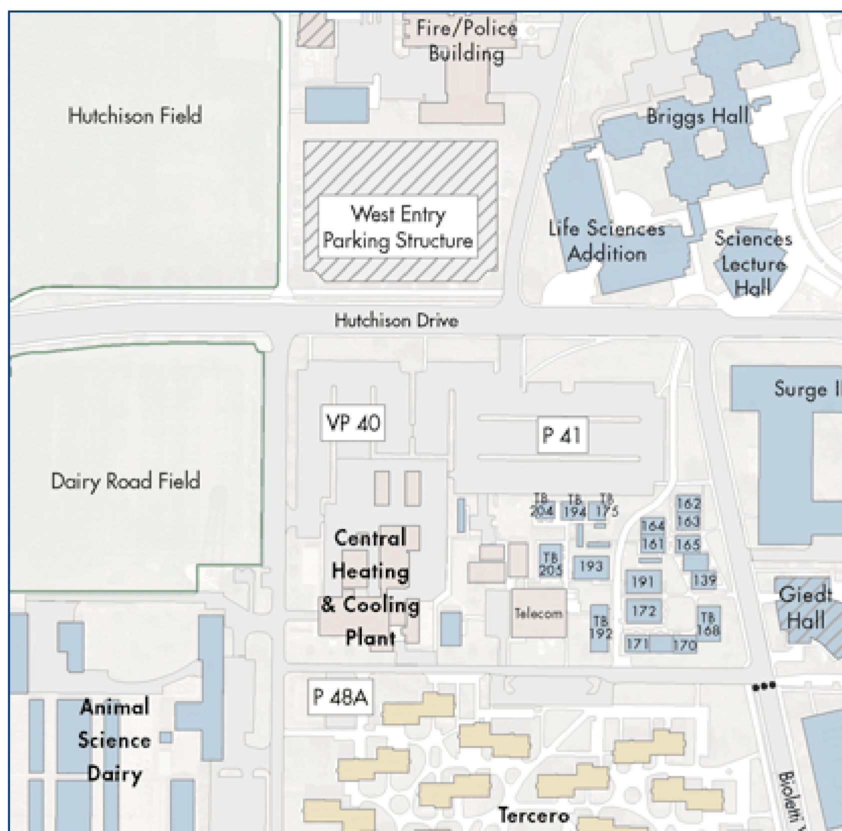
After the Sessions…

Join us at the Dairy Road Intramural Field for our Annual Student/Faculty Softball Game!



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Schedule

8:30 AM: Light Breakfast and Coffee

Session I

9:00 AM: **Mark Wogulis**
Identification and structural characterization of *S. cerevisiae* fromyl kynurenine formidase and kynurenine aminotransferase

9:25 AM: **Matt Schenauer**
Mass Spectrometry Defines Chemokine-binding Epitopes of Heparan Sulfate

9:50 AM: **Dr. Oliver Fiehn**
Metabolomics for biochemical phenotyping and classification

10:15 AM: Coffee Break

Session II

10:30 AM: New Student Introductions

10:45 AM: *Presentation of the Teaching Award*
Dr. Enoch Baldwin
Modulation of recombination specificity and reaction outcome through engineered protein-protein contacts in Cre-LoxP

Notes

Notes

recombination.

11:10 AM: *Keynote Speaker*
Dr. Kenneth Burtis
Serendipity in science: the twisting path from 237 Briggs to 207 Life Sciences

11:35 AM: *Presentation of the Lifetime Achievement Award*
Dr. James DeVay

11:50 AM: Faculty Introductions

12:00 PM: Lunch and Poster Session

Session III

2:00 PM: **Ed Seikel**
Voltage Gated Potassium Channel Modulation by Diverse Auxiliary Subunits

2:25 PM: **Ed Kraft**
Functional Analysis of the ACH family of E3 ligases in *Arabidopsis*

2:50 PM: **Dr. Bruce Draper**
Zebrafish gemetogenesis: A model system for the study of stem cell biology

3:40 PM: Batting Practice

4:00 PM: Softball Game

Notes

remains largely in the cytoplasm. These results suggested that decreased nuclear expression of FlnA16-24 in prostate cancer cells is associated with androgen independence. Transfection of FlnA16-24 in C4-2 cells restored nuclear localization of the 90kDa fragment. Unlike LNCaP cells, C4-2 cells are not growth inhibited by Casodex, but transfection of FlnA16-24, not FlnA1-15, resulted in Casodex-induced growth inhibition in C4-2 cells. In addition, downregulation of FlnA in LNCaP cells by FlnA specific siRNA induced resistance to Casodex in these cells. Our data suggest that expression of FlnA16-24 in the nuclei of androgen-independent C4-2 cells reverses these cells to an androgen-dependent phenotype. Thus FlnA may be a promising novel target of therapy in prostate cancer.

Expression Pattern and Domain Analysis of UNC-83, a Nuclear Envelope Component Required for Nuclear Migration

E. Ladwig-Scott, R. Rillo, M. Meyerzon, D. A. Starr.
313 Briggs Hall

Characterization of enhancers of unc-83/unc-84 in nuclear migration

M. Meyerzon, K. Warltier, D. A. Starr.
313 Briggs Hall

in trans for inner membrane fusion. These observations indicate that the role of the Fzo1/Ugo1/Mgm1 complex is to temporally couple outer and inner membrane fusion via some signaling/conformational changes.

