

**26TH MBI LAKE ARROWHEAD
RESEARCH CONFERENCE
& ANNUAL RETREAT**



OCTOBER 15-17, 2004

**UCLA LAKE ARROWHEAD CONFERENCE CENTER
LAKE ARROWHEAD, CA**

26TH MBI LAKE ARROWHEAD RESEARCH CONFERENCE
OCTOBER 15-17, 2004

Friday

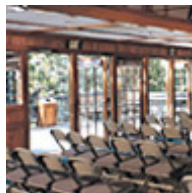
4:00 Check-in
5:30 Social Hour with hors d'oeuvres

6:30 - 7:45	DINNER
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7:45 Welcome

Session I: Immunology, Microbiology & Virology (Chairs: Wen Shi & Janine Bekker)

- 8:00-8:15 **Peter Bradley (new MIMG Faculty, former MBIDP grad)**
How does toxoplasma invade and survive in human host cells? The role of novel secretory organelles called rhoptries
- 8:20-8:30 **Dawn Hower (Shi lab, MBIDP grad)**
Identifying chemotaxis protein interactions using a Treponema denticola yeast two-hybrid genomic library
- 8:35-8:45 **Lamei Chen (C. Lee lab, Chem grad)**
Positive selection detection in 40,000 HIV-1 sequences automatically identifies drug resistance and positive fitness mutations in HIV protease and reverse transcriptase
- 8:50-9:00 **June Round (Miceli lab, MIMG grad, C&MB trainee)**
Dlgh1 coordinates actin polymerization, synaptic TCR and lipid raft aggregation, and effector function in T cells
- 9:05-9:15 **Brian Zarnegar (Genhong Cheng lab, MIMG grad)**
Comparing the contribution of type 1 (p50-dependent) and type 2 (p52-dependent) NF- κ B activation pathways in cell survival, proliferation, homotypic aggregation and specific gene regulation of murine primary B-lymphocytes
- 9:20-9:30 **Vladimir Ramirez-Carrozzi (Smale lab, MIMG postdoc)**
The SWI/SNF chromatin-remodeling complex mediates LPS-inducible expression of IL-12 p40
- 9:35-9:45 **Ashley Forbes (Braun lab, MBIDP grad)**
Enhancing Membrane Possibilities: EMP2 regulates membrane composition



10:00-MIDNIGHT	SOCIAL TIME (POKER - MUSIC - CONVERSATION - ETC.)
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Saturday

8:00 - 8:45	BREAKFAST
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Session II: Signal Transduction and Disease (Chairs: Fuyu Tamanoi & Parthive Patel)

- 9:00-9:30 **Bruce Torbett (guest speaker from Scripps)**
Role of dmp1 isoforms in cellular regulation
- 9:40-10:10 **Steve Young (new Medicine/Cardiology Faculty, formerly UCSF)**
Mouse models for precocious aging
- 10:20-10:30 *Break*
- 10:30-
10:40 **John Streicher (Y. Wang lab, Anesthesiology grad, MCIP trainee)**
A role for MAPKAPK-2 in mediating the p38 regulation of cardiomyopathy
- 10:45-
10:55 **Gang Lu (Y. Wang lab, Anesthesiology grad)**
An alternative p38 activation pathway by TAB1 mediates different downstream effects in cardiac myocytes from MKK3
- 11:00-
11:10 **Janine Bekker (Crosbie lab, MBIDP grad)**
Novel mechanisms for microtubule-based protein interactions
- 11:15-
11:25 **Jessica Russell (Hill lab, MIMG grad)**
GAS11, the mammalian homolog of a dynein regulatory complex subunit, colocalizes with dynein 2 and the Golgi apparatus
- 11:30-
11:40 **Woj Wojtowicz (Zipursky lab, Biol Chem grad)**
Alternatively spliced drosophila Dscam axon guidance receptors exhibit isoform-specific homophilic binding

12:00-1:00	LUNCH
1:00-4:30	FREE TIME

- 4:30 **Poster Session (All posters need to be up by 4:30 for judges)**
and Social Hour with hors d'oeuvres

6:30-8:00	DINNER
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- 7:30-7:45 **MBIDP Awards Presentation (during dessert)**
Presented by Mike Carey, MBIDP Faculty Advisor
Paul D. Boyer Outstanding Teaching Awards (2)
Amgen Dissertation Year Awards (2)
- 8:00-8:10 **Seung-Hye Jung (Banerjee lab, MBIDP grad)**
Characterization of signaling pathways and organ structure in Drosophila hematopoiesis
- 8:15-8:25 **Mike Strong (Eisenberg lab, MBIDP grad)**
Genomes, maps and modules: navigating the M. tuberculosis genome

Saturday continued

Session III: Genes, Genomics and Gene Regulation (Chairs: Arnie Berk & Mike Strong)

- 8:30-8:45 **Arnie Berk (MIMG Faculty)**
Mediator requirement for a post-recruitment step in transcription initiation
- 8:50-9:05 **Guillaume Chanfreau (Chem & Biochem Faculty)**
Genomic studies of gene expression control by eukaryotic RNase III
- 9:10-9:20 **Sarah Villa Dolan (S. Clarke lab, Chem & Biochem grad, C&MB trainee)**
First evidence in eukaryotes of active protein L-isoaspartate/D-aspartate O-methyltransferase enzymes encoded by two genes is found in Arabidopsis thaliana
- 9:25-9:40 **Jon Braun (Path & Lab Medicine Faculty)**
Dressing up: targeting proteins to the plasma membrane with EMP2
- 9:45-10:00 **Siavash Kurdistani (new Biol Chem Faculty, formerly Grunstein lab)**
Mapping global histone acetylation patterns to gene expression
- 10:05-10:15 **Sean Thomas (Campbell lab, MBIDP grad)**
Transcription initiation complexes in kinetoplastids
- 10:20-10:30 **Yue Liu (Berk lab, Postdoc)**
Overexpression of adenovirus 2/5 E1B 55K oncoprotein leads to aggresome formation and sequestration of the MRN complex to the aggresome

10:30-MIDNIGHT	SOCIAL TIME (POKER - MUSIC - CONVERSATION - ETC.)
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Sunday

8:00 - 8:45	BREAKFAST
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8:50 **Poster Awards Announcement & Presentation**

Session IV: Bioinformatics, Structural & Chemical Biology and Evolution

(Chairs: Stan Nelson & Brian O'Connor)

- 9:00-9:15 **Julian Whitelegge (Psychr & Biobehav Sci Faculty)**
Subtle modification of isotope ratio proteomics; an integrated strategy for expression proteomics
- 9:20-9:30 **Feng Qiao (Bowie lab, Chem & Biochem grad)**
Derepression by depolymerization: structural insights into the regulation of Yan by Mae
- 9:35-9:50 **Steve Cole (Med-Hemat & Onc Faculty)**
Moving from which to why: computational analysis of the determinants of gene expression
- 9:55-10:05 **Janel Laidman (Yeates lab, Chem & Biochem grad)**
Two strategies for self-assembling nanoscale structures from protein

Sunday continued

- 10:10-10:25 *Break*
- 10:30-10:45 **Feng Guo (new Biol Chem Faculty, formerly U Colorado-Boulder)**
Structure of the Tetrahymena ribozyme: base triple sandwich and metal ion at the active site
- 10:50-11:00 **Celia Goulding (Eisenberg lab, Postdoc)**
From outside to inside or vica versa! Assembling systems with TB structural genomics
- 11:05-11:20 **Clifford Brunk (Ecology & Evolutionary Biology Faculty)**
Identification of protein coding genes based on codon utilization
- 11:25-11:30 **Closing Remarks**

11:30-1:00	CHECK OUT - LUNCH
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Session Abstracts

(Listed Alphabetically by Speaker's Last Name)

Janine M. Bekker^{1,2}, Jessica N. Russell³, Kent L. Hill^{2,3}, and Rachelle H. Crosbie^{1,2}

¹Department of Physiological Science, ²Molecular Biology Institute

³Department of Microbiology, Immunology and Molecular Genetics, UCLA

Novel mechanisms for microtubule-based protein interactions

The human Growth Arrest Specific 11 protein (*Gas11*) is homologous to a novel family of cytoskeletal proteins that are instrumental in microtubule-based cellular locomotion. The *Chlamydomonas reinhardtii* ortholog, PF2, is an essential component of the dynein regulatory complex where it is tightly associated with axonemal microtubules and is required for proper cell motility. Trypanin, the *Gas11* homolog in *Trypanosoma brucei*, is an integral component of the flagellar cytoskeleton and is required for directional, flagellum-based cell motility. Although *Gas11* is expressed along the length of sperm flagella, we have discovered that it is also expressed in cells that lack a motile flagellum. We show that *Gas11* is expressed prior to muscle cell differentiation in non-flagellated C2C12 mouse muscle cells. This is the first evidence that *Gas11* expression is not dependent on growth arrest as previously thought. Using *Gas11* isolated from C2C12 myotubes, we demonstrate for the first time that *Gas11* co-sediments with microtubules, and we further show that *Gas11* directly binds microtubules through a novel microtubule association domain, which we have named BMAD. Using deletion analysis, we identified an inhibitory domain in *Gas11* (IMAD) that blocks this interaction. In addition, we show that *Gas11* is phosphorylated and that this modification appears to regulate the interaction between *Gas11* and microtubules. Our data represent the first report of a direct interaction between *Gas11* and microtubules. Furthermore, our discovery of two novel regulatory protein domains is suggestive of new mechanisms for microtubule-based protein interactions.

Arnold J. Berk

Microbiology, Immunology & Molecular Genetics, and Molecular Biology Institute, UCLA

Mediator requirement for a post-recruitment step in transcription initiation

The MED23(SUR2) subunit of mammalian mediator complexes is required for induction of the *EGR1* immediate early gene in response to MAP kinase pathway activation in embryonic stem (ES) cells. ChIP assays were performed on wt and *sur2*^{-/-} cells to determine the step in transcription that requires SUR2. Transcription factors SRF and ELK1 were bound to serum response elements in the *EGR1* control region and histone H3 had a methylation pattern characteristic of active chromatin before addition of serum in both wt and mutant cells. ELK1 was phosphorylated at MAP kinase sites and lysines of histones H3 and H4 were acetylated rapidly in response to serum in both wt and mutant cells. Before addition of serum when there was no *EGR1* transcription, GTFs and Pol II were nonetheless bound to the promoter region to the same extent as for a highly transcribed gene. In response to serum, there was a significant increase in association of mediator complexes with the promoter region in wt cells, but not in *sur2*^{-/-} cells, and this was associated with and the appearance of Pol II in the coding region. These results indicate that the *EGR1* promoter region is assembled into transcriptionally competent chromatin with a pre-initiation complex bound to the promoter and poised to respond to activation of MAP kinase pathways. MAP kinase phosphorylation of ELK1 results in an interaction with mediator complexes via the SUR2 subunit that stimulates a post-recruitment step in transcription initiation. Similar results were observed for the closely related *EGR2* gene. Serum also increased Pol II, TFIIB, E, and H binding to the promoter region, without causing a significant increase in TFIID or TFIIA.

