outline the opportunities for enzyme applications in commercial processes featuring the enzymes advantages of modest temperature, intermediate pH, and minimal side reactions. Specific examples will be discussed in the detergent, baking and animal feed industry sectors to describe how enzymes can function as environmentally-friendly substitutes for harsh traditional chemicals thereby changing the ways in which people live and work.

OVERVIEW OF RESEARCH AND DEVELOPMENT AT ROCHE BIOSCIENCES

Jim Barnett
Roche Bioscience

Roche Bioscience is a research-driven, product focused company located in Palo Alto, California, in the heart of Silicon Valley. Formed following the acquisition of Syntex by global pharmaceutical giant Roche Holding Ltd, Roche Bioscience is one of the newest enterprises in human pharmaceutical research on the West Coast. Our research is focused on the discovery of innovative pharmaceutical products to treat neurobiological disorders and inflammatory diseases. We are currently working on new drugs for diseases including arthritis and osteoporosis as well as pain and urinary tract disorders.

Our mission is to discover innovative pharmaceutical products that significantly improve the quality of life for people throughout the world. In the Neurobiology Unit, which has a strong pharmacology base, we are focused primarily on peripheral nervous system disorders including prostate disease, incontinence and pain. Employees in our Inflammatory Diseases unit have expertise in cell biology and endocrinology. They are working on advanced compounds to treat rheumatoid arthritis and other inflammatory conditions as well as osteoporosis and osteoarthritis.

Headquartered in Basel, Switzerland, the international Roche Group is a world leader in research-based healthcare with activities in pharmaceuticals, diagnostics, vitamins, and fine chemicals and fragrances and flavors. Roche has a long tradition of innovative breakthroughs in drug development and is a pioneer in pharmaceutical and other applications of gene technology. Founded in 1896, Roche today has one of the largest market capitalizations in the world. In 1995, Roche's global sales were 14.7 billion Swiss francs. We believe that the Roche Bioscience formula for success is a powerful one: an innovative approach, a heritage of scientific excellence, a premier geographic location
and the financial backing of Roche.

Palo Alto was home to Syntex Corporation for many years. Established in 1944, Syntex was best known for innovation in the synthesis of steroidal and nonsteroidal compounds. Syntex scientists were world leaders in the research and development of corticosteroids to treat dermatological conditions and for the synthesis of compounds leading to the development of oral contraceptives. Subsequently, Syntex research in pain and inflammation research led to the discovery of nonsteroidal anti-inflammatory drugs (NSAIDs) including Naprosyn® 174; (naproxen), which became one of the top selling drugs in that class with sales in excess of one billion dollars annually. ALEVE® 174;, a non-prescription form of Naprosyn, was introduced in the United States in 1994 and quickly became one of the best-selling over-the-counter NSAIDs. Prescription products discovered by Syntex are now marketed by Roche entities worldwide.

CELLULAR REGULATION OF BOVINE LEUKEMIA VIRUS (BLV) REPLICATION

Kathryn Radke
Department of Avian Sciences
University of California, Davis

Our research focuses on how retroviral gene expression alters host cell function and on how host cells regulate viral gene expression. Bovine leukemia virus (BLV) is closely related to human T-cell leukemia viruses-1 and -2, and is an important model for learning how retroviral gene expression is regulated in cells that host silent infections *in vivo*. Knowing how viral latency is maintained and then is broken is necessary to understand how these viruses spread within the host and how they induce cancers. We investigate the regulation of viral gene expression and the production of viral particles using peripheral blood mononuclear cells with silent infections - the characteristic state of most infected cells in the infected individual. The stimuli encountered by host lymphocytes when participating in immune responses are thought to be important regulators of viral gene expression. Since B lymphocytes are the predominant host cells for BLV *in vivo*, signal transduction pathways specific to B cells are likely to play key roles in viral expression. Our long-term goal is to understand how the state of cellular activation supports BLV replication, expression, and virus production. Peripheral blood mononuclear cells from sheep in known stages of infection are manipulated in culture to elicit BLV expression. The genetic basis for altered envelope protein function of a natural BLV variant is being determined and the signal-transducing capacity of the BLV envelope protein complex is being explored. The research Ed will discuss is directed toward determining whether cells already activated *in vivo* preferentially support BLV expression in culture and whether BLV infection of the host animal alters the activation state of both infected and uninfected B cells.