Alteration of mitochondrial morphology has been implicated in a variety of pathological conditions. Understanding the mechanisms that govern the cellular processes of fission and fusion has implications for understanding the pathology of these diseases. By examining the biochemical characteristics of the fusion and fission orthologs in mammalian cells we can better understand the molecular mechanisms of these conditions.

A 90kDa fragment of Filamin A promotes Casodex-induced growth inhibition in the Casodex-resistant androgen-receptor positive C4-2 prostate cancer cell line

Yu Wang[1], Roble Bedolla[2], Xiao-Hua Lu[1], Margarita Mikhailova[2], Jeffrey I. Kreisberg[2] and Paramita M. Ghosh[1,2,3]

[1] Department of Urology, University of California Davis, School of Medicine, Sacramento, CA; [2]Department of Surgery, University of Texas Health Science Center at San Antonio, San Antonio, TX and [3]Research Services, VA Northern California Health Care System, Mather, CA

Prostate tumors are initially dependent on androgens for growth, but the majority of patients treated with anti-androgen therapy progress to an androgen-independent state which involves resistance to such therapy. This study investigates the resistance of androgen-independent prostate cancer (AIPC) cells to the anti-androgen Casodex. Filamin A (FlnA) is a 280kDa membrane-associated protein consisting of an actin-binding domain (ABD) followed by 24, 96-residue repeats. FlnA is cleaved intracellularly to a 170kDa fragment consisting of the ABD and repeats 1-15 (FlnA1-15) and a 110kDa fragment consisting of repeats 16-24 (FlnA16-24) which is further cleaved to a 90kDa fragment. Comparison of FlnA expression in the androgen-dependent LNCaP prostate cancer cell line and its androgen-independent subline C4-2 showed that in LNCaP cells, the 90kDa fragment translocates to the nucleus, whereas in C4-2 cells, FlnA

Session I

9:00 AM

Mark Wogulis
Dr. David Wilson's Lab
Section of Molecular and Cellular Biology
3 Briggs Hall

Identification and structural characterization of *S. cerevisiae* formyl kynurenine formamidase and kynurenine aminotransferase

The essential enzymatic cofactor NAD can be synthesized in many eukaryotes, including yeast and mammals, using tryptophan as a starting material. Metabolites along the pathway have important biological functions such as kynurenic acid which can act as a NMDA antagonist, thereby functioning as a neuroprotectant in a wide range of pathological states. N-formyl kynurenine formamidase (FKF) catalyzes the second step of the NAD biosynthetic pathway by hydrolyzing N-formyl kynurenine to produce kynurenine and formate. The S. cerevisiae FKF had been previously identified as Bna3, a pyridoxal phosphate-dependent enzyme. Combined crystallographic and biochemical methods identify Bna3 as the yeast kynurenine aminotransferase, which converts kynurenine to kynurenic acid. Additionally, we identify YDR428C, a yeast ORF coding for an á/â hydrolase with no previously assigned function, as the FKF. An interpretation of prior structural genomics results along with sequence homologies predicted its function as an FKF. Biochemical and in vivo metabolomics data derived from LC-MS demonstrate that YDR428C, which we have designated BNA7, is the yeast formylkynurenine formamidase.

Notes

major pathway acting in the restart of stalled replication forks. Deletion of the *Rad5* gene in a *rad55-S2,8,14A* background leads to an increase in MMS sensitivity compared to the single mutants. Thus, phosphorylation of these residues in the Rad55 protein becomes vital in the absence of Rad5.

In summary, we think that Rad55-S2,8,14A phosphorylation plays an important role for the protein's function and further research will show the nature of this role.

Dynamics of Mitochondrial Fusion and Fission

Laura Lackner
Dr. Jodi M. Nunnari's lab
3215 Life Sciences

Mitochondria are dynamic organelles, which continually undergo fission and fusion. Regulation of mitochondrial copy number, morphology, and distribution is important for cellular differentiation and function. In *Saccharomyces cerevisiae*, mitochondria maintain a continuous reticular structure along the cell cortex. The establishment and maintenance of this structure requires a balance of fission and fusion events. These processes are mediated by two distinct sets of conserved proteins. Among these proteins members of the dynamin related GTPase family (DRP) play important roles. Dnm1 is the master regulator of mitochondrial fission and two DRPs, Fzo1 and Mgm1, are required for fusion.

Our data suggest that Dnm1 drives membrane constriction during mitochondrial fission by self-assembly and conformational changes. Fis1 and Mdv1 are two additional components required for Dnm1-dependent mitochondrial fission. Fis1 targets and sequesters Mdv1 on the mitochondrial outer membrane, where Mdv1 interacts with Dnm1 to trigger mitochondrial division. We hypothesize that the Fis1/Mdv1 complex triggers division by regulating assembly of Dnm1 spirals on the mitochondrial surface.