Peter Bradley

Microbiology, Immunology & Molecular Genetics, UCLA

How does toxoplasma invade and survive in human host cells? The role of novel secretory organelles called rhoptries

Toxoplasma gondii is an obligate intracellular parasite in the phylum Apicomplexa that causes severe central nervous system disorders of immunocompromised (AIDS/transplant/lymphoma) individuals and birth defects to congenitally infected neonates worldwide. In addition to being a globally important pathogen in its own right, *Toxoplasma* serves as a model system for studying Apicomplexan parasites which cause a number of diseases of medical and veterinary importance. The most notable of these is *Plasmodium falciparum*, the causative agent of malaria which kills 1-2 million people each year. Other members include important pathogens such as *Cryptosporidia* (also causing an opportunistic infection in humans) and *Neospora* and *Eimeria*, which cause disease in cattle and poultry (respectively).

One defining feature of these parasites is the presence of rhoptries, unique secretory organelles that are involved in host cell invasion and establishment of the parasitophorous vacuole (PV) for intracellular survival. While secretion of the rhoptries has long been implicated in the establishment of infection, only a few rhoptry proteins have been reported and little is known about their precise role in this process. In order to understand the role of the rhoptries in invasion/PV formation, we have developed a method for isolating highly purified rhoptries and analyzed these organelles using both proteomic and monoclonal antibody approaches. Our proteomic approach has identified a large number of novel genes encoding the major protein constituents of the rhoptry fraction and we are confirming their

localization using anti-peptide antibodies and immunofluorescence staining. Several of these proteins have clear homologues in other Apicomplexans while others appear to be unique to *Toxoplasma*. In addition, our monoclonal antibody approach has highlighted a rhoptry protein that traffics from the rhoptries to the host cell nucleus rapidly after invasion. This is the first *Toxoplasma* protein shown targeted to the host nucleus and its putative function suggests a novel role for the rhoptries in modulating the host cell during infection.

Jonathan Braun

Pathology & Laboratory Medicine, UCLA

Dressing up: targeting proteins to the plasma membrane with EMP2

Epithelial membrane protein 2 (EMP2) is a representative of a distinct class of four-transmembrane proteins exemplified by PMP/22, the common genetic target of Charcot-Marie-Tooth disease. EMP2 is highly expressed in several cell types, including epithelial cells of the endometrium and retina. Our laboratory with its collaborators have demonstrated that EMP2 plays an important role in the function of each of these cell-types. In the endometrium, EMP2 expression is strictly required for competence in blastocyst implantation. Moreover, EMP2 expression is hormonally regulated in endometrium, providing a physiologic control of the episodic function of endometrium during estrus. In the retina, the pigmented epithelium maintains retinal health by reprocessing photoreceptor lamella, and EMP2 expression is required for proper levels of integrins required for this function.

Our current projects address basic and translational issues of EMP2 function. EMP2 is remarkable among tetraspan proteins because it not only selectively associates with integrins, but actually regulates integrin surface expression and receptor function. Recently, we have previously shown that EMP2 colocalizes with beta 1 and alpha 6 integrins, but not alpha 5 integrin, and that EMP2 reciprocally regulates cell surface expression and cognate functions of alpha 6 and alpha 5 integrin (Wadehra, 2002, JBC). Recently, we have also shown that EMP2 levels profoundly affect expression of caveolin-1, with over-expression of EMP2 decreasing caveolin-1 protein levels and reduction of EMP2 levels increasing caveolin-1 expression (Wadehra, 2004, Mol Bio Cell). Additionally, EMP2 increases surface expression of GPI-linked proteins, a membrane component selectively associated with lipid rafts. Collectively, these findings indicate a role for EMP2 in plasma membrane trafficking and formation of lipid rafts. Our studies are presently addressing the biochemical mechanism by which EMP2 contributes to the regulation of integrin trafficking and caveolin dynamics. Since EMP2 also regulates expression of other classes of surface proteins, including class I MHC molecules like H-2D, this mechanism may constitute an important but unappreciated mode of cellular control of receptor repertoire. We have formulated a mechanistic model in which EMP2 facilitates selective transport of receptors and address-bearing molecules from post-Golgi sorting vesicles to the cell surface.

As described above, EMP2 mediates important functions of epithelial cell-types at the center of reproductive conception and photoreceptor health. These insights suggest that EMP2 may be a useful target for diagnosis or therapy of corresponding medical problems, including infertility and macular degeneration. Translational studies in the lab are devoted to mouse model systems to assess the role of EMP2 in these disease states, and strategies to target EMP2-related functions as the basis of new therapeutic interventions.

Cliff Brunk, Jill Skoczylas and Shahe Soghomonian

Ecology & Evolutionary Biology, UCLA

Identification of protein coding genes based on codon utilization

The identification of genes in genomic sequences is challenging. The most popular method for identifying genes involves a hidden Markoff model approach. However, protein coding genes are unique among DNA sequences of the genome in that they are composed of codons which invariably have special constraints. In fact, the relative codon utilization is characteristic of an organism. Thus it is plausible that protein coding sequences can be identified in a genome based primarily on the match of the potential codons in a region of DNA to the codon utilization table (CUT) characteristic of the organism. Generally this relationship is believed to be insufficiently robust for the reliable identification of genes. However, in an organism with a highly skewed CUT such as *Tetrahymena thermophila*, it may be possible to use this approach for gene identification. The complete genome sequence of *T. thermophila* has been determined, but not yet annotated.

Toward the end of identifying *T. thermophila* genes, we developed an algorithm capable of scanning a DNA region and returning a potential of the presence of a protein coding gene at each point along the genome. Our algorithm presents the match of the codons in a specific region to the CUT characteristic of the organism. A "hamming" window is scanned along the genome sequence comparing the observed codons in each of the six reading frames to the characteristic CUT of the organism. This match is then normalized to the match expected for random codons having the base composition found in the window. Finally, the match occurring in each frame is compared to the matches occurring in the sum of the other frames. This algorithm is capable of providing visual identification of many of the known genes in the *T. thermophila* genome.

A further refinement of this approach is provided by a weighting of the various codons. Several codons have much higher informational content than the average codon. Using a properly weighted CUT significantly enhances the ability of the algorithm to identify protein coding genes. An examination of the available genes suggests that there may be several distinct CUTs in *T. thermophila*. The presence of different CUTs for highly expressed and infrequently expressed genes have been demonstrated for bacteria and yeast. Our approach is via a statistical analysis of the CUT for individual genes rather than a grouping of genes on supposed level of gene expression, and then the production of a composite CUT for each group.

The combination of a sensitive algorithm for detecting the match of a specific reading frame in a region of the genome to an

appropriately weighted CUT of a specific class of genes appears to provide a robust means of identifying the genes in the *T. thermophila*.

Guillaume Chanfreau

Chemistry & Biochemistry, UCLA

Genomic studies of gene expression control by eukaryotic RNase III

Rnt1p is the only member of the RNase III family of endonucleases in *S. cerevisiae*. In contrast to other members of this family of endonucleases, it recognizes and cleaves double-stranded RNA capped by RGNN-type tetraloops. We study the mechanisms of the specificity of RNA recognition by Rnt1p and its roles in the regulation of gene expression pathways. To exhaustively identify the gene expression pathways regulated by Rnt1p, we have used a combination of experimental gene expression studies by microarrays, and of whole-genome theoretical folding to search for dsRNA regions capped by RGNN-type tetraloop. These approaches have led to the identification of several novel function for Rnt1p. We will present results showing that Rnt1p participates in the degradation of unspliced pre-mRNAs coding for ribosomal proteins, of several mRNAs coding for proteins involved in the iron starvation response, as well as of a novel acireductone dioxygenase encoding mRNA.

Lamei Chen, Alla Perlina, Christopher J. Lee

Chemistry & Biochemistry, UCLA

Positive selection detection in 40,000 hiv-1 sequences automatically identifies drug resistance and positive fitness mutations in HIV protease and reverse transcriptase

Drug resistance is a major problem in the treatment of AIDS, due to the very high mutation rate of human immunodeficiency virus (HIV) and subsequent rapid development of resistance to new drugs. Identification of mutations associated with drug resistance is critical for both individualized treatment selection and new drug design. We have performed an automated mutation analysis of HIV Type 1 (HIV-1) protease and reverse transcriptase (RT) from approximately 40,000 AIDS patient plasma samples sequenced by Specialty Laboratories Inc. from 1999 to mid-2002. This data set provides a nearly complete mutagenesis of HIV protease and enables the calculation of statistically significant *Ka/Ks* values for each individual amino acid mutation in protease and RT. Positive selection (i.e., a *Ka/Ks* ratio of >1 , indicating increased reproductive fitness) detected 19 of 23 known drug-resistant mutation positions in protease and 20 of 34 such positions in RT. We also discovered 163 new amino acid mutations in HIV protease and RT that are strong candidates for drug resistance or fitness. Our results match available independent data on protease mutations associated with specific drug treatments and mutations with positive reproductive fitness, with high statistical significance (the *P* values for the observed matches to occur by random chance are $10_{-5.2}$ and $10_{-16.6}$, respectively). Our data indicate that positive selection mapping is an analysis that can yield powerful insights from high-throughput sequencing of rapidly mutating pathogens.

Steve Cole

Medicine/Hematology & Oncology, UCLA

Moving from which to why: Computational analysis of the determinants of gene expression

We have developed several new bioinformatic tools to identify up-stream influences on microarray gene expression profiles. In the context of cancer, major determinants include aberrant transcription factor activity and alterations in chromosomal structure that lead to gene amplification or deletion. To monitor transcription factor activity, we have developed the Transcription Element Listening System (TELiS: <http://www.telis.ucla.edu>) - a database mapping the incidence of 193 vertebrate transcription factor-binding motifs in the upstream sequences of all human, rat, and mouse genes. Population-based statistical tests allow TELiS to identify specific transcription factors activated in both in vitro and in vivo model systems. To map chromosomal alterations involved in aberrant cell growth, we have developed the Expression-based Inference of Chromosomal Alteration (EICA) algorithm. EICA utilizes recursive binary partitioning of microarray differential expression data to map the spatial bounds of chromosomal amplifications and deletions. In studies of known chromosomal aberrations, EICA has detected amplifications and deletions with greater precision than conventional cytogenetic techniques. EICA may also be useful in assessing regional epigenetic influences. After presenting the analytic models underlying TELiS and EICA and some simple validation studies, I will survey some recent analyses of genome-regulatory processes in cancer.