HOST CELL STATE AND BLV EXPRESSION

B. Edward Fulton Jr.* and Kathryn Radke
Microbiology Graduate Group* and Department of Avian Sciences,
University of California, Davis

Bovine leukemia virus (BLV), an oncogenic retrovirus related to the human T-cell leukemia viruses (HTLVs), infects cattle and by experimental injection can infect sheep. BLV and HTLV have the unique feature of appearing to be silent in host lymphocytes *in vivo*, where little or no viral mRNA or protein is expressed. We are trying to determine how BLV infection is maintained in this seemingly dormant state and what conditions favor its expression. Therefore, we assessed the mitotic cell cycle state and lymphocyte lineage of host cells to test the hypothesis that activated B-lymphocytes preferentially support virus expression. In experiments performed by pulse-labeling PBMCs (peripheral blood mononuclear cells) from BLV-infected sheep with 5-bromo-2'-deoxyuridine (BrdU) via direct injection, PBMCs that had recently traversed S phase did not show greater expression of BLV protein in culture than non-cycling cells, and B-cells were not more likely than other mononuclear cells to have cycled through S phase *in vivo*. When BrdU pulses were performed in culture with addition of lipopolysaccharide (LPS), a polyclonal activator of B cells, the number of cells that entered S phase increased as did the expression of the BLV capsid protein (CA). The additional cells that entered S phase were largely B-cells with immunoglobulin M (IgM) expressed on the cell surface. However, only a few of the additional cells that entered S phase expressed BLV CA protein. Treatment with LPS therefore activates viral expression in cells from BLV-infected sheep, and also increases the early proliferation of B cells, many of which may be uninfected. BLV may be maintained in non-cycling B-cells, and viral spread may be limited to episodic periods of stimulation of host cells by foreign antigens. Further analysis is planned which will use flow cytometric techniques to look at three parameters simultaneously.

OVERVIEW OF RESEARCH

Alan Bennett
Vegetable Crops
University of California, Davis

Current research interests focus on the molecular mechanisms underlying changes in plant cell wall architecture. Plants are structurally defined by their cell wall and developmentally-related changes in cell form require disassembly and rearrangement of the cell wall polymers. Endo-b-1, 4-glucanases have been implicated in plant cell wall disassembly and we have been examining their specific role by isolating the gene family from tomato, assessing their patterns of expression and producing transgenic plants with altered gene expression in order to assess their physiological function. In general terms, the ability to relatively easily modulate endo-b-1, 4-glucanase levels by expression of sense and antisense genes provides a useful means to address the function of individual members of a divergent gene family.

Related research focuses on the interaction between the plant cell wall and its oligomeric disassembly products with plant pathogens. Ectopic expression of a transgene encoding a cell wall hydrolytic enzyme in a mutant tomato plant has demonstrated that cell wall fragments can induce pathogen virulence. In addition, we have isolated a plant gene that encodes a proteinaceous inhibitor of fungal pectin hydrolases, indicating the presence of a complex interplay between pathogen-derived cell wall hydrolases and plant proteins that modulate these activities. Preliminary observations indicate that overexpression of a plant-derived inhibitor of fungal pectin hydrolases decreases fungal susceptibility of transgenic plants, suggesting that it may be possible to modify these interactions to enhance plant pathogen resistance.

CELL WALL DISASSEMBLY IN RIPENING MELON FRUIT

Kristen A. Hadfield*, Jocelyn K.C. Rose and Alan B. Bennett
Department of Vegetable Crops
University of California, Davis

Cell wall disassembly underlies a number of developmental and physiological processes in plants including cell expansion, organ abscission, pathogen resistance/susceptibility, fruit ripening and fruit softening. Our laboratory has shown that genes encoding a number of cell wall localized enzymes are differentially expressed in tissues where cell wall modifications are taking place and that altering the level of expression of some of these genes in transgenic plants can have an effect on one or more physiological processes. We are currently characterizing the regulation of cell wall mediated fruit softening in Charentais melons which provide an excellent model system due to the rapid and extensive softening that accompanies ripening. Cell wall analysis has revealed that both the non-cellulosic hemicellulose and pectin components of the cell wall are extensively modified during fruit softening. Therefore, we have targeted enzymes whose activities are predicted to mediate these modifications for further study. Xyloglucan endotransglycosylase (XET) is a hemicellulose modifying enzyme that has been proposed to play a role in cell expansion and fruit softening. XET activity has been detected in softening melon fruit and two distinct cDNAs encoding XET have been cloned and each is expressed in expanding and softening melon fruit. The role of XET in fruit softening and its potential cooperativity with other hemicellulose modifying enzymes in currently being investigated. Ripening-associated pectin metabolism indicative of polygalacturonase (PG) activity is present in melon and we have identified a large multi-gene family that encodes PG. Three members are expressed at the mRNA level in softening melon fruit and the expression of three other members has been detected in other tissues. The activity of PG in melon is undetectable, however, and we intend to resolve these potentially conflicting results by overexpressing the three ripening-regulated clones in *Aspergillus* sp. and functionally analyzing their gene products *in vitro*. The overexpression experiments will be done in collaboration with researchers at Novo Nordisk Biotech.