Through *in vitro* mitochondrial fusion assays established in our lab, we have shown that fusion of the outer and inner mitochondrial membranes are separable events, each having distinct energetic and protein requirements. Specifically, we have shown that Fzo1 is required in trans for outer membrane fusion, while Mgm1 is required

Regulation of Homologous Recombination through Rad55-Rad57 phosphorylation

Kristina Herzberg [1], Vladimir Bashkirov [2], Wolf-Dietrich Heyer [1]
[1] University of California Davis, Section of Microbiology, 1 Shields Ave., Davis, CA 95616, [2] Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404
3143 Life Sciences

Homologous recombination (HR) plays a major role in the repair of DNA double-stranded breaks (DSBs). These breaks can be inflicted due to exposure of the cell to energy-intensive radiation or to chemicals, but can also result from the activity of metabolic products inside the cell. Additionally, HR is implicated in the process of restarting stalled replication forks involving either repair or tolerance of the DNA damage. The critical step in homologous recombination is the invasion of a homologous DNA duplex by the single-stranded ends formed at the site affected by the DNA damage. Successful execution will lead to the restoration of the original information using the duplex as a template. This process requires the action of Rad51 protein, which forms a filament on the single-stranded ends. The resulting nucleoprotein filament is active in homology search and strand invasion. However, the formation of the filament requires the assistance of mediator proteins, e.g. Rad52 and the obligate heterodimer Rad55-Rad57.

Rad55 and Rad57 were found to be phosphorylated in response to DNA damage in *Saccharomyces cerevisiae*. This phosphorylation requires the active checkpoint kinases Mec1 and Rad53. We mapped Rad55 phosphoylation sites and conducted a mutant analysis to determine the physiological role of the phosphorylation events. Here we present data acquired for the *rad55-S2,8,14A* mutant. The mutant is sensitive to the alkylating agent methylmethane-sulfonate (MMS). Results from Yeast Two Hybrid assays show that the mutant protein is not impaired in its interaction with Rad51. Genetic analyses of combination mutants with deletions in checkpoint genes confirm the dependency of the phosphorylation on checkpoint activity. We also analyzed the relationship between *Rad55* and genes required for post-replication repair (PRR), e.g. *Rad18*, *Rad5*, *Rev3* and *Rad30*. PRR is a

9:25 AM

Matt Schenauer
Dr. Julie Leary's Lab
Section of Molecular and Cellular Biology
1300 Genome and Biological Sciences Facility

Mass Spectrometry Defines Chemokine-binding Epitopes of Heparan Sulfate

Recent advances in mass spectrometry (MS), including the development of "soft ionization" techniques such as electrospray ionization (ESI) and MALDI, have led to the application of MS for the detection of noncovalent macromolecular assemblies. To date, complexes incorporating proteins, nucleic acids, carbohydrates, and lipids have all been observed using ESI MS.

Chemokines are a large class of proteins in humans and other mammals that direct the migration of leukocytes from the vasculature during routine immune surveillance, infection, inflammation, and disease. A prerequisite for chemokine function is their retention on cell surface glycosaminoglycans (GAGs) prior to signaling. Heparan sulfate (HS) is the primary cell surface GAG. HS polysaccharides may be composed of at least 23 distinct disaccharides, which when linked in the proper sequence, constitute protein binding sites.

We are using ESI MS to screen a library of HS oligosaccharides for those capable of binding to inflammatory chemokines. The results are verified using an affinity enrichment strategy, followed by MS detection of the unbound carbohydrate. Stoichiometry of each noncovalent complex is obtained, as well as exact mass identification and characterization of bound oligosaccharides.

Notes

Elucidating the roles of the structure-selective endonuclease Mus81-Mms4

William Wright, Erin Schwartz, Zach Whitfield, Kirk Ehmsen, and Wolf-Dietrich Heyer.
Section of Microbiology and Center for Genetics and Development
3143 Life Sciences

Mus81-Mms4(Eme1) cuts branched DNA structures arising from as yet unknown origin(s) within DNA metabolism. Many models of DNA repair and recombination have been proposed which include the involvement of Mus81-Mms4, yet its distinct role is difficult to demonstrate *in vitro* because the endonuclease possesses activity against several different branched structures (see below). A major goal of our laboratory is to employ diverse techniques in order to find evidence of Mus81-Mms4's primary substrates and how it is employed by the cell to facilitate DNA damage repair and recombination. Regulation of this complex is controlled through multiple phosphorylation sites on both Mus81 and Mms4. Work is currently underway to characterize the phenotypes of various phosphorylation site mutants. We are also employing the use of multiple biochemical techniques to elucidate areas of ambiguity in the field, including determining the functional consequences of interactions with proteins such as Rad54, explore Mus81-Mms4 activity towards uncharacterized substrates such as paranemic joints and define the subunit composition of the active complex through hydrodynamic experiments. Another method being used to determine subunit composition and overall structure is cryo- and negative stain-electron microscopy where images collected can be used to reconstruct a 3-D image of the Mus81-Mms4 complex. Chromatin immunoprecipitation (IP) and *in vivo* co-IP techniques will be employed to monitor the kinetics of Mus81-Mms4 localization to the sites of DNA lesions and stalled replication forks. Ultimately, these subprojects will culminate into a clearer picture of the function of this enzyme in DNA metabolism.