Ashley Forbes

Molecular Biology IDP, UCLA

Enhancing Membrane Possibilities: EMP2 regulates membrane composition

Cell surface proteins mediate a vast array of cell functions, including adhesion, proliferation, signal transduction, and uptake of molecules. The composition of plasma membrane receptors is dynamically controlled both during development and during physiologically induced hormonal changes. However, the mechanisms regulating these inducible changes are poorly understood. We have established epithelial membrane protein 2 (EMP2) as a key regulator of surface expression for variety of membrane proteins, including integrins, MHC class I, GPI-linked proteins, and caveolins (Wadehra, 2002, JBC; Wadehra, Int Immunol, 2003, Wadehra, MBC, 2004). As a result, we have formulated a mechanistic model in which EMP2 facilitates selective transport of receptors and address-bearing molecules from post-Golgi sorting vesicles to the cell surface. Biologically, we are focusing on EMP2 in the uterine endometrium (its critical role in progesterone-regulated delivery of endometrial surface proteins in the uterine window of implantation competence), and retinal pigmented epithelium (its role in maintaining photoreceptor health). Biochemically, I will show that in the model NIH 3T3 mouse fibroblast cell line, EMP2 complexly regulates expression of alpha 5, alpha 6, and beta 1 integrins, and profoundly suppresses expression of Caveolin-1. EMP2 selectively affects Caveolin-1 protein, as over-expression of EMP2 decreases Caveolin-1 mRNA 3-fold while protein levels decline 17-fold. Currently we are examining how Caveolin-1 protein levels are being modulated. This work validates and refines our model of EMP2 as a functional regulator of a large class of cell membrane proteins, and further defines a role for EMP2 in maintaining and controlling endometrial function and photoreceptor health.

Celia W. Goulding, Marcin Apostol, Soyeon Im, Angie Parseghian, Michael R. Sawaya and David Eisenberg

UCLA-DOE, Center for Genomics and Proteomics

From outside to inside or vice versa! Assembling systems with TB structural genomics

Tuberculosis (TB) is the most deadliest infectious disease in the world and is caused by the bacterial pathogen, *Mycobacterium tuberculosis*. TB is responsible for the death of over three million people every year! In light of these devastating numbers, the TB structural genomics consortium was formed with the aims to determine *M. tuberculosis* protein structures to provide a broad structural foundation for understanding its biology and to investigate potential drug targets. Structural genomics is the large-scale determination of three-dimensional protein structures on a genome-wide scale.

Protein targets for structure determination at UCLA fall into two main categories: 1) Individual Proteins, such as proteins which are involved in essential metabolic pathways and secreted proteins whose functions include bacterial-host interactions and factors involved in virulence and pathogenicity.; 2) Protein complexes and systems, some of these complexes and networks are predicted by bioinformatic approaches and others from literature searches.

Two protein systems that are representative of both categories will be described, both bridge the cytosolic and extracellular space across the *M. tuberculosis* membrane will be described. One is a disulfide bond isomerase system, which transports electrons across the membrane to ensure correctly folded proteins in the extracellular space. The second is a novel heme-uptake pathway which potentially transports scavenged heme from the host into the cytosolic space of *M. tuberculosis*. Proteins in both these systems may serve as potential drug targets.

Feng Guo¹, Anne R. Gooding, Thomas R. Cech

Howard Hughes Medical Institute, Department of Chemistry and Biochemistry

University of Colorado, Boulder, CO 80309-0215

¹ Present address: Department of Biological Chemistry, UCLA School of Medicine***Structure of the tetrahymena ribozyme: base triple sandwich and metal ion at the active site***

The *Tetrahymena* self-splicing intron was the first ribozyme identified. As part of its splicing reaction, the ribozyme catalyzes phosphoryl transfer between guanosine and a substrate RNA strand. Here we report the first refined crystal structure of an active *Tetrahymena* ribozyme in the absence of its RNA substrate at 3.8-Å resolution. The 3'-terminal guanosine (wG), which serves as the attacking group for RNA cleavage, forms a coplanar base triple with the G264-C311 base pair, and this base triple is sandwiched by three other base triples. In addition, a metal ion is present in the active site, contacting or positioned close to the ribose of the wG and five phosphates. All of these phosphates have been shown to be important for catalysis. Therefore, we provide a picture of how the ribozyme active site positions both a catalytic metal ion and the nucleophilic guanosine for catalysis prior to binding its RNA substrate.

Dawn Hower

Molecular Biology IDP, UCLA

Identifying chemotaxis protein interactions using a *Treponema denticola* yeast two-hybrid genomic library

Treponema denticola is an anaerobic spirochete implicated in periodontal disease. Signaling cascades, functioning via protein-protein interactions, are likely important in its pathogenicity. To identify new interacting proteins and to verify hypothesized protein-protein interactions, a complete and representative *T. denticola* genomic library has been constructed for use in the yeast two-hybrid system.

Chemotaxis is implicated in the virulence of this motile pathogenic bacterium. Analysis of the completed genome of *T. denticola* has revealed new potential chemotaxis proteins, including twenty putative methyl-accepting chemotaxis proteins (MCPs). Chemotaxis protein CheW is known to interact in a ternary complex with the chemosensory MCPs, as well as with the kinase CheA. Using CheW as bait in the yeast two-hybrid system, one previously known MCP and twelve new putative MCPs were pulled out from the *T. denticola* genomic library, providing the first evidence for those twelve as functional MCPs. The known protein-protein interaction between CheW and CheA was also confirmed through this assay. The seven other putative *T. denticola* MCPs, not pulled out from the initial yeast two-hybrid screening experiments, are now being tested in one-on-one yeast two-hybrid experiments with CheW to determine if they are also likely to be functional MCPs.

In addition to new MCPs, there are other proteins identified in *T. denticola* genome analysis that may also be important for chemotaxis. Disrupting the function of the unique spirochetal CheX protein results in a chemotaxis deficient phenotype; however, the involvement of CheX in the chemotaxis signal transduction pathway is not yet known. Using the genomic library in the yeast two-hybrid system, the role of CheX may soon be uncovered by identification of its interacting protein partner(s). Other potential spirochetal chemotaxis proteins are also being studied to determine their protein interactions and potential roles in chemotaxis signal transduction.

As shown by these chemotaxis protein studies, the *T. denticola* yeast two-hybrid genomic library is proving to be a useful tool to investigate unknown protein interactions in order to better understand the pathogenesis of this motile spirochete.

Seung-Hye Jung

Molecular Biology IDP, UCLA

Characterization of signaling pathways and organ structure in *Drosophila* hematopoiesis

Drosophila serves as a good genetic model system to study hematopoiesis since the roles of important transcription factors and signal transduction pathways have been conserved through evolution. *Drosophila* has only 3 types of blood cells: plasmatocytes, crystal cells and lamellocytes, which are similar to the mammalian myeloid lineage. Our lab has shown various signaling pathways contribute to cell fate determination during hematopoiesis. Whereas crystal cell lineage is dependent on Notch signaling, plasmatocyte lineage requires both PDGF/VEGF receptor and Jak/Stat pathways. In order to better understand blood development, we have used a variety of genetic tools to study the lymph gland, the hematopoietic organ in *Drosophila*. Our studies have established that the lymph gland has 3 distinct zones that differ in the expression of molecular markers. We are extending this study to better understand how blood cells develop in the *Drosophila*.

Siavash K. Kurdistani

Biological Chemistry, UCLA

Mapping global histone acetylation patterns to gene expression

Histone acetyltransferases and deacetylases with specificities for different sites of acetylation affect common chromatin regions. This could generate unique patterns of acetylation that may specify downstream biological processes. To search for existence of these patterns and their relationship to gene activity, we analyzed the genome-wide acetylation profiles for eleven lysines in the four core histones of *Saccharomyces cerevisiae*. We find that both hyper- and hypo-acetylation of individual lysines are associated with transcription, generating distinct patterns of acetylation that define groups of biologically-related genes. The genes within these groups are significantly co-expressed, mediate similar physiological processes, share unique cis-regulatory DNA motifs, and are enriched for binding of specific transcription factors. Our data also indicate that the in vivo binding of the transcription factor Bdf1 is associated with acetylation on most lysines but relative deacetylation on H4 lysine 16. Thus certain acetylation patterns may be used as surfaces for specific protein-histone interactions, providing one mechanism for coordinate regulation of chromatin processes that are biologically related.

Janel Laidman

Chemistry & Biochemistry, UCLA

Two strategies for self-assembling nanoscale structures from protein

Nanoscale assemblies are a first step in construction of nanoscale devices. We present here a method for creating assemblies from protein building blocks using two different symmetry strategies. The first strategy takes advantage of the naturally occurring symmetry built into oligomeric proteins. Fusing oligomeric domains of proteins whose structures are known allows us to create a chimera with self assembling properties driven by the high affinity and specificity of the protein interfaces. Controlling the angle of the fusion determines the type of structure that can be built from the subunits, i.e. cages, layers, filaments and crystals. The second strategy takes advantage of the crystallographic symmetry of monomeric proteins. By using a protein with known structure and crystallization parameters, crystal contacts can be exploited for assembly of proteins into filaments and layers. We will present our latest results towards the production of self-assembling protein layers, cages and crystals from these two strategies.

Yue Liu,¹ Anna Shevchenko,² Andrej Shevchenko² and Arnold J. Berk¹Molecular Biology Institute, University of California, Los Angeles, CA90095, USA¹Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany²***Overexpression of adenovirus 2/5 e1b 55k oncoprotein leads to aggresome formation and sequestration of the mrn complex to the aggresome***

Adenovirus E1B 55K oncoprotein plays an important role in both early and late stages of virus infection. Recent studies showed that in the presence of E4orf6, E1B forms a complex with E4orf6, Cul5, Elongin B, C and Rbx1. This complex serves as an E3 ubiquitin ligase that targets p53 and the Mre11-Rad50-NBS1 (MRN) complex for protein degradation. In order to learn the role of E1B 55K in this complex and to identify new substrates, we performed immunoprecipitation assays (IP) and MALDI mass spec assays on 293 cells stably transformed with E1B 55K and found that in mock infected but not Ad5 infected 293 cells, E1B pulled down p53, Mre11 and Rad50. This implies that E1B 55K is the substrate recognition unit of the E1B/E4orf6 ubiquitin ligase and confirms that the MRN complex is a new substrate. In this assay, we also found that cyto skeleton proteins such as Drebrin E, Eplin, β -tubulin and Vimentin coimmunoprecipitated with E1B 55K. Combined with previous studies and our immunofluorescence results which showed that E1B 55K forms a large cytoplasmic complex in the absence of E4orf6, we suspected that Ad2 E1B 55K is an aggregation-prone protein, and that the large cytoplasmic complex of E1B 55K is an aggresome. We can prove this hypothesis by the following: 1. E1B 55K colocalizes with γ -tubulin, the centrosome marker in 293 and E1B 55K transfected IMR90 (human primary fibroblast) cells. 2. The E1B 55K complex is surrounded by a vimentin cage in E1B 55K transfected A549 cell and H1299 cell. 3. Treatment with the microtubule depolymerizing agent Nocodazole leads to the disruption of the E1B complex in both 293 cell and A549 cell transfected with E1B. 4. Over expression of p50/dynaminin disrupts the formation of E1B 55K aggresome. We also found that both p53 and the MRN complex colocalize with E1B 55K aggresome. Overexpression of E1B 55K leads to complete sequestration of the MRN complex in the aggresome and inhibition of Mre11 foci formation post γ -irradiation. Furthermore, E1B 55K retrovirus stably infected IMR90 cells are more sensitive to γ -irradiation than its mother cell line. These results indicate that E1B 55K might inhibit the MRN complex function in the absence of E4orf6.