PURSUING EMERGING PROTOZA

Patricia Conrad
Vet Med: Pathology, Microbiology and Immunology
University of California, Davis

Research efforts focus on protozoal parasites of veterinary and human health importance, specifically *Babesia, Toxoplasma, Neospora and Cryptosporidium*. We have identified what appears to be a new species of human *Babesia* and are currently studying the molecular pathogenesis and epidemiology of this parasite. This work involves in vitro cultivation, as well as antigenic and genotypic characterization of new *babesial* isolates from humans and wildlife. In addition, we have a significant colloborative research program on *Neospora*, a newly recognized and important cause of abortion in cattle which we have also shown to be infective for primates. Our *Neospora* research (CMI) in cattle and primates, parasite biology, development of serologic and molecular diagnostic assays and evaluation of potential vaccines. *Cryptosporidium* research is currently focused on the molecular characterization of isolates.

ROLE OF CYTOKINES IN PATHOGENESIS OF HUMAN BABESIA ISOLATES IN MICE

Ruth Hemmer
Vet Med: Pathology, Microbiology and Immunology
University of California, Davis

Babesia is a tick-transmitted parasite which infects erythrocytes and induces hemolytic anemia. Human babesiosis is emerging as an important public health problem with increasing reports of clinical and even fatal cases. In the U.S. human babesiosis is caused by at least two different parasites. The most common agent is *B. microti*, a parasite that is endemic in the northeast and midwest. The other is a newly recognized species, WA1, which has been transmitted by ticks and blood transfusions to patients in Washington and California. While most *B. microti* infections are mild or asymptomatic, infection with WA1 and related parasites have required hospitalization and treatment. The striking differences in the pathogenesis of these two *Babesia* species in rodent models provides a unique system to examine the inflammatory response. In our study in hamsters it was found that infection with either isolate resulted in 50-70% parasitized erythrocytes. However, WA1 infections were fatal, while *B. microti* infections were always resolved. Upon histopathological analysis of tissues, it was observed that WA1-infected animals showed pulmonary edema, vasculitis (particularly in the lungs and heart), and margination and infiltration of leukocytes into the interstitium of the lungs. These lesions were not seen in *B. microti*-infected animals. This study is designed to test the hypothesis that an overproduction of inflammatory mediators is contributing to the death of WA1-infected animals. The kinetics of cytokine production, (specifically TNF-a, IFN-g IL-12, IL-10, IL-4 and nitric oxide synthase) were measured during WA1 and *B. microti* infections in rodents using RT-PCR and flow cytometry. WA1-infected mice showed increased production of TNF-a, IFN-g and nitric oxide synthase. Analysis of the results of *B. microti*-infected mice are in progress. These results will identify inflammatory mediators central to babesiosis, and will aid in assessing the importance of these factors in producing or limiting inflammation.

OVERVIEW OF RESEARCH

Dewey Ryu
Chemical Engineering
University of California, Davis

Major research interests are in the area of biochemical and biomolecular process engineering dealing with genetically engineered recombinants, biologically active molecules, enzymes , and other gene products. Specific examples of current research programs include:

1) Biomolecular process engineering and recombinant bioprocess technology: Improvement of gene expression and secretion with a focus on efficient transcription and post-translational protein processing (i.e., vector system design, analysis of promoter regulation, protein folding, kinetics of peptide assembly). Genetic and metabolic flux analyses of recombinant bioprocess systems for optimal design and process improvement based on quantitative physiology and metabolic regulation.