Elucidating the functions of Rad51 and Rad54

Shannon J. Ceballos, Xiao-Ping Zhang, Xuan Li, Wolf-Dietrich Heyer
Section of Microbiology and Center for Genetics and Development
3143 Life Sciences

Homologous recombination (HR) is important for high-fidelity repair of DNA double strand breaks (DSB) and recovery of stalled or collapsed replication forks. Two key players in the HR pathway in *Saccharomyces cerevisiae* are the Rad51 and Rad54 proteins, and it is believed that their interaction is crucial. Rad51 protein catalyzes homology search and DNA strand invasion, similar to its *E. coli* homolog, RecA. RecA protein, unlike Rad51, displays a high ATPase activity which allows RecA to turn over and release the heteroduplex DNA product after strand invasion. We hypothesize that Rad51 turnover after DNA strand exchange requires not only its intrinsic ATPase activity but also an extrinsic factor, the Rad54 motor protein. We were able to show that Rad54 promotes the disassembly of the Rad51-dsDNA complex. To further test this model we are taking several approaches. Based on structural information on Rad51 and Rad54, we are testing point and deletion mutants in a variety of biochemical assays, to identify specific mutations in both proteins that affect their interaction and their function in the HR pathway. Another approach we are taking is a genetic screen to find a mutant form of the Rad51 protein that can function independently of Rad54's ATPase activity. We speculate that such a Rad51 mutant would resemble its *E. coli* homolog RecA in its biochemical properties.

9:50 AM

Dr. Oliver Fiehn
Genome Center
1315 Genome and Biological Sciences Facility

Metabolomics for biochemical phenotyping and classification

Metabolomic data provide a comprehensive signature of the physiological state and reflect specific biochemical processes that were altered when comparing different sets of perturbations (G x E) : the 'metabolic phenotype'. Accurate phenotype assessments by metabolite analysis are helpful or mandatory for various applications in plant biotechnology, e.g. bioengineering or breeding metabolic traits, functional genomics, food safety and GM substantial equivalence, mode-of-action detection or fundamental plant biochemistry.

Some 200,000 different metabolites have been estimated alone for the plant kingdom. In the tissues of any particular organism, thousands of these may be present under given biological conditions. With respect to this complexity, what are the techniques used to ascribe metabolic phenotypes, what are the major applications, and how does this relate to progress in bioinformatics and theoretical biology? This talk will cover recent developments in these fields. The talk will highlight methods reported from recent publications as well as in the Fiehn laboratory at the UC Davis Genome Center. Specifically, strengths and weaknesses of each method will be discussed, and the need for further method improvements will be highlighted.

Use of metabolomic technology is briefly exemplified for studies on long distance transport in plants, G x E effects on hybrid vigor in Arabidopsis crosses, and investigating unintended effects in GM versus non-GM plants. The generation and theoretical background of metabolic correlation networks is demonstrated by simulation experiments. As an outlook, results are presented for correlation of metabolite contents with transcript or protein levels which ultimately might give clues to regulation of plant responses on the systems level.

Notes

Butterflies to Bloodpressure: Inhibitors of the soluble epoxide hydrolase as therapeutic agents to treat blood pressure and inflammation

Chin-Min Ho, Bora Inceoglu, Cindy Tsai, Pasha Aronov, Christophe Morisseau, Bruce D. Hammock
Department of Entomology
90 Briggs Hall

Basic research in the regulatory biology of insect metamorphosis led to insights in chemical mediation across species. Based on this information we initiated a study of a mammalian enzyme which turned out to be a key regulator of a new branch of the arachidonic acid cascade. A combination of techniques in molecular biology and protein chemistry led to the discovery of transition state inhibitors of the enzyme which are being optimized for clinical trials by medicinal chemistry approaches. These potent inhibitors and analytical tools have allowed us to explore the arachidonic acid cascade and find that the resulting inhibitors not only reduce hypertension but transcriptionally regulate cyclooxygenase 2 and other key enzymes in the arachidonic acid cascade to shift the cascade from propagation of inflammation and pain to its resolution. From a practical standpoint we hope to have the compoiunds in clinical trials in a year. The compounds are more potent in models than classical NSAIDs like Advil, celebrex and Vioxx. They also synergize these common therapeutics and shift the plasma eicosanoid profile from 'Vioxx like' which may encourage cardiac problems to 'aspirin like' or a more normal balance of thromboxanes to prostacyclins. From a basic standpoint they help us take a metabolomic approach to understanding the regulatory biology of inflammation.

cannot increase their function by increasing cell division. Rather, they meet the increased demands on the heart by increasing cell mass. This results in short-term improvement in cardiac output, but such a response cannot be sustained and in the long term, congestive heart failure ensues. Pharmacological inhibition of sEH has resulted in decreased blood pressure in rat models of hypertension. Furthermore, male sEH knock-out mice had blood pressure reduced to female levels. In cultured rat primary cardiomyocytes, hypertrophy can be induced by the introduction of -adrenergic receptor agonists to the cell media. Under these conditions, the activity of endogenous sEH is elevated, and when small molecular inhibitors of this enzyme are incubated with the cells, the endogenous activity drops significantly. Furthermore, the drop in sEH activity correlates with a decrease in the cellular markers of the hypertrophic response in cultured cells. It is proposed that the decrease in hypertrophy is due to an increase in endogenous EETs resulting from the inhibition of the enzyme that is responsible for converting these EETs to the less active diols. The epoxide and diol fatty acid profile has yet to be determined. Future work is planned to determine if the EETs are involved in a molecular pathway that regulates the hypertrophic response, and whether these endogenous chemical mediators are cardioprotective in hypertrophy and heart failure.