Gang Lu¹, Harvey Herschman¹, Jiahuai Han², Yibin Wang¹¹Division of Molecular Medicine and Molecular Biology Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095²Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.***An alternative P38 activation pathway by TAB1 mediates different downstream effects in cardiac myocytes from MKK3***

Previous studies have shown that activation of stress-activated MAP kinase, p38 can be triggered by TGF β activated kinase binding protein 1 (TAB1) through auto-phosphorylation independent of upstream MAP kinase kinases (MKKs). Yet, the difference of downstream signaling effects between MKK and TAB1 mediated p38 activity has not been established. By overexpressing in cultured myocytes, TAB1 induced p38 phosphorylation and kinase activity. Overexpression of MKK3bE, a constitutively activated p38 specific upstream MKK, induced MKK2 phosphorylation and activity, elevated the expression level of COX-2 and TNF α . In contrast, TAB1 expression showed no significant effects compared to LacZ control. In addition, MKK3bE induced hypertrophic changes in cultured myocytes, including increase in ANF and decrease in β MHC, as well as induction of sarcomere organization, while TAB1 failed to induce significant features of hypertrophy. Furthermore, co-expression of TAB1 with MKK3bE could attenuate the downstream signaling of p38 by MKK3bE, although total intracellular p38 phosphorylation level is increased. The p38 binding domain of TAB1 was necessary and sufficient to exert this inhibitory function. By cell fractionation and immunostaining, we found TAB 1 was localized exclusively in cytosol and prevented nuclear distribution of phosphorylated p38. These data suggest that TAB1 is a novel functional modulator of p38 pathway and may play an important role in regulating the stress-signaling to different cellular compartments.

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Derepression by depolymerization: Structural insights into the regulation of Yan by Mae

Yan, an ETS-family transcriptional repressor, is regulated by receptor tyrosine kinase signaling via the Ras/MAPK pathway. Phosphorylation and down regulation of Yan is facilitated by a protein called Mae. Yan and Mae interact through their SAM domains. We find that repression by Yan requires the formation of a higher-order structure mediated by Yan-SAM polymerization. Moreover, a crystal structure of the Yan-SAM/Mae-SAM complex shows that Mae-SAM specifically recognizes a surface on Yan-SAM that is also required for Yan-SAM polymerization. Mae-SAM binds to Yan-SAM with ~1000 fold higher affinity than Yan-SAM binds to itself and can effectively depolymerize Yan-SAM. Mutations on Mae that specifically disrupt its SAM domain dependent interactions with Yan disable the derepression function of Mae *in vivo*. Depolymerization of Yan by Mae represents a novel mechanism of transcriptional control that sensitizes Yan for regulation by receptor tyrosine kinases.

Reference: Qiao, F. et. al, Cell, 2004, Derepression by depolymerization: Structural insights into the regulation of Yan by Mae, in Press

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The SWI/SNF chromatin-remodeling complex mediates LPS-inducible expression of IL-12 p40

IL-12 is a pro-inflammatory cytokine produced by activated macrophages and dendritic cells in response to microbial pathogens. Proper regulation of IL-12 expression is critical for inflammatory responses and for the development of the T helper 1 (Th1) immune response. Previous studies of the induction mechanisms for the IL-12 p40 gene in murine macrophages revealed that binding sites for NF- κ B, C/EBP, and AP-1 transcription factors are present in the p40 promoter and contribute to transcriptional induction by lipopolysaccharide (LPS) and other bacterial products. In resting macrophages, the p40 promoter is wrapped around a positioned nucleosome. This positioned nucleosome is remodeled following macrophage stimulation by LPS and appears to be essential for IL-12 p40 transcriptional activation. The remodeling event appears to be independent of c-Rel, C/EBP β and AP-1. Recently, we have identified an enhancer region approximately 10kb upstream of the p40 transcription start site that undergoes changes in chromatin structure during macrophage stimulation. Thus, critical unresolved issues include the identities of the chromatin-modifying complex and of the DNA binding proteins that recruit the remodeling complex to the promoter and enhancer regions. To determine the factors required for inducible remodeling of the positioned nucleosome at the IL-12 p40 promoter and enhancer region, we have abolished the expression of the catalytic ATPase subunits of SWI/SNF complexes BRG1 and BRM in macrophage cell lines. The silencing of both BRG1 and BRM remodelers in J774 macrophages was mediated through delivery of siRNA hairpins using a retroviral approach; and resulted in a significant decrease in restriction enzyme accessibility at both the IL-12 p40 promoter and enhancer regions, and a decrease in IL-12 p40 expression in response to LPS. Similarly, over-expression of a dominant negative BRG1, that is unable to bind and hydrolyze ATP, led to a decrease in chromatin remodeling activity at the IL-12 p40 promoter and enhancer regions and a decrease in IL-12 p40 gene expression. We have analyzed the global effects of BRG1/BRM deletion and dominant negative inhibition on LPS-inducible gene expression through microarray and real-time PCR analysis in J774 macrophages. The kinetic analysis of LPS-stimulation has revealed that the SWI/SNF complex is required for the expression of late LPS-inducible genes whereas SWI/SNF is not required for the expression of early LPS-inducible genes.

June L. Round, Tamar Tomassian, Min Zhang, Viresh Patel, Stephen P. Schoenberger, and M. Carrie Miceli
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Dlgh1 coordinates actin polymerization, synaptic TCR and lipid raft aggregation, and effector function in T cells

Lipid raft membrane compartmentalization and MAGUK-family molecular scaffolds function in establishing cell polarity and organizing signal transducers within epithelial cell junctions and neuronal synapses. Here we elucidate a role for the MAGUK protein, Dlgh1, in polarized T-cell synapse assembly and T-cell function. We find that Dlgh1 translocates to the immune synapse and lipid rafts in response to TCR/CD28 engagement and that LckSH3-mediated interactions with Dlgh1 control its membrane targeting. TCR/CD28 engagement induces the formation of endogenous Lck:Dlgh1:Zap70:WASp complexes in which Dlgh1 acts to facilitate interactions of Lck with Zap70 and WASp. In addition, suppression of Dlgh1 expression in T-cells leads to defective antigen-induced actin polymerization, synaptic raft and TCR clustering, and T-cell function. We propose Dlgh1 is imperative in the coordination of TCR/CD28-induced actin-driven T-cell synapse assembly and effector function. These findings highlight common molecular strategies used to regulate cell polarity and synapse assembly and organization in immune, neuronal and epithelial cell systems.

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GAS11, the mammalian homolog of a dynein regulatory complex subunit, colocalizes with dynein 2 and the Golgi apparatus

The mammalian protein GAS11 is part of a recently discovered family of cytoskeletal proteins represented in organisms ranging from *Giardia lamblia* to humans. GAS11 homologs in *Trypanosoma brucei* (trypanin) and *Chlamydomonas reinhardtii* (PF2) are flagellar proteins that are required for regulating flagellar beat. PF2 was previously demonstrated to be part of a dynein regulatory complex that controls axonemal dynein activity in *C. reinhardtii*. This suggests that GAS11 might similarly be involved in dynein regulation in mammalian cells and we have set out to investigate this. Interestingly, unlike trypanin and PF2, GAS11 is expressed in several mammalian cells and tissues that lack motile flagella. In COS7 cells, GAS11 is primarily associated with the detergent-insoluble cytoskeleton and exhibits a juxtanuclear localization that colocalizes with the Golgi apparatus around the centrosome. This localization is cell-cycle dependent; specifically, when cells progress through mitosis, GAS11 becomes dispersed throughout the cell much like the Golgi apparatus. Nocodazole and Brefeldin A treatments demonstrate that GAS11 localization is microtubule-dependent and imply a functional interaction between GAS11 and the Golgi. Based on the role of PF2 in dynein regulation in *C. reinhardtii*, we hypothesized that GAS11 may associate with cytoplasmic dynein 2, which has previously been implicated in Golgi assembly. Consistent with this idea, we find that GAS11 colocalizes with dynein 2, but not dynein 1 by immunofluorescence. The Golgi apparatus in COS7 cells is pericentrosomal, corresponding to the (-) ends of cytoplasmic microtubules. Bidirectional traffic along microtubules to and from this position presumably requires coordinated activation and inactivation of microtubule-dependent motors. The discovery that GAS11, the mammalian homolog of a dynein regulatory complex subunit, colocalizes with dynein 2 and the Golgi apparatus at the (-) end of cytoplasmic microtubules is consistent with the possibility that GAS11 may be involved in dynein regulation in mammalian cells.

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The role of MAPKAPK-2 in mediating the p38 regulation of cardiomyopathy

Heart failure is the number one cause of death in the United States, and the second leading cause of death world wide. Heart failure is characterized by a progressive loss of contractility, resulting in an inability to maintain cardiac output and death. Progression to heart failure involves specific changes in cardiomyocytes, including electrophysiological properties, morphology and metabolism. There is no cure for heart failure, and few effective treatments due to a lack of understanding of the mechanisms of the disease. Recent research in heart failure, however, has revealed a complex network of intracellular signaling molecules that regulate the onset and progression of this disease, yielding many exciting new insights for the mechanism of heart failure. The mitogen-activated-protein kinases (MAPK) are highly conserved signaling molecules responsible for a wide variety of cellular processes, from proliferation and motility to differentiation and apoptosis. One of three main MAPK branches is the p38 pathway, which is known to be activated by different stressors (osmotic stress, UV irradiation, endotoxin etc.) and mediates stress responses, including the production of cytokines such as TNF- α . Increased activation of p38 has been associated, with some controversy, with end-stage failing human hearts, as well as animal models of heart failure. More specifically, we have shown that activation of p38 in a mouse model leads to profound cardiomyopathy without overt hypertrophy. However, little is known about the up- and downstream activators and effectors of p38 that would mediate the role of p38 in heart failure.