2) Biotransformation and enzymatic synthesis of biologically active compounds, antibodies, amino acids, fine chemicals, pharmaceuticals, and agrochemicals of economic importance based on biocatalysis using enzymes and whole cells

3) Cell cultivation technology and optimization of staged (i.e., two-stage) continuous or fed batch bioreactor systems as applied to cultivation of recombinant cells including immobilized cells and enzymes, microorganisms, and plant and animal cell lines for production of gene products and secondary metabolites of medical and economic importance. Also, design and optimization of novel bioreactor systems for plant, animal and insect cell cultivation.

4) Bioseparation of biologically active high value gene products in low concentration using immunoaffinity purification methods with a focus on interactions between the monoclonal antibody and antigen, ligand matrix, their binding kinetics, distributed equilibrium parameter, elution techniques, and scale-up problems.

SYNTHESIS OF GLYCEROL-3-PHOSPHATE (AND OTHER HIGH VALUE PHOSPHOLIPIDS) COUPLED WITH ATP REGENERATION

Rajesh Krishnan,
Chemical Engineering and Materials Science
University of California, Davis

The development of a novel bioreactor for the enzymatic synthesis of high value phospholipids was investigated. Using glycerol-3-phosphate (G3P), the primary phospholipid precursor, as a model product, we are currently developing two bioreactor systems, based on enzymatic synthesis and metabolic extension of phospholipid pathways in yeast, to accomplish our goal. Enzymatic synthesis of the G3P will be coupled with co-factor (ATP) regeneration , in an immobilized enzyme system, based on enzyme retention on one side of a semi-permeable membrane (hollow fiber) across which substrates and products may diffuse. The hollow fiber membrane system was selected since it will allow for retention and preservation of the native enzymes in the reactor, without requiring surface immobilization or gel entrapment, which would compromise the overall enzyme activity and loading requirements. From a series of batch reactor studies, I have measured the kinetic parameters for the glycerokinase enzymatic reaction, for synthesis of glycerol-3-phosphate. The reaction follows normal Michaelis-Menten kinetics, with regards to the substrates, glycerol and ATP. Magnesium chloride is required at an optimal ratio of 640 mM $MgCl_2$ to 1 Unit/ml of enzyme; times the concentration of ATP for the reaction; the glycerokinase enzyme reacts with the Mg-ATP complex, rather than pure ATP. I have determined the reaction conditions (temperature, pH, substrate ratios) that will maximize glycerol-3-phosphate production, for a given enzyme concentration. From comparative studies of the ATP regeneration reaction, catalyzed by acetate kinase, I have shown suitable compatibility, in terms of reaction systems, between the G3P synthesis and ATP regeneration reactions. I have experimentally measured the diffusion coefficients of the principal substrates and products in the G3P and ATP regeneration reactions: Glycerol, ATP, ADP, Acetyl Phosphate, and G3P. The enzymes, acetate kinase and glycerokinase, were completely retained by the membrane, as expected. The diffusion coefficients were measured from substrate flux data, obtained using a diffusion cell, fitted with a membrane representative of the hollow fiber membrane to be used. Based on these results, and measurement of the reaction rates with native enzyme, I have shown that the mass transfer limitations on substrate/ product diffusion across the hollow fiber membrane is minimal, compared to the overall reaction rate at desirable enzyme concentrations. A preliminary scheme for the hollow fiber reactor design has been developed, based on the above findings, and will now be tested with the coupled enzyme reactions.

OVERVIEW OF RESEARCH

Marty Privalsky
Microbiology
University of California, Davis

Oncogenes, the regulation of cell proliferation and differentiation, hormone receptors and transcriptional regulation. Research focuses on two oncogenes, v-erb A and v-erb B, involved in retroviral induced neoplasia. The v-erb A oncogene is derived from a cellular locus encoding a thryoid hormone receptor (a hormone regulated transcription factor). The v-erb B oncogene is derived from a cellular gene for the epidermal growth factor receptor, a tyrosine-specific protein kinase. A combination of molecular biology, genetic, and biochemical analyses to elucidate the roles these genes play in normal and in cancer cells is utilized.