Session II

10:45 AM

Presentation of the Teaching Award

Dr. Enoch Baldwin
Section of Molecular and Cellular Biology
4B Briggs

Dr. Enoch Baldwin is Associate Professor of Molecular and Cellular Biology, and is affiliated with the Section of Molecular and Cellular Biology and the Department of Chemistry. Dr. Baldwin is a professor for three core courses in the Biochemistry and Molecular Biology program of study. An engaging lecturer, Dr. Baldwin explains complicated concepts of physical biochemistry and molecular genetics clearly and enthusiastically. We are proud to present Enoch Baldwin as this year's recipient of the annual Biochemistry and Molecular Biology Teaching Award.

Modulation of recombination specificity and reaction outcome through engineered protein-protein contacts in Cre-LoxP recombination.

Substrate specificity results from energy differences between cognate and non-cognate protein-ligand contacts in reaction complexes. For enzyme-DNA interactions, we typically envision specific hydrogen bonds and vanderwalls contacts between protein sidechains and DNA nucleotides. However, protein-protein interactions in multisubunit protein-DNA complexes also can contribute to specificity. For example, Cre recombinase prefers to recombine identical 34 bp LoxP DNA substrates that contain a symmetric dyad of 13 bp protein-binding sequences because it is a homotetramer. Cre-LoxP recombination has been extensively utilized to promote chromosome rearrangements in living cells, but recognition sites must be inserted synthetically because of the lack of naturally-

Notes

Zinc finger-based therapeutics for the genome

M. Bhakta, K. Brayer, V. Brondani, A.T. Ooi, and D.J. Segal
Department of Pharmacology
4513 Genome and Biological Sciences Facility

How can we use the information in the human genome to improve human health? Our lab is developing DNA recognition tools based on modified zinc finger DNA-binding domains for use in functional genomics and gene therapy. There are more than 4,500 zinc finger domains encoded in our genome. Our studies try to understand the purpose of all these domains, learn how they accomplish their function, then try to use their abilities to create new tools that benefit human health and biological studies. Current applied work aims to engineer targeted DNA endonucleases and sequence biosensors. These reagents are being applied in the diagnosis and treatment of cardiovascular disease and HIV/AIDS.

Pharmacological Inhibition of Soluble Epoxide Hydrolase in Cultured Cardiomyocytes Attenuates the Hypertrophic Response: Potential Role for Epoxyeicosatrienoic Acid in Hypertrophy

Kathy Tran
Dr. Bruce Hammock's lab
Department of Entomology
90 Briggs Hall

The soluble epoxide hydrolase (sEH) is a homodimeric enzyme with a monomeric unit of 62.5 kDa. It is a ubiquitous enzyme that is involved in the metabolism of arachidonic acid epoxides such as epoxyeicosatrienoic acid (EET). EETs are important endogenous mediators of blood pressure regulation, cell growth and inflammation. Upon hydrolysis by sEH, the epoxides are converted to the corresponding diols which are significantly less active in regulating blood pressure and inflammation. Many cardiovascular diseases result from malfunctions in the regulatory process of blood pressure control. One such disease is cardiac hypertrophy. Hypertrophy is initially a compensatory reaction to stresses on the heart (such as pressure overload). Since cardiac cells are terminally differentiated, they

Early pathfinding in chick melanoblasts is Ednrb2 dependent

Melissa.L. Harris and Carol A. Erickson
Section of Molecular and Cellular Biology
3133 Life Sciences

Melanoblasts, a population of neural crest-derived cells, migrate along a dorsolateral route between the ectoderm and the somite while other neural crest cells are inhibited from entering this path. This pathway choice has been attributed to unique cell autonomous migratory properties acquired by neural crest cells upon their restriction to the melanoblast lineage. In mouse trunk, melanoblasts expressing the c-Kit receptor tyrosine kinase undergo chemotaxis toward the ligand, steel factor, produced by the dermamytome. Once in the dorsolateral path, mouse melanoblasts then require the G-protein-coupled receptor, EDNRB, in order to maintain migration past the dorsal lip of the dermamyotome. We show in chick trunk that the requirement for these two receptors is temporally different. ShRNA directed against *c-kit* during early pathfinding does not result in aberrant dorsolateral migration. However, shRNA targeting chick *Ednrb2*, a melanocyte-specific endothelin receptor subtype, results in an absence of affected cells entering the dorsolateral path, suggesting that it is required for directed migration. This hypothesis is corroborated by our expression studies showing that early migrating, non-melanogenic neural crest cells can be induced to migrate dorsolaterally if they express *Ednrb2.* We propose that in chick, Ednrb2 is required for early migration from the neural tube to the dorsolateral path while c-Kit may be important in regulating the later movement of melanoblasts into the epidermis. Assaying the function of these two receptors during different times in development through additional shRNA and chemotaxis studies should solidify the roles of c-Kit and EDNRB2 in avian melanoblast pathfinding.