One of the main downstream signaling molecules of p38 is MAPK Activated Protein Kinase-2 (MAPKAPK-2 or MK2). MK2 has been shown to be critical for stress induced production of interleukin-6 and TNF- α as well as an inducer of injury during ischemic insult to the brain. The role of MK2 in myocytes was first analyzed *in vitro* in rat neonatal cardiomyocytes. Infection of the myocytes with an adenovirus encoding a constitutively active form of MK2 was found to induce the expression of cyclooxygenase-2 and related cytokines. Furthermore, a dominant negative MK2 construct blocked the constitutively activated MKK3 (via p38) induction of Cox-2 expression and Hsp27 phosphorylation. MK2 overexpression also increased the level of p38 protein, while loss of MK2 expression led to a significant decrease. The role of tristetraprolin as the effector of MK2 induction was also examined.

In order to determine the *in vivo* role of MK2 in heart failure, MK2^{-/-} and wild type controls were subjected to chronic infusion of the β -adrenergic agonist isoproterenol or trans-aortic constriction as two different approaches to induce hypertrophy and heart failure. Isoproterenol treatment was found to induce a compensated cardiac hypertrophy without activation of the fetal gene program, while pressure overload induced hypertrophy transition to heart failure with extensive changes in the cardiac gene expression profile. The impact of MK2 knockout on the development of hypertrophy and heart failure was analyzed at the whole heart level, and at the molecular level with gene expression profiling and analysis of protein expression. Our study suggests an important role for MK2 in mediating the effects of p38 during cardiac hypertrophy and failure, thus representing a potential target for future therapy.



Michael Strong

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Genomes, maps and modules: navigating the *M. tuberculosis* genome

10 years ago, the introduction of high-throughput genome sequencing revolutionized the biological sciences. While the identification of the complete set of genes and corresponding proteins was indeed a remarkable achievement, one question remains: "How do the encoded proteins function together within their cellular environment?" Over the past decade a number of methods have been developed to answer such a question.

We have applied some of these methods to identify functionally linked genes and proteins throughout the *Mycobacterium tuberculosis* genome. Using a combination of the Rosetta Stone, Phylogenetic Profile, conserved Gene Neighbor, and Operon computational methods, we have been able to identify networks of interconnected genes in this deadly pathogen. We have also developed novel methods for the visualization of genome-wide functional linkages. Specifically, we have developed a method to visualize functional linkages on a two-dimensional matrix, which we have termed a genome-wide functional linkage map. The use of these maps has enhanced our understanding of protein connectivity in relation to genome organization, and subsequent hierarchical clustering of these maps has led to the identification of functional modules within the genome of *M. tuberculosis*. We have also developed a novel expression system, which we have employed to identify physically interacting proteins. This method mimics bacterial operon organization, and has been used to demonstrate physical interactions among *M. tuberculosis* proteins. We have also extended our methods to identify global protein domain modularity. Such a holistic approach to microbial genomics has facilitated our investigations of species-specific protein networks, and has provided new means to investigate global protein domain organization and modularity.

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Transcription initiation complexes in kinetoplastids

Like many protozoans, kinetoplastids differ in drastic ways from the memetic ideal of eukaryotic biology. Nuclear protein-coding genes are transcribed polycistronically, with individual messages resolved by trans-splicing by which a 39-nt exon from the Spliced Leader (SL) RNA is ligated to the 5' end of each message. In an organism with few known promoters, the SL RNA promoter in *Leishmania tarentolae* is well characterized. Two proteins were identified as candidates for involvement in initiation of transcription from the SL RNA promoter: TBP (TATA-Binding Protein), and SNAP50 (Small Nuclear Activating Protein). In model eukaryotes, TBP is a global factor involved in almost every single transcription initiation event, and kinetoplastid SNAP50 has been shown to interact with the SL RNA promoter in *Trypanosoma brucei* and *Leptomonas seymouri*. Electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) data were collected for a range of promoter regions, including the SL RNA promoter, with the goal of characterizing the roles that TBP and SNAP50 play in transcription initiation in *Leishmania tarentolae*.

Bruce Torbett

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Role of *dmp1* isoforms in cellular regulation

The cyclin D-interacting Myb-like Protein (DMP1) transcription factor regulates p14^{ARF} and CD13/Aminopeptidase N (APN) expression, thus playing a role in cell-cycle control and differentiation and function of hematopoietic and non-hematopoietic cells. We have recently identified two novel and developmentally expressed human DMP1 splice variants (termed α and β), of which one of these proteins (β) functions as a dominant-negative regulator of the originally reported DMP1 protein (now termed α). DMP1 β positively regulates human p14^{ARF} (also referred to as ARF or p16 in humans and p19^{ARF} in mouse) human tumor suppressor and CD13/APN genes. ARF is critical for the positive regulation of p53, which in turn, controls cellular proliferation and modulates apoptosis. CD13/APN is necessary for dendritic and endothelial cell development and has been implicated in tumor metastasis. Loss of *dmp1* in the mouse increases the risk of solid and hematopoietic tumors and loss of human *DMP1* on chromosome 7q21 is associated with human acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). We have shown in primary human myeloid cells that an increased β/α ratio is associated with terminal differentiation of monocytes and neutrophils and ectopic DMP1 β , but not DMP1 α , expression in U937 cells resulted in increased proliferation of terminally differentiated monocytes. Lastly, increased DMP1 β expression or knockdown of DMP1 promotes decreased serum dependency in selected cell lines. These findings implicate DMP1 β in the regulation of DMP1 α function, and in turn, strongly support a role for DMP1 β in cellular growth/proliferation.

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First evidence in eukaryotes of active protein L-isoaspartate/D-aspartate O-methyltransferase enzymes encoded by two genes is found in Arabidopsis thaliana

As proteins age, deamidation, isomerization, and racemization of aspartyl and asparaginyl residues contribute to the accumulation of spontaneous protein damage. The repair of age-damaged L-isoaspartyl residues is catalyzed by the protein L-isoaspartate/D-aspartate O-methyltransferase (PIMT). Using S-adenosyl-L-methionine as a methyl donor, PIMT initiates the conversion of damaged residues back to L-aspartyl via a methyl esterification reaction. It is thought that this reaction is an important repair mechanism in a wide variety of prokaryotes and eukaryotes, including higher plants. In *Arabidopsis thaliana*, two PIMT genes are found on chromosomes 3 (*PIMT1*) and 5 (*PIMT2*). We expressed *Arabidopsis* recombinant protein splicing variants of *PIMT2* in *Escherichia coli* and we used vapor diffusion assays to show they methylate both L-isoaspartyl and D-aspartyl residues. These PIMT2s share a similar pH and temperature activity profile with PIMT1. This is the first evidence that two genes are found in an eukaryote that produce active PIMT enzymes, and that D-aspartyl residues are substrates for plant PIMT, with the human form being the only other eukaryotic PIMT to recognize such residues.

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Subtle modification of isotope ratio proteomics; an integrated strategy for expression proteomics

Quantitation is a critical aspect of modern proteomics. All relative expression approaches to date rely upon mixing of isotope-coded samples yielding peptide pairs separated by several mass units in the mass spectrometer. Use of minor modification of isotope ratio to code samples for expression proteomics is being investigated for coding within a single isotopic envelope. Alteration of ¹³C abundance to ~ 2 % yields a measurable effect on peptide isotopic distribution and inferred isotope ratio. Elevation of ¹³C abundance to 4 % lead to extension of isotopic distribution and background peaks across every unit of the mass range. A better understanding of natural measurement variability and the contribution of sequence dependence will be necessary before meaningful mixing experiments for relative expression proteomics are performed.

Subtle modification of isotope ratio (~1 - 2 % increase in ¹³C) had no effect upon either the ability of data-dependent acquisition software or database searching software to trigger tandem mass spectrometry or match MSMS data to peptide sequences. More severe modification of isotope ratio caused a significant drop in performance of both functionalities. Development of software for deconvolution of isotope ratio concomitant with protein identification using LC-MSMS, or any other proteomics strategy, is underway (Isosolv). The identified peptide sequence is then used to provide elemental composition for accurate isotope ratio decoding and the potential to control for specific amino-acid biases should these prove significant. It is suggested that subtle modification of isotope ratio proteomics (SMIRP) offers a convenient and inexpensive approach to in vivo isotope coding of living organisms that could potentially be extended to living humans.

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Alternatively spliced Drosophila Dscam axon guidance receptors exhibit isoform-specific homophilic binding

Dscam is an immunoglobulin (Ig) superfamily protein required for the formation of neuronal connections in *Drosophila*. Through alternative splicing, Dscam potentially gives rise to 19,008 different extracellular domains linked to one of two alternative transmembrane segments resulting in 38,016 isoforms. These isoforms share the same domain structure, but contain variable amino acid sequences within 3 Ig domains. Here we demonstrate that individual isoforms exhibit surprising binding specificity. Each isoform bound to itself, but less, if at all, to other isoforms. This specificity is determined by the amino acid sequences in all 3 variable Ig domains. Even closely related isoforms sharing nearly identical amino acid sequences exhibit preferential isoform-specific binding. While two isoforms differing by 7 amino acids exhibited low levels of binding to one another, no binding was observed between isoforms differing in only 9 amino acids. This homophilic binding specificity may regulate interactions between cells required for the formation of complex patterns of neuronal connections.

Stephen G. Young

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Mouse models for precocious aging

Over the past few years, our laboratory has studied the enzymes that carry out the posttranslational processing of prenylated proteins, relying heavily on insights from genetically modified mice. One of these enzymes, Zmpste24, is an ER metalloproteinase required for the processing of prelamin A to lamin A, a structural component of the nuclear lamina. *Zmpste24* deficiency results in an accumulation of prelamin A within cells, a complete loss of mature lamin A, and misshapen nuclear envelopes. *Zmpste24* knockout mice (*Zmpste24*^{-/-}) exhibit retarded growth, alopecia, micrognathia, dental abnormalities, osteolytic lesions in bones, and osteoporosis—phenotypes shared with Hutchinson-Gilford progeria syndrome (HGPS), a human precocious aging syndrome caused by the synthesis of a mutant prelamin A which cannot undergo processing to lamin A. *Zmpste24*^{-/-} mice also develop muscle weakness. We hypothesized that prelamin A might be toxic and that its accumulation in *Zmpste24*^{-/-} mice is responsible for all of the disease phenotypes; we further hypothesized that *Zmpste24*^{-/-} mice with half-normal levels of prelamin A (*Zmpste24*^{-/-} mice with one *Lmna* knockout allele) would be subjected to less toxicity and would be protected from disease. Thus, we bred and analyzed *Zmpste24*^{-/-}*Lmna*^{+/-} mice. As expected, prelamin A levels in *Zmpste24*^{-/-}*Lmna*^{+/-} cells were reduced by 50%. *Zmpste24*^{-/-}*Lmna*^{+/-} mice were entirely normal—lacking all disease phenotypes. Also, misshapen nuclei were less frequent in *Zmpste24*^{-/-}*Lmna*^{+/-} cells than in *Zmpste24*^{-/-} cells. These data suggest that prelamin A is toxic and that reducing its levels by as little as 50% provides striking protection from disease.