DISSECTION OF TETRAMER FORMATION BY RETINOID X RECEPTORS, A NOVEL MODE OF DNA RECOGNITION BY NUCLEAR HORMONE RECEPTORS

Benjamin C. Lin*, Hong-Wu Chen, Chi-Wai Wong, and Martin L. Privalsky
Microbiology
University of California, Davis

Nuclear hormone receptors function as hormone-regulated transcription factors that serve to regulate cell growth and differentiation, homeostasis, metabolism, and reproduction in multicellular organisms. Most nuclear hormone receptors can bind to DNA as protein homodimers or heterodimers and are, therefore, traditionally viewed as being able to regulate the transcription of adjacent target genes in this manner. We have previously demonstrated, however, that DNA recognition by retinoid X receptors (RXRs), a member of the nuclear receptor superfamily, can occur by an unanticipated mechanism in which receptor tetramers and higher order oligomers are cooperatively formed on hormone response elements (HREs) containing suitably reiterated half-sites (specifically the CRBP-II element which contains four half-sites). We report here that RXR tetramers form with high cooperativity on a number of response elements with a variety of nucleotide spacings between half-sites as well as a variety of half-site orientations, as determined by electrophoretic mobility shift assay (EMSA). Intriguingly, the spacing and orientation requirements for tetramer formation are non-equivalent at the different half-sites in the HRE. These results suggest that at least two protein-protein interfaces may be responsible for RXR tetramer formation and that at least one of these interfaces is likely to be non-identical to those previously implicated in RXR dimer formation.

EXTRACTION AND CONTROLLED RELEASE TECHNOLOGIES USING MICELLES AND MICROEMULSIONS

Stephanie Dungan
Chemical Engineering
University of California, Davis

Amphiphilic molecules will often form molecular aggregates known as micelles and microemulsions in solution, and these aggregates will extensively solubilize substances that are otherwise quite insoluble in the bulk solvent. This ability of surfactant aggregates to preferentially solubilize certain molecules can be utilized to design effective separation methods for valuable food/pharmaceutical components, or to enhance the controlled release rate of therapeutic molecules. In our laboratory we are measuring and modeling the ability of micelles and microemulsions to incorporate solutes such as whey proteins, cholesterol, and a variety of hydrophobic molecules, in order to employ these aggregates for extraction purposes. In addition, we are studying the solubilization behavior of these aggregates within complex systems such as emulsions and gels, with results impacting on the development of novel controlled release technologies.

MICELLAR PROPERTIES OF QUILLAJA SAPONIN

Shuman Mitra* **and Stephanie R. Dungan**
Chemical Engineering
University of California, Davis

Quillaja saponins are naturally occurring surfactants used extensively in the food industry, and with interesting potential applications as pharmaceutical agents and in cholesterol extraction technology. In this study, the ability of quillaja saponins to form micelles in aqueous solution is investigated. We have used surface tension measurements to demonstrate that these molecules aggregate above a critical concentration, and have also found that above the same concentration the presence of these aggregates enhance the solubility of a hydrophobic dye. Both of these observations are consistent with micelle formation above a critical micelle concentration (cmc). The influence of temperature, pH and aqueous salt concentration on the cmc, size and aggregation number of these saponin micelles is explored, and their molecular properties at the air-water interface are discussed. Despite the fact that quillaja saponin is often regarded as a nonionic surfactant, its aggregation properties exhibit a significant influence of charge interactions.

This information on the micellar properties of quillaja saponin is further used in a study of aqueous saponin solutions containing cholesterol. To this end, we have investigated the surface behavior of cholesterol saturated saponin solutions and found clear evidence of micellar aggregates. Interestingly, the properties of these micelles are very different from those of pure saponin as far as the cmc, size and effect of temperature on the cmc are concerned. The knowledge gained from these studies of quillaja saponin solutions is useful in exploring their ability to extract solutes of biological significance, like cholesterol from animal fats and oils, and in understanding their use as pharmaceutical agents.