occurring LoxP sequences. The problem has been partially overcome by use of altered-specificity Cre mutants that recognize different 13 bp repeats, but the requirement for symmetric identical sites remains. If the homotetramer symmetry could be broken, then recombination could be carried between non-symmetric non-identical sequences, like those that are more likely to occur in natural genomes. In my lab, we study substrate specificity and reaction mechanism in Cre-LoxP recombination. In our previous work, we studied the structural mechanisms of DNA recognition at the Cre-LoxP interface. More recently, we began to probe the influence of the symmetric intersubunit contacts and harnessed them to change Cre's substrate specificity. Using engineered "exclusive" subunit interfaces in combination with altered DNA binding mutants, we can direct recombination to specific asymmetric Lox sites. In addition, unexpected changes in reaction directionality were also realized because of the altered interface affinities. These data both aid in understanding specificity mechanisms in multimeric DNA binding proteins and in obtaining useful Cre variants for chromosome manipulations.

Notes

Arcadia Biosciences
Dr. Claire M. McCallum
202 Cousteau Place, Suite 200
Davis, CA 95616

The genomes of increasing numbers of organisms have been entirely sequenced, from single-celled microbes (Eisen et al., 2002) to man (Lander et al., 2001; Venter et al., 2001). The availability of this genomic information has resulted in a shift of research emphasis away from gene identification to determination of gene function. Thus reverse genetic strategies have become increasingly important. Reverse genetics is broadly defined as identifying gene function from the starting point of gene sequence. A number of transgenic methods have been developed to facilitate reverse genetics. Arcadia is using a non-transgenic method of reverse genetic screening called TILLING® (Targeting Induced Local Lesions In Genomes). TILLING combines mutagenesis of seeds with an alkylating agent such as ethyl methanesulfonate (EMS) with the identification of induced alterations in target genes of interest (Colbert et al., 2001; McCallum et al., 2000). TILLING has several advantages as a reverse genetic method. First an allelic series in each target gene can be rapidly generated. Second, the identified mutation is the molecular marker. Third, the DNA library is a valuable long-term resource. Finally, because the modified plants are not transgenic, there is a reduced time to market and reduced regulatory costs. We have prepared mutagenized populations of several important crops, including tomato, wheat, soybean, rice and lettuce. The application of our method in all of these organisms has resulted in the discovery of many novel single nucleotide polymorphisms (SNPs). We have performed more than 2.5 million TILLING assays for induced novel alleles in commercially relevant gene targets and have identified novel variants of all of these targets. To date, we have identified 2,062 mutations in 72 genes in six crop species. We will describe this work in more detail and present phenotypic data on a selection of target genes.

Analysis of close stable homolog juxtaposition during meiosis in mutants of *saccharomyces cerevisiae*

Doris Y. Lui, Tamara L. Peoples-Holst, Joshua Chang Mell, Hsin-yen Wu, Eric W. Dean and Sean M. Burgess
Section of Molecular and Cellular Biology
132 Briggs Hall

Meiosis is the process by which a single round of DNA replication is followed by two successive nuclear divisions to generate haploid gametes. During the first division, homologous chromosomes are segregated. To prevent aneuploidy, accurate segregation of homologous chromosomes is promoted by the pairing of homologous chromosomes. We have used a site-specific recombination (Cre/*loxP*) assay in *Saccharomyces cerevisiae* to examine the close juxtaposition of homologous chromosome pairs during meiosis in a series of mutants defective in recombination, chromatin structure, or intracellular movement. Red1, a component of the chromosome axis, and Mnd1, a chromosome binding protein that facilitates interhomolog interaction, are critical for achieving high levels of allelic interaction. Homologous recombination factors (Sae2, Rdh54, Rad54, Rad55, Rad51, Sgs1) aid in varying degrees in promoting allelic interactions, while the Srs2 helicase appears to play no appreciable role. Ris1 (a *SWI2*/*SNF2* related protein) and Dot1 (a histone methyltransferase) appear to play minor roles. Surprisingly, factors involved in microtubule-mediated intra-cellular movement (Tub3, Dhc1 and Mlp2) appear to play no appreciable role in homolog juxtaposition, unlike their counterparts in fission yeast. Taken together these results support the notion that early to mid stages of meiotic recombination contribute to high levels of homolog interaction during budding yeast meiosis. We have also developed a physical assay that reports on Cre/*loxP* interactions between chromosomal loci in real time by using quantitative PCR to analyze mutants with reduced return-to-growth viability.