Brian Zarnegar

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Comparing the contribution of type 1 (p50-dependent) and type 2 (p52-dependent) NF-κB activation pathways in cell survival, proliferation, homotypic aggregation and specific gene regulation of murine primary B-lymphocytes

Ligation of CD40 on B-lymphocytes plays crucial roles in the progression of T cell-dependent humoral immune responses. Still, the mechanisms responsible for the signaling and functional specificities of CD40 remain to be elucidated. As B-cells can be activated by multiple stimuli, comparison of differential biochemical pathway activation potential via such stimuli can facilitate the identification of signaling events unique to CD40. With this approach, we have found that CD40, unlike other B cell stimuli, strongly activates both the type 1 and type 2 NF-κB activation pathways. Here, we have compared the contribution of the type 1 (p50-dependent) and type 2 (p52-dependent) NF-κB activation pathways in cell survival, proliferation, homotypic aggregation and specific gene regulation of murine primary B-lymphocytes. Rescue of spontaneous apoptosis is diminished in p52^{-/-} B-cells after BAFF (a type 2 activator) stimulation and in p50^{-/-}c-Rel^{-/-} B-cells after LPS (a type 1 activator) stimulation. Interestingly, significant CD40-induced B-cell survival is still observed even in p50^{-/-}c-Rel^{-/-} p65^{-/-} B-cells, which is correlated with the ability of CD40L to upregulate Bcl-xL expression in these cells. CD40L and LPS-induced B-cell proliferation as well as upregulation of proliferation-related genes, however, are greatly reduced in c-Rel^{-/-} or p50^{-/-}c-Rel^{-/-} B-cells but are normal in p52^{-/-} B-cells. We have further demonstrated that both c-Rel and p52 are required for CD40-mediated B-cell homotypic aggregation, which explains well why neither LPS nor BAFF has this function. Overall, our studies suggest that both type 1 and type 2 NF-κB pathways contribute to the gene expression and biological program unique for CD40 in B-cell activation.



Poster Abstracts

(Listed Alphabetically by Retreat Participant's Last Name)

Copper dependent localization of the aerobic oxidative cyclase (CHL27) in chlamydomonas

Michael Allen (C&MB), Maryse Block, and Sabeeha Merchant

Chlorophyll synthesis requires the conversion of Mg Protoporphyrin monomethyl ester to protochlorophyllide using the oxidative cyclase enzyme. This three step, six electron oxidation requires molecular oxygen, iron, and NADPH. We have recently identified CHL27 as being required for this reaction in Arabidopsis. The green alga *Chlamydomonas reinhardtii* contains two homologs of this protein, Crd1 and Cth1, which are both targeted to the chloroplast, but reciprocally regulated based on copper. During copper deficiency, both CRD1 and a 3kb CTH1 mRNA are produced, whereas in copper replete conditions only a 2kb CTH1 mRNA is produced. Here we show that the 3kb CTH1 mRNA is not translatable and that Crd1 and Cth1 are differentially localized based on copper nutrition.

Conversion of recombinant prion protein into a disease-like state

Marcin I. Apostol (C&MB), Sangho Lee and David Eisenberg

Misfolded and aggregated prion protein is thought to be the transmissible agent responsible for prion diseases such as Mad Cow. We have converted monomeric recombinant hamster prion into an aggregated state resembling that of the protein extracted from diseased tissues. Upon mild denaturation along with a cycle of reduction and oxidation the prion protein was found to form long fibrillar assemblies. These assemblies are characteristic of amyloid in that they display increased stability to denaturation, exclusive ability to bind the dyes congo red and thioflavin T, and exhibit a characteristic x-ray fiber diffraction pattern. Moreover, biochemical characterization of our prion fibrils suggest that covalent bonds between monomers play a role in the stability and assembly of the fibers. We propose a domain-swapping model for prion fibers, involving intermolecular disulfide bonds that can account for the stability and coexistence of two molecular forms of the protein.

Mediator, ETS factors, and Egr1 expression: a civil war of cellular proportions

Michael Balamotis (Berk lab, Postdoc)

The regulation of eukaryotic gene transcription is highly dependent on the Mediator, a massive 20-30 protein subunit complex. The Mediator acts as a molecular bridge to physically relay activation signals from promoter-bound transcription factors to the general transcription machinery at a given gene. Mouse embryonic stem (ES) cells with a knock-out in one subunit of the Mediator, Sur2, show reduced expression of only a small subset of genes. Within this subset, Egr1 gene expression is down 10-fold compared to WT cells following serum stimulation. This deficiency is due to the dependence of Elk1, an ETS factor TCF subgroup member, on Sur2 for gene activation. However, in Sur2 knock-out mouse embryonic fibroblasts (MEF), Egr1 expression is only reduced 3-fold. This suggests that Elk1 is not the sole factor regulating Egr1 in MEF cells. Other TCFs may regulate Egr1, and may be less dependent on Sur2 for activation. Indeed, Elk1 and Sap1 display different activation potentials in knock-out cells despite sharing nearly identical activation domains. Quantitative real-time PCR in ES and MEF cells showed that Sap1 and Sap2 are expressed 3-fold and 10-fold higher in MEF cells, respectively. The absolute expression of Sap2 was greater than Elk1 in MEF but not ES cells. Our current model is that TCF factors compete for binding to the Egr1 promoter. The expression level of each TCF determines which factors will influence transcription, and thus dictate the degree of Sur2 dependence for a gene. To confirm this, ES and MEF knock-out cells will be transfected with Elk1, Sap1, and Sap2 expressing constructs, and their ability to circumvent the loss of Sur2 will be determined by a co-transfected luciferase reporter driven by an Egr1 promoter fragment. Chromatin-IP with antibodies against Elk1, Sap1, Sap2, Mediator, and RNAP2 will be used to determine the extent of factor recruitment to the

Egr1 promoter in ES and MEF knock-out and WT cells. Data thus far suggests that TCFs can activate transcription through different Mediator subunits and can compete for binding within a promoter. Disparate activation potential and promoter binding competition between otherwise similar factors may be one mechanism which allows for more complex transcriptional regulation.

The mediator acts as a conduit to promote chromatin remodeling in vitro

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Eukaryotic transcriptional activation involves the coordinated recruitment of coactivators and chromatin remodeling factors. The 30-subunit mammalian mediator is one of the major co-activators in a cell and plays roles in both assembly and function of a preinitiation complex. Our lab has previously employed immobilized naked DNA templates to study mediator recruitment in vitro. We have shown that cooperative recruitment of purified Mediator and TFIID by GAL4-VP16 generates a rate-limiting intermediate in the assembly of a preinitiation complex. In the context of chromatin, ATP dependent remodeling enzymes and histone acetyltransferases (HATs) are required in addition to Mediator and TFIID for efficient gene activation. The role of Mediator in chromatin remodeling is not well understood. To elucidate the role of mediator in chromatin remodeling, we have analyzed interactions between purified chromatin remodeling complexes and the Mediator using immobilized GAL4-responsive chromatin templates. Kinetic experiments demonstrated that two major HATs, p300 and STAGA, display similar recruitment kinetics to Mediator in crude HeLa extracts. Mediator and HAT recruitment were dependent upon the binding of GAL4-VP16 to the immobilized template. Remarkably, purified Mediator bound to GAL4-VP16 greatly stimulated recruitment of the purified HATs. Furthermore, the increased HAT recruitment resulted in increased acetylation of the chromatin templates. Additional evidence suggests different HATs may interact sequentially with Mediator, undergoing a controlled switch. Our results highlight a new possible role for Mediator in chromatin remodeling.

Intracellular disulfide bond abundance in hyperthermophilic microbes: genomic and biochemical revelations

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Recent research has identified a number of hyperthermophilic microbes predicted to contain an abundance of cytosolic protein disulfide bonds [Mallick, *et al.*, (2002)], challenging the long-held view that such bonds are prohibited in the cytoplasm. Here, we provide further experimental data and computational analyses that expand upon these findings and reveal surprising results. 1D and 2D diagonal gel electrophoresis experiments confirm that the hyperthermophilic archaean *Pyrobaculum aerophilum* contains a large fraction of disulfide-bonded intracellular proteins, including a number of protein complexes held together by intermolecular disulfide bonds. Furthermore, thermal denaturation studies of proteins in cell lysate reveal that the disulfide bonds contribute to protein stability. Though several factors have been identified as potentially contributing to thermal stability in individual proteins, no single characteristic has been found to be a major stabilizing factor of thermophilic proteins in general. Our findings suggest that, in at least some hyperthermophilic organisms, disulfide-bonds constitute a single major contributing factor to the structural stability of a significant fraction of cytosolic proteins. Various related computational studies are also underway. These studies aim to (1) illuminate from a bioinformatics perspective how these cells can maintain intracellular disulfide bonds, (2) develop special amino acid substitution tables for disulfide rich organisms, and (3) improve protein fold prediction methods on the basis of disulfide bond information.

Investigating roles of covalent histone modifications on transcriptional activation and repression.

Janet Choi (Carey lab, MBIDP)

We are investigating the coordinated events required for chromatin transcription in a cell-free system, particularly those involving histone modifications. To this end, we have constructed an immobilized GAL4-VP16-responsive chromatin template using recombinant *Xenopus* histones. We have shown that GAL4-VP16 binds to the chromatin template and actively recruits components necessary for transcription from HeLa cell nuclear extract. Recruitment of chromatin remodeling enzymes, the multisubunit mediator coactivator complex, and the general transcription factors (GTFs) results in activated transcription from the chromatin templates as measured by *in vitro* transcription assays. Furthermore, activator-directed histone acetylation and methylation of the chromatin templates by nuclear extract was detected, although details regarding which enzymes are acting on the template remain to be elucidated. We are also studying the role of histone methylation in transcription silencing *in vitro*. Methylation of histone H3K9 and K27 has been implicated in the establishment and maintenance of heterochromatin.

Utilizing the immobilized chromatin templates, we are in the process of developing an artificial system to recreate siRNA-mediated transcriptional silencing followed by heterochromatin formation and spreading to better understand this important aspect of gene regulation in cells.