POSTERS

GENERATION OF A SOLUBLE RECEPTOR FROM AN IMMUNOADHESIN BY SPECIFIC CLEAVAGE: THE EXAMPLE OF INTERFERON γ RECEPTOR

Steven Chamow
Senior Scientist
Genentech

Interferon-γ (IFN-γ) binds to a cell surface protein known as the IFN-γ receptor (IFN-γR). To produce soluble IFN-γR (sIFN-γR) for structural studies, we constructed a molecular fusion of the IFN-γR ectodomain with immunoglobulin G heavy chain that contains a protease-specific sequence joining the two portions of the molecule (IFN-γR-G-IgG). We introduced the hexapeptide sequence AAHY:TL (where ":" denotes the scissile bond) at the junction of IFN-γR and IgG regions for specific cleavage by an H64A variant of subtilisin BPN' (Genenase I), an endoprotease that cleaves selectively at this sequence. A plasmid encoding the fusion protein was used to transfect human embryonic kidney 293 cells transiently; and secreted IFN-γR-G-IgG was purified from cell supernatants by protein A chromatography. From the purified fusion protein, sIFN-γR was then generated by enzymatic cleavage with immobilized Genenase I. Genenase I was immobilized on Sepharose 4B by coupling a unique sulfhydryl group of the enzyme to oxidized beads via a heterobifunctional crosslinker. Incubation of IFN-γR-G-IgG with immobilized Genenase I resulted in rapid, specific cleavage at the target site. Reaction conditions, including enzyme:substrate ratio, time and pH were optimized. The resulting sIFN-γR was separated from the immunoglobulin Fc cleavage product by re-chromatography on protein A. The purified sIFN-γR showed both the ability to bind to its ligand, IFN-γR, and demonstrated anti-viral activity. These results, taken together with similar results using interleukin-1 receptor, confirm the potential utility of this approach for the production of isolated receptor domains for structural or biological studies. To date, several investigators at Genentech have already used this approach successfully for a variety of proteins of interest.

ORGANIZATION AND LINKAGE OF THE CHICKEN SARCOMERIC MYOSIN HEAVY CHAIN MULTIGENE FAMILY

I. Analysis of 5 fast and 4 slow cardiac genes expressed in skeletal muscles

Q. Chen*, L.A.Moore, Q. Zhang, and E. Bandman
Food Science and Technology
University of California, Davis

We have analyzed 9 myosin heavy chain (MyHC) genes that are expressed in developing and mature chicken skeletal muscle using fluorescent in situ hybridization (FISH), pulsed-field gel electrophoresis (PFGE), and Southern blotting. Using cDNA, oligonucleotide, and genomic probes we show that multiple fast myosin heavy chain genes are linked within 400 kb on a chicken microchromosome. This result is analogous to the linkage observed for the mammalian fast myosin heavy chain gene cluster. While there appear to be only 2 slow cardiac myosin genes in mammals, there are at least 4 slow/cardiac myosin heavy chain genes expressed in chicken skeletal and cardiac muscles. We demonstrated that there is a separated slow myosin heavy chain gene cluster of 80 kb that is constructed of sm1, sm2 and an atrial myosin heavy chain genes. The genes encoding the SM1 and SM2 proteins expressed in embryonic and adult slow muscles respectively, are closely linked and are separated by a *Not* I site. FISH data showed that sm2 gene hybridized to another pair of microchomosomes. The two myosin heavy chain genes expressed in cardiac muscle, atrial myosin (AMHC1) and a ventricular myosin (VMHC1), show no close linkage, as confirmed by VMHC1 which are mapped to chromosome 1. The atrial gene is linked to sm1 as both reside on a 50 kb fragment. Lastly, we have recently identified a series of yeast artificial chromosome (YAC) clones that contain chicken fast myosin heavy chain genes. One clone, with a 600 kb YAC, contains all 5 fast myosin genes that we and others have characterized. We conclude that there are at least three MyHC gene loci, fast, slow and ventricular MyHC gene clusters respectively, in the chicken genome.

CHARACTERIZATION OF MUTANTS IN MID TO LATE STAGE *ARABIDOPSIS THALIANA* OVULE INTEGUMENT DEVELOPMENT

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Molecular and Cellular Biology
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As part of a project to understand regulation of the *Arabidopsis* ovule development pathway, we have isolated four novel mutants with aberrant mid to late stage integument development. In comparison to oblong wild type ovules the *ste 147* mutant has abnormally round ovules. Using scanning electron microscopy, we determined that the *ste 147* inner integument appears normal in growth; however, a generally dwarfed appearance of ovule structures including the outer integument and funiculus is observed. In contrast, the *ste 130* mutant appears to have an unusually elongated inner integument. The *347-8* mutant appears to have aberrant cell differentiation of the outer integument. From SEM analysis, *347-8* ovule primordia appear normal; however, at anthesis, cell elongation and division appear irregular. The fourth isolate, *461-5*, appears to have normal cell number but reduced cell size of the outer integument. We are currently conducting complementation tests to determine allelic relationships between these and other ovule mutants isolated in the lab. Moreover, we are creating double homozygous mutants to discern the epistatic interactions between these genes to address the gene order in the ovule development pathway. Results describing the characterization of these putative regulatory ovule determinants will be discussed.