11:10 AM

Keynote Speaker

Dr. Kenneth Burtis
Dean, College of Biological Sciences

Serendipity in science: the twisting path from 237 Briggs to 207 Life Sciences

An alumni of UC Davis, Kenneth Burtis earned his B.S. in biochemistry in 1976, and worked as a research associate for Professor Roy Doi. After earning his doctorate in biochemistry from Stanford Medical School in 1985 and completing postdoctoral research in molecular genetics at Stanford, Burtis returned to UC Davis in 1988 as an assistant professor of genetics.

Burtis' main research interest is DNA repair in the model organism Drosophila. *He was a participant in the* Drosophila *Genome Project. Burtis has served as associate director of the UC Davis Genome Center, vice chair of the Section of Molecular and Cellular Biology, and chair of the Genetics Graduate Group. Burtis also served as associate dean for undergraduate academic programs.*

The College of Biological Sciences was created in July 2005 from the Division of Biological Sciences. After serving one year as interim chair, Burtis was appointed the first dean of the College of Biological Sciences.

Notes

***In vitro* assays reveal multiple pathways for the import of chloroplastic β-barrel membrane proteins**

Shih-Chi Hsu
Dr. Kentaro Inoue's Lab
Department of Plant Sciences
131 Asmundson

Beta-Barrel proteins are found in the outer membranes of Gram-negative bacteria, and mitochondria and chloroplasts of eukaryotic cells[1]. They play roles essential for viability of these organisms[2,3]. The mechanisms of their targeting and insertion in Gram-negative bacteria and mitochondria have been elucidated recently[2,3], whereas those of chloroplastic proteins remain largely unexplored. Five putative β-barrel membrane proteins, Toc75, OEP21, OEP24, OEP37 and OEP80, have been identified in chloroplasts[4-7]. Among them, the protein translocation channel Toc75 has a unique bipartite transit peptide in its N-terminus and its targeting to chloroplasts partly depends on the general pathway[5]. By contrast, other four proteins lack apparent transit peptides and thus may not depend their targeting on the general pathway[4-7]. In order to better understand the molecular details of chloroplast development and also membrane protein biogenesis, we examined the *in vitro* import requirements of the chloroplastic β-barrel membrane proteins. First, targeting of OEP37 was found dependent on the general pathway. Second, we showed that import of OEP24 and OEP80 was independent of the general pathway but required nucleotide triphosphates (NTPs). Finally, OEP21 import was found independent of both the general-pathway and NTPs, but required proteinaceous components in the outer membrane. These results suggest the presence of multiple pathways for β-barrel membrane protein insertion into chloroplasts.

1. Wimley, 2003, *Curr Opin Struct Biol.* 2. Voulhoux *et al*., 2003, *Science*. 3. Kozjak *et al*., 2003, *JBC.* 4. Bölter and Soll, 2001, *EMBO J.* 5. Inoue and Potter, 2004*, Plant J.* 6. Hinnah *et al.*, 1997, *EMBO J.* 7. Goetze *et al.*, 2006, *JBC.*

Poster Abstracts

Cellular and Proteomic Probes for Detecting Redox-Regulated Signal Transduction Pathways

Christina Takanishi
Dr. Matthew Wood's lab
Department of Environmental Toxicology
4330 Meyer Hall

Oxidative damage by reactive oxidative species (ROS) poses a common threat for all aerobic organisms and is at the root of many structural and metabolic defects that can lead to cellular dysfunction. Though it is known that ROS plays a major role in oxidative damage, there is increasing evidence that ROS has a larger cellular effect due its role in many signal transduction pathways. The association of oxidative stress in the aging process and human diseases has elicited great interest in developing an *in vivo* ROS biosensor that can be used to measure and monitor the impact of oxidative stress on biological systems. I am currently developing two novel ROS sensing probes using the redox sensitive yeast transcription factor Yap1. The first ROS probe exploits the observation that Yap1 contains reactive cysteine residues that form reversible and transient disulfide bonds in response to increased levels of ROS. I am expressing Yap1 protein fragments in vivo, trapping the disulfide bonded protein complexes that form in response to ROS and using proteomics to identify the protein complexes. The second ROS probe exploits the observation that Yap1 undergoes dramatic conformational changes in response to disulfide bond oxidation and reduction. I am using the three-dimensional structure of Yap1 to engineer a fluorescence resonance energy transfer (FRET)-based activity reporter that can be used to monitor oxidative stress in live cells and intact organisms. Preliminary data for both probes show promising results and further characterization is underway.

11:35 AM

Presentation of the Lifetime Achievement Award

Dr. James DeVay
Professor Emeritus of Plant Pathology

James DeVay earned his B.S. in plant pathology with a specialization in biochemistry at the University of Minnesota in 1949. He continued his education at the University of Minnesota, where he earned his doctorate in plant pathology with a specialization in biochemistry in 1953. After graduating, DeVay was appointed as a faculty member of the Department of Plant Pathology at the University of Minnesota, where he quickly rose to the rank of associate professor.