Crosstalk between MyD88-dependent and MyD88-independent signaling pathways: TLR9 transcriptional activation vs. TLR3 transcriptional repression

Edward Chow (G. Cheng lab, MBIDP)

Toll-like Receptors (TLRs) are receptors that recognize conserved motifs on bacteria and virus. Previously, we reported that TLR3 and TLR4 specifically induced a MyD88-independent Type I IFN anti-viral gene program through combinatorial activation of NF- κ B and IRF3. Here we show that TLR9 specifically induces a set of MyD88-dependent genes through combinatorial activation of NF- κ B and Smads. Microarray comparing TLR3 and TLR9 gene activation in bone marrow-derived macrophages (BMMs) show a series of TLR9 specific genes. One of these genes, PDGF-B, is known to be activated by TGF- β through Smads. Electrophoretic mobility shift assays (EMSA) show that TLR9 can also potentially induce Smad binding to a Smad3/4 consensus binding sequence, where TLR3, 4 are unable to induce potent binding. Furthermore, CpG stimulation of TLR9 activates the Smad-consensus luciferase construct, 3TP-Luc, where poly I:C stimulation of TLR3 cannot. In Smad3-/- BMMs, induction of these TLR9 specific genes is either lost or greatly impaired. This loss of TLR9 specific gene induction is also seen in NF- κ B-defective Raw 264.7 cells. Besides showing TLR9's ability to activate a transcription factor previously thought to be solely mediating TGF- β /BMP gene induction, we show that TLR3, 4 inability to activate Smads is through MyD88-independent activation of Stat1 by Type I IFNs. We show that activation of the Type I IFN pathway through TLR3 or recombinant IFN- β leads to repression of TLR9 specific gene induction and TLR9 induced Smad binding to Smad3/4 consensus sequence. In Stat1-/- BMMs, this repression of TLR9 specific genes no longer occurs. Furthermore, in Stat1-/- BMMs, TLR3 and TLR4 can now activate these TLR9 specific genes to varying degrees. Thus, we show a novel TLR9 specific gene program that is mediated through NF- κ B and Smads. Furthermore, we show that this specificity is actually due to TLR3, 4's self regulation of inflammatory gene induction through Type I IFN-Stat1 mediated repression of Smads. We now have evidence of crosstalk between the MyD88-dependent and MyD88-independent pathways of TLRs, as well as crosstalk between TLRs and the TGF- β Superfamily that has implications in autoimmune disorders, such as SLE-related glomerulonephritis.

Understanding the dynamics of activator-mediator interactionsCraig Crockett (MBIDP) and Michael Carey
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The Mediator coactivator complex is a necessary adapter between activators and general transcription factors. The potent transcriptional activator VP16 interacts with Mediator both *in vitro* and *in vivo*. However, the exact mechanism by which Mediator interacts with VP16 is not completely understood. VP16 activation domain can be subdivided into the N- and C-domains, both of which interact with Mediator. I will describe biochemical identification of Mediator subunits interacting with VP16 subdomains through the use of an immobilized DNA template. *In vivo* functional interactions will be addressed through the use of overexpressing subdomains of Med subunits interacting with VP16. I find that VP16 N-domain interacts with the MED25 subunit of the Mediator, correlating with previously reported results. In addition, a previously uncharacterized interaction between VP16 C-domain and MED14 was identified. Specifically, the C-terminal 166 amino acids of MED25 are necessary for interaction with VP16. Together, this data will provide a paradigm for how a specific activator recruits Mediator to a transcriptional pre-initiation complex.

Dip3, a member of the MADF/BESS domain transcription factor family, is a regulator of eye/antennal fate in *Drosophila*

Hao A. Duong (Chem & Biochem) and Albert J. Courey

Dip3 is a representative of a novel family of *Drosophila* transcription factors. In addition to Dip3, this family includes *Adf1*, *Stonewall*, and 11 uncharacterized gene products. These family members contain an N-terminal MADF-domain, which is a variant of the more widespread SANT domain and a C-terminal BESS domain. In Dip3, the MADF domain mediates binding to a DNA sequence consisting of multiple trinucleotide repeats, while the BESS domain mediates interactions with a number of regulatory proteins. Transient transfection assays in S2 cells demonstrate that Dip3 can activate transcription directly. Dip3 can also be recruited to the template by protein:protein interactions with other DNA bound factors, in this case functioning as a coactivator. Antibody staining shows that Dip3 is highly expressed in the embryo and the eye discs of third instar larvae posterior to the morphogenetic furrow, and localizes to discrete punctuate spots in the nucleus. Over-expression of Dip3 in the eyes using the Ey-Gal4 driver produces antennal duplications as well as eye-to-antennal transformation phenotypes. These two phenotypes are produced by distinct mechanisms. The antennal duplication results from growth inhibition in the eye disc and concurrent over-proliferation of the antennal disc. In contrast, the eye-to-antennal transformation involves the actual transformation of eye tissue to antennal tissue. Both phenotypes are rescued by over-expression CycE or constitutively active Notch (N^{act}). Currently, we are attempting to determine the critical stage of the eye/antennal fate decision. In addition, we are attempting to generate and study Dip3 loss-of-function alleles.

Stable transgene expression from a Helper Dependent Adenovirus-Epstein-Barr virus hybrid vector system: potential as a therapeutic for genetic diseases of the liver

Sean Gallaher (Berk lab, MIMG)

Helper Dependent Adenovirus (HDA) vectors, which lack viral coding regions, are highly efficient gene transfer vehicles, but are limited by the fact that their linear, non-integrating genomes are not well maintained in mitotic cells. We have addressed this limitation by incorporating elements from Epstein-Barr Virus (EBV), whose genome persists as an extrachromosomal, circular episome in mitotic B lymphocytes. We created an HDA-EBV hybrid in which a linear HDA vector genome is circularized into an EBV episome upon transduction of target cells. This

system uses Cre recombinase expressed from one HDA vector (HDA.Cre) to recombine and excise a loxP flanked episome sequence on a second “target” vector (HDA.target) in co-infected cells. The excised episome contains sequences from EBV for maintenance, a human origin for replication, and a transgene that is expressed only after recombination.

In the present study, we demonstrate that this system is capable of forming episomes *in vivo* with high efficiency. An HDA.target vector bearing a luciferase reporter gene was injected intravenously into mice, and luciferase expression was assayed non-invasively by cooled CCD camera. The data show that the vector targets hepatocytes, where episome formation is rapid and robust, and transgene expression persists for at least 36 days. This pilot experiment suggests that the HDA-EBV system is ideally suited to treating genetic diseases of the liver such as alpha-1 antitrypsin deficiency; a major cause of emphysema and liver disease. An HDA-EBV vector carrying the human alpha-1 genetic locus will be administered to mice. Expression of the antitrypsin will then be assayed by ELISA of peripheral blood. Based on the encouraging results with the luciferase reporter, it is expected that alpha-1 antitrypsin expression will be robust and persistent, thus establishing the utility of the HDA-EBV vector as tool for the treatment of genetic disease.

Development of a Single Vector system for gene delivery via Hybrid Helper Dependent Adenovirus - Epstein-Barr Virus vector for long term persistence

Joey Gil (Berk lab, MBIDP)

Helper Dependent Adenoviral (HDA) vectors are an effective method for *in vivo* gene transfer. The lack of viral genes and replication competent virus greatly diminishes the immune response and cytotoxicity of transduced cells. They are relatively easy to grow to high titers, appropriate for *in vivo* gene therapy. Though, like typical Adenoviral vectors, HDAs have no mechanism to persist in dividing cells. Epstein-Barr viral (EBV) episomes, on the other hand, confer stability upon themselves with the use of the Epstein-Barr Nuclear Antigen-1 (EBNA-1), acting upon the OriP region. EBNA-1 induces episomal replication at S phase, ensuring dilution does not occur, and tethers the episome to metaphasic chromosomes during mitosis, to minimizing episome lost during cell division. We have previously demonstrated effective recombination of our HDAs into EBV episomes *in vivo*, through optical imaging. This work is based upon hybrid vectors previously developed that required coinfection of the target vector, to be recombined into an episome and the recombinase expressing vector. The separation of the vectors has been necessary to prevent premature recombination of the target vector upon propagation. To circumvent the problem of premature self recombination, Cre recombinase must not be expressed during viral propagation, but must be expressed upon infection of the target tissue. As we are targeting liver, we have opted to use a hepatocyte specific promoter/enhancer, consisting of the minimal Alpha Anti-trypsin promoter and 3 repeats of the ApoE enhancer. The single vector is to be propagated in 293FLP cells, expressing FLP recombinase to restrict packaging of the helper virus, through the FRT flanked packaging region. After several rounds of serial infection with addition of helper virus, the vector is purified through cesium chloride buoyant density centrifugation. Experiments previously performed with the binary system, both *in vitro* and *in vivo* will be duplicated with the single vector system. The single vector system promises to prove more practical, as coinfection is not required, reducing the MOI required for efficient coinfection.



The role of anaerobic bypass metabolism in lifespan extension of *Caenorhabditis elegans*

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Several eukaryotic organisms including the soil-dwelling nematode *Caenorhabditis elegans* can survive and reproduce in low oxygen environments. They do so by utilizing terminal electron acceptors other than oxygen in the electron transport chain to maintain the mitochondrial membrane potential and to keep the pool of nicotinamide adenine dinucleotide predominantly oxidized. When aerobic respiration is inhibited in *C. elegans* via RNA interference (RNAi) of components of the electron transport chain (ETC), a significant extension in lifespan results (Lee, S. S. et al. (2003) Nat Genet 33, 40-48). This phenotype may be the result of a metabolic shift in the animal leading to reduced oxygen consumption and a decrease in damaging reactive oxygen species (ROS). An assay measuring survival of RNAi-treated nematodes in a hypoxic chamber will elucidate whether these animals are better adapted to function anaerobically. Quantitative PCR will then be used to measure mRNA transcript levels to determine the nature of the metabolic adaptation if present. The results will shed light on the aging process in nematodes and perhaps give insights into the same process in humans.

Functional role of Ras activation in the development of heart failure

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The Ras/Raf/MEK/extracellular signal-regulated kinase (ERK) signaling MAP kinase pathway has been implicated in cellular proliferation and differentiation, gene transcription and the development of cardiac hypertrophy and myopathy. Previous transgenic models, which employed cardiac specific chronic activation of Ras, demonstrated hypertrophy, calcium cycling defects and early mortality due to heart failure. In order to identify the primary effects of Ras signal in the adult heart we developed a transgenic model with tamoxifen induced gene-switch approach. Ras activation in adult hearts led to transient hypertrophy with an increase in the left ventricular weight to body weight ratio 4 days after induction which was ablated by day 14. Correlating with transient hypertrophy, ERK activity was also significantly upregulated on day 4 but returned to control levels by day 7. These findings suggest that Ras/Raf/MEK/ERK signaling is potently regulated by a negative feedback mechanism in the adult heart. However, significant increases in the mRNA levels of ANF, BNP and β -MHC with a corresponding decrease in SERCA and phospholamban were detected even at later stages when compared to wild type littermate controls. In addition, the transgenic hearts displayed significant arrhythmia suggesting alterations of the electrophysiological properties within the heart. The arrhythmic activity persisted throughout the course of the observation and could be attenuated by β -blocker treatment. These results suggest that downstream MAP kinase signaling is responsible for Ras induced hypertrophy but is subject to potent negative feed-back regulation. Meanwhile, other downstream signaling are more likely responsible for gene regulation, remodeling and arrhythmia. Studies from this unique inducible transgenic model will help to dissect specific signaling pathways related to different aspects of cardiac pathologies and contribute new insights to the disease mechanism signal transduction in human diseases.