PHENOTYPIC CHARACTERIZATION OF AN ALLELIC SERIES OF *DRAGON* MUTANTS REVEALS THAT THIS LOCUS IS ESSENTIAL FOR EARLY STAGES OF OVULE DEVELOPMENT

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We have identified a new genetic locus, *DRAGON (DRG)*, essential for ovule development in *Arabidopsis thaliana*. The strongest allele of a series of seven *drg* mutants we have isolated forms ovule primordia but lacks the normal two integuments, which are instead replaced by a single ridge of cells. In the weakest allele, this ridge of cells expands and divides, forming a structure which may represent a fusion of the inner and outer integuments. By examining serial optical sections of several *drg* ovules with a laser confocal microscope, it has been determined that a megaspore mother cell forms, but meiosis does not occur and, consequently, an embryo sac is never formed. In addition to this ovule phenotype, *drg* plants also have petals which are small compared to WT. The *DRG* gene has been mapped to the southern half of chromosome IV, 2.6 cM south of the molecular marker PG11. In an effort to place *DRG* in the developmental genetic pathway of ovule formation, double mutant analysis has been carried out with a number of other ovule development mutants.

CHARACTERIZATION OF PROTEINS SECRETED BY *TRICHOSANTHES KIRILOWII* PLANT CELL CULTURES

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Trichosanthes kirilowii, a Chinese medical cucumber, contains an active protein substance called trichosanthin (TCS). TCS has been found to be a ribosome inactivating protein (RIP) due to its N-glycosidase activity against procaryotic and eukaryotic ribosomes. RIPs, basic proteins in the 25-35 kDa range, are believed to be a part of plant defense mechanisms against bacteria, fungus and plant virus. Recently, it is also reported that TCS possesses anti-HIV and anti-tumor activity. TCS can be found intracelluarly in the *T. kirilowii* root tuber. In this poster we are reporting discovery of TCS-like protein that is excreted by agrobacterium transformed *T. kirilowii* callus cultures. Agrobacterium transformed *T. kirilowii* callus plant cultures were incubated in shaking flasks for 21 days at room temperature. The resultant harvest culture fluid was filtered and dialyzed against 20 mM phosphate buffer, pH 7.0. The dialyzed samples were applied onto a Hewlett Packard HPLC system using a perfusion column HS/M. The adsorbed proteins were eluted with a linear increase of NaCl in 20 mM phosphate buffer, pH 7.0. Four fractions were collected and their chitinase activity and in vitro protein translation inhibition ability were detected. F1 exhibited strong in vitro protein translation inhibition whereas F3 also demonstrated some activity. F2 and F4 essentially have no in vitro protein translation inhibition ability but showed very strong chitinase activities. SDS-PAGE analysis of these fractions demonstrated that F1 contains three major protein, 36, 30, and 28 kDa whereas a protein of estimated 15 kDa was the majority protein in F3. F2 and F4 contain a 28 kDa protein. With goat anti-TCS serum, we were able to detect the presence of a TCS-like protein in F1, which possesses identical molecular weight with standard TCS. This small amount of extracelluar TCS-like protein may due to cell lysis. We also found a strong cross-reaction between goat anti-TCS serum and the 28 kDa protein. However, this protein also cross-reacted with rabbit anti goat serum in a control blot, but to a lesser degree. Thus no definite confirmation that the 28 kDa protein also share similar structures with TCS.

ENZYMATIC HYDROLYSIS OF PRETREATED RICE STRAW

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California rice straw is being evaluated as a feedstock for production of power and fuel. The process under consideration involves pretreatment of rice straw, enzymatic hydrolysis of the polysaccharides, microbial fermentation of mixed carbohydrates to ethanol, membrane separation of solids and liquids, and solids combustion to power. Three distinct state-of-the-art pretreatments, a process developed by Swan Biomass Company, dilute-acid hydrolysis (US DOE National Renewable Energy Laboratory), and ammonia fiber explosion (Texas A&M University) and six commercial cellulases, products of Genencor International (USA), Novo (Denmark), logen (Canada) and Fermtech (Russia) were evaluated. Enzymatic hydrolysis was evaluated on the basis of glucose and reducing sugars production. The changes in carbohydrate composition of rice straw after pretreatment and after enzymatic hydrolysis were monitored using various methods of carbohydrate analysis.