DeVay was recruited to the relatively new Department of Plant Pathology at UC Davis in 1959 where he became full professor in 1965. His early research interests at Davis focused on diseases of stone fruits, and along with key collaborators he is credited for characterizing the bacterial canker disease of stone fruits caused by Pseudomonas syringae *pv.* syringae. *This research resulted in industry changes to reduce yield losses and tree mortality. Later in his career at Davis, DeVay focused on diseases of cotton including the epidemiology of wilt of cotton, and disease management.*

In 1970, the UC Davis Division of Biological Sciences was established as an inter-college unit, under the College of Letters and Sciences and the College of Agricultural and Environmental Sciences. Shortly after its establishment, James DeVay was appointed to associate dean of the division, holding the position from 1975-1979. After has tenure as associate dean DeVay continued his research until becoming professor emeritus in 1991. Amassing over 280 publications his contributions to research, administration, and teaching have been invaluable to the growth of biological sciences at UC Davis. We are proud to present James DeVay as the first recipient of the Biochemistry and Molecular Biology Lifetime Achievement Award.

Notes

2:50 PM

Dr. Bruce W. Draper
Department of Molecular and Cellular Biology
Life Science 3129

Zebrafish gametogenesis: A model system for the study of stem cell biology

Our lab is interested in the genetic mechanisms that regulate germline development and function in vertebrates. We use the zebrafish to study fundamental aspects of germ cell biology, with an emphasis on germline stem cell (GSC) formation and maintenance. Zebrafish have several advantages for these studies. First, adult zebrafish females can produce hundreds of eggs every week, so GSC are enormously active. Second, unlike many systems investigating stem cell biology, it is possible in zebrafish to study GSC in the context of their normal environment or niche, the gonad. Third, forward genetic screens for genes that regulate germline development are feasible in zebrafish and we anticipate identifying both genes that function cell autonomously in germ cells as well as those that are required for niche formation and/or function. Lastly, we have developed a reverse genetic resource for zebrafish that makes it possible to knock-out candidate genes with potential roles in germline development- an approach that has so far led to the determination that *nanos1* and *ziwi* have essential and evolutionarily conserved roles in maintaining germline stem cells in zebrafish. Our approach promises to enrich our understanding of germ cell biology and to elucidate both conserved and novel aspects of the genetic pathway regulating germline formation and function in vertebrates.

Notes

Session III

2:00 PM

Edward Seikel
Dr. James Trimmer's Lab
School of Medicine, Pharmacology
3617A Genome and Biological Sciences Facility

Voltage Gated Potassium Channel Modulation by Diverse Auxiliary Subunits

Kv4 potassium channels regulate action potentials in neurons and play a role in the molecular mechanism underlying learning and memory. Kv4 channel activity is regulated through phosphorylation of the channel. In neurons, Kv4 channels are constitutively phosphorylated, but in heterologous cells this phosphorylation is dependent upon co-expression of Kv4 with at least one of its identified auxiliary subunits. Modulation of Kv4.2 by the Ca^{2+} binding, cytoplasmic KChIP family of auxiliary subunits is well established, but little is known of the role for another auxiliary subunit, the Type II transmembrane protein, DPPX. We co-expressed DPPX with Kv4.2 and found a dramatic redistribution of Kv4.2, releasing it from the endoplasmic reticulum and allowing trafficking of the channel to the plasma membrane. We found that co-expression of DPPX with Kv4.2 also leads to fundamental changes in phosphorylation, detergent solubility, and stability that reconstitute the molecular properties of the channel in native cells. These effects parallel the changes caused by the co-expression of Kv4.2 with KChIP1-3. KChIP4, which negatively influences the impact of other KChIPs through what is possibly a direct competition with the KChIP binding site, also inhibits the effects of DPPX on Kv4.2 despite DPPX having a different binding site on Kv4.2. The association of Kv4.2 with either KChIP 1-3 or DPPX may lead to many similar changes in channel properties, however, the vastly different structures of these two auxiliary subunits, along with our results, show that they may be regulating Kv4.2 through different mechanisms.

Notes

2:25 PM

Edward Kraft
Dr. Judy Callis's Lab
Section of Molecular and Cellular Biology
103 Briggs Hall

Functional Analysis of the ACH family of E3 ligases in *Arabidopsis*

Ubiquitin-mediated proteolysis is one of the primary means for protein turnover in eukaryotic cells. As much as 5% of the *Arabidopsis* proteome is predicted to be involved in ubiquitination processes. Ubiquitination involves an enzymatic cascade including an E1, E2, and E3. One type of E3 protein involved in this process is the RING domain-containing E3 ligases. One subgroup of RING E3 ligases in *Arabidopsis*, the ACHs, contain a C-terminal RING domain and a previously uncharacterized and evolutionarily conserved N-terminal domain. To date all five members of this family have been cloned and tested for activity as E3 ligases *in vitro*. All five members are active in ubiquitination with E2s of the UBC8 family of Arabidopsis. Yeast-two hybrid analysis identified six interacting proteins. One of the interacting proteins, IXI, lacks any previously characterized domains and is targeted for ubiquitination by ACH family members. In addition, another identified interacting protein, CHN, dramatically enhances the ubiquitination activity of E3 ligases in general. CHN does not have any previously characterized domains. CHN also enhances the ubiquitination of IXI mediated by ACH5. Efforts are currently underway to determine the cellular function of this family of E3 ligases, the function of the target protein IXI, and the ubiquitination rate enhancing properties of CHN.