The role of galectin-1 in development of T regulatory cells

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CD4⁺CD25⁺ T regulatory cells (Treg) represent a newly identified subpopulation of T cells believed to be essential in maintaining peripheral tolerance to self antigens. Treg cells are generated (positively selected to develop) in the thymus by interactions with agonist ligand. These cells are thought to be selected for their high affinity interactions with self antigens and function upon TCR stimulation to inhibit the expansion of activated effector T cells in the periphery. However, relatively little is known regarding the molecular requirements for Treg selection and development. We have developed a mouse model of agonist driven Treg cell development using 5CC7 TCR transgenic mice. Because this TCR includes variable region 3 (V β 3) in the beta chain, it is engaged by the superantigens encoded by DNA sequence from mouse mammary tumor viruses (MMTV) 3 and/or 13. A line of 5CC7 positive B10.A mice (negative for MMTV 3/13 sequence) have been crossed with mice of the 129 s/v strain (which are positive for MMTV 3/13 sequence), then backcrossed to B10.A. Our preliminary findings indicate that the presence of the superantigen 5CC7 agonist promotes development of Treg cells; superantigen positive 5CC7 mice (MP-5CC7) have a significantly higher percentage of CD4⁺ cells which are CD25⁺5CC7⁺ (10% versus 2%) than 5CC7 mice which are negative for the superantigen (MN-5CC7).

Previous studies from our laboratory examining requirements for agonist driven negative selection of conventional CD8⁺ T cells and positive selection of CD8⁺ intraepithelial lymphocytes (IELs) in the HY- TCR transgenic model indicate that galectin-1 functions to promote agonist driven selection of thymocytes. Galectin-1 is an endogenous lectin expressed throughout the thymus with known capacity to regulate apoptosis of thymocyte subsets alone and in the context of TCR engagement. To examine the role of galectin-1 in agonist selection of Treg cells we have generated MP-5CC7 mice which are galectin-1 null.

Preliminary data indicates that galectin-1 null mice in our MP-5CC7 colony have a lower percentage of CD4⁺ T cells which are CD25⁺5CC7⁺ (7% versus 12%) than Galectin-1 wild types.

SUMO in *Drosophila* embryo and larva development.

Minghua Nie (MBIDP), Pinmanee Boonheung, Joseph Loo, & Albert Courcy

Small ubiquitin-related modifier protein (SUMO) belongs to a family of ubiquitin-like proteins that covalently attach to other proteins and alter certain properties of these targets, such as stability, activity, and subcellular localization. Since its discovery in 1996, SUMO has been found to conjugate to more than 50 proteins, involved in diverse cellular processes. SUMO proteins are highly conserved from yeast to beast to human, and SUMO conjugation takes place in all tissues at all developmental stages. We use *Drosophila* as a model organism to study the role of SUMO in *Drosophila* embryonic development and to identify additional SUMO target proteins. We have investigated the effects of SUMO mutations on embryogenesis through analyses of the cuticle phenotypes of homozygous SUMO mutant germ line clone embryos generated using the FRT/hsFLP system. Two P-element insertion mutant lines, *sumo*⁰⁴⁴⁹³ and *sumo*¹⁰¹²¹¹, representing two SUMO mutant alleles, exhibit different levels of lethality and a broad spectrum of cuticular defects, including mild to severe head defects, segment deletions, and weakly dorsalized phenotypes. Both mutant lines also exhibit, to different degrees, various eggshell defects. While the difference in lethality between the two SUMO mutant alleles might be due to differences in the strength of the alleles, the broad range of embryonic patterning and egg shell patterning defects could be reflective of the different sensitivities of multiple SUMO targets to SUMO modification. In addition to the genetic

analyses, two proteins containing consensus sumoylation sites, bicoid and hunchback, have been examined in transient transfection assays to determine if they are functional SUMO targets.

Type I interferon production enhances susceptibility to *Listeria monocytogenes* infection

Ryan O'Connell (Cheng lab, MIMG/C&MB)

Numerous bacterial products such as LPS potently induce type I interferons (IFNs); however, the contribution of this innate antiviral response to host defense against bacterial infection remains unclear. While mice deficient in either interferon regulatory factor 3 (IRF3) or the type I interferon receptor (IFNAR1) are highly susceptible to viral infection, we show that these mice exhibit a profound resistance to infection caused by the Gram-positive intracellular bacterium *Listeria monocytogenes* (LM) compared to wild-type controls. Furthermore, this enhanced bacterial clearance is accompanied by a block in LM-induced splenic apoptosis in IRF3- and IFNAR1-deficient mice. Thus, our results highlight the disparate roles of type I IFNs during bacterial versus viral infections and stress the importance of proper IFN modulation in host defense.

Extreme susceptibility to infection by *F. tularensis* in the absence of MyD88

Eric Pietras (Cheng lab, MIMG)

Francisella tularensis is a Gram negative intracellular bacterium that is highly infectious in humans causing a disease termed Tularemia. Although *F. tularensis* is known to infect and replicate within macrophages, the role of innate immune receptors and signaling pathways in the host response against *F. tularensis* has been left relatively unexplored. To address this topic, we have challenged both wild-type mice and mice deficient in the TLR signaling adaptor molecule MyD88 with a live vaccine strain (LVS) of *F. tularensis*. Results from these studies indicate that MyD88-deficient mice are highly susceptible to an intravenous *F. tularensis* LVS challenge relative to wild-type controls. Upon closer analysis, our data demonstrate that MyD88 plays a critical role during the immune response against *F. tularensis* LVS by regulating both cytokine production and accumulation of activated macrophages in infected tissues.

Identification of genes expressed in cone photoreceptor cells

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Purpose: To identify genes expressed exclusively or preferentially in cone photoreceptors that may be candidates for the cause of retinal degeneration in animals and humans. **Methods:** The retina of adult *cd* dogs is totally devoid of cone photoreceptors due to an inherited retinal disease. Previously, we subtracted *cd* dog retinal mRNAs from those of the normal dog retina using two rounds of RDA. The output of RDA was then shotgun cloned into a plasmid vector to create a mini-library in a bacterial host. 2000 cDNA clones generated from the subtracted library were microarrayed and screened. **Results:** 80 differentially expressed, non-redundant clones were sequenced. After BLAST analyses using different databases, we identified several clones that had been described as cone-specific, and few that did not correspond to any known characterized gene or to genes that have not been described in retina. Mouse and human orthologs of some of the dog clones were obtained and Northern blots confirmed their expression in mouse retinas. Developmental expression of two isolated cDNAs (15A15 and 12A11) in the normal mouse retina was compared with that of rod- (PDE α), cone-

(PDE α'), and glial Müller- (Carbonic anhydrase) specific genes using real-time RT-PCR. Expression patterns of 15A15 and 12A11 were more similar to those of cone- rather than rod- or Müller- specific genes. Both genes' mRNAs were already detected by 4 days; 15A15 mRNA had its maximal expression by 40 days, whereas the levels of 12A11 mRNA increased gradually until 355 days. Also using real-time RT-PCR, we compared the expression patterns of 15A15 and 12A11 cDNAs with those of the same cDNAs in *rd* mouse retinas. The *rd* mouse is characterized by rapid photoreceptor degeneration, although photoreceptor cells develop normally until postnatal day 7. Rods degenerate first and by 30 days they are no longer present in the *rd* retina, while cones start degenerating later and many are still viable by 80 days. Our results confirmed that 15A15 and 12A11 cDNAs must be present in cone photoreceptors since their expression slowly decreases after 40 postnatal days and it is still significant by 80 postnatal days. **Conclusion:** Identification of several cDNAs known to be in cones confirmed the validity of our subtractive hybridization and microarray screening. Northern blots and real-time RT-PCR of cDNAs that did not correspond to any known characterized gene or genes found in retina confirmed their differential expression in normal and *cd* retinas. Characterization of two of these clones is in progress.

Two functions of the *Saccharomyces cerevisiae* Coq7 protein in Coenzyme Q biosynthesis

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Coenzyme Q (ubiquinone or Q) is an essential prenylated benzoquinone lipid that functions in the respiratory electron transport chain and serves as a lipophilic antioxidant. In the budding yeast *S. cerevisiae*, Q biosynthesis involves eight identified Coq proteins (Coq1- Coq8). Characterized *coq* mutant strains are Q deficient and can't grow on medium containing a non-fermentable carbon source (e.g. ethanol or glycerol). Previous work suggested both an enzymatic activity and a structural role for the Coq7 protein. Here we present evidence for the dual function of the Coq7 protein. The *Escheria coli ubiF* gene encodes a flavin dependent monooxygenase that shares no homology to the yeast Coq7 protein and is required for the final monooxygenase step of *E. coli* Q biosynthesis. In the present study we test whether *E. coli ubiF* can functionally substitute for yeast COQ7. The *E. coli ubiF* gene expressed from a low copy yeast shuttle vector can restore growth on medium containing a non-fermentable carbon source of a *coq7* mutant point mutant, but fails to rescue a *coq7* null mutant. Expression of *ubiF* from a multicopy vector rescues both classes of mutants, although this cross complementation is more effective in the yeast *coq7* point mutant than in the *coq7* null mutant. The results indicate that the yeast Coq7 protein functions as a hydroxylase catalyzing the last monooxygenase step of Q biosynthesis and as an essential component of the putative yeast biosynthetic Coq protein complex.

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Identifying potential sSubstrates of the adenovirus E4orf6/E1B55K ubiquitin ligase

Jennifer Woo (MBIDP) and Arnold Berk

Upon infection into cells, adenovirus early proteins E4orf6 and E1B55K form an E3 ubiquitin ligase with cellular proteins, Elongin B, C, Cullin 5, and Rbx1. This E3 ligase ubiquitinates p53, which is then degraded by the 26S proteasome. Recent evidence suggests that this ligase also targets the DNA repair complex Mre11-Rad50-Nbs1 for degradation. We postulate that there are other substrates of this E3 ligase that are necessary for viral infection. This is supported by the fact that E4orf6/E1B55K is involved in other aspects of viral infection, such as stimulation of late viral mRNA nuclear export and inhibition of host cell mRNA transport.

However, the biochemical mechanism(s) of how E4orf6/E1B55K exerts its control in these activities are not known. The deletion of either E4orf6 or E1B55K in the virus results in decreased viral infectivity and deletion of both creates a nonviable virus. Identifying other substrates of this E3 ligase complex requires a block in ubiquitin-mediated degradation. Here we propose a modified immunoprecipitation approach to identify other possible substrates. We also created Adenoviral vectors over expressing either flag-tagged wild type human cullin 5, cullin 5 deleted in its Nedd8 site (cul5 Δ Nedd8), or N-terminal truncation of