All three pretreatment techniques substantially increased the enzymatic digestibility of rice straw. Two enzyme preparations - Cellulase 100L (Iogen) and *Trichoderma reesei* A1 (Fermtech) - demonstrated a higher saccharification activity on pretreated rice straw compared to others. The conditions for hydrolysis of rice straw with Cellulase 100L have been optimized. Considerations were given to the efficacy of the pretreatment based on the combined yield of soluble sugars produced by pretreatment step and by enzymatic hydrolysis.

The best results for conversion of complex carbohydrates into soluble sugars (98%) were obtained during hydrolysis of washed, dilute-acid pretreated rice straw (5 g/l) using Cellulase 100L (1 FPU/ml, FPU - Filter Paper Unit). The best yield of RS (43 g/l) was obtained during hydrolysis of the same substrate (150 g/l) using Cellulase 100L (1 FPU/ml) supplemented with Novozyme 188 (1 Cellobiase Unit/ml). 99% of RS were represented in this case by glucose.

CALIFORNIA INSTITUTE OF FOOD AND AGRICULTURAL RESEARCH

Sharon Shoemaker, Director
CIFAR

California Institute of Food and Agricultural Research (CIFAR) is an independent, multidisciplinary program in the College of Agricultural and Environmental Sciences at UC Davis. CIFAR's mission is to create and further enhance channels for research collaboration, program sponsorship and technology exchange between UC Davis and the food and agricultural industries.

Industrial and governmental affiliates identify areas of interest and CIFAR staff identifies individuals, initiates discussions, provides contracting assistance, and in some cases, assists in the execution and reporting of project results.

CIFAR has been highly responsive to the needs of the food and agricultural industries, since its inception five years ago, and has contributed to faculty research, technology transfer and information services, especially related to environment and energy issues.

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Remi Shih	Chemical Engineering
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Martina McGloughlin

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Vic Knauf	Vice President of Research	Calgene
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Jean C. Kridl	Senior Scientist	Calgene
Randall Mrsny	Senior Scientist	Genentech, Inc.
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UNIVERSITY OF CALIFORNIA, DAVIS
GRADUATE TRAINING PROGRAM
IN BIOTECHNOLOGY

TRAINEES 1995-96

Trainee	Preceptor	Department
Continuing Trainees		
Ed Fulton	Kathryn Radke	Avian Science
New Trainees		
Kristen Hadfield	Alan Bennett	Vegetable Crops
Ruth Hemmer	Patricia Conrad	VM: Immunology
Rajesh Krishnan	Dewey Ryu	Chemical Engineering
Benjamin C. Lin	Martin Privalsky	Mol Cell Biology
Shuman Mitra	Stephanie Dungan	Food Science & Technology

UNIVERSITY OF CALIFORNIA, DAVIS
GRADUATE TRAINING PROGRAM
IN BIOTECHNOLOGY

First Year Fellowship Awards Fall 1995

NAME	GRADUATE GROUP	UNDERGRADUATE INSTITUTION	FELLOWSHIP
Colussi, Thomas	Chemical Engineering	UC Santa Barbara	UC Davis
Flieder, Laurie M.	Plant Biology	UC San Diego	Calgene
Frampton, Gordon	Biochem/Mol Biol	Tufts - MS	Roche Bioscience
Gates, Brian	Chem Eng & Mat Sci	Johns Hopkins	UC Davis
Santos, Jennifer L.	Biochem/Mol Biol	CSU Hayward	Genentech
Song, Yujuan	Plant Biology	Lanzhou Univ.-China	UC Davis
Stone, Remington J. S.	Biochem/Mol Biol	Berkeley	UC Davis
Vijay, Kamni	Food Science & Tech	Dartmouth	Novo Nordisk Biotech
Weigel, Andrea	BioChem	USC	Chiron
Wong, Francis Chi Yuen	Microbiology	Berkeley	Entotech

Biotechnology Training Grant
Executive Committee
1996-97

Doi, Roy H. Director	Professor	Molecular & Cellular Biology
Ryu, Dewey, Co-Dir.	Professor	Chemical Engineering
Anderson, Gary	Professor	Animal Science
Bruening, George	Professor	Plant Pathology
Carlson, Don M.	Professor	Molecular & Cellular Biology
Hanley, Michael R.	Professor	MED: Biol Chem
Meares, Claude	Professor	Chemistry
Privalsky, Marty	Professor	Microbiology
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Nedwin, Glenn	President	Novo Nordisk Biotech
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Barnett, James	Research Sec. Ldr.	Cancer & Dev. Biol., Roche Bioscience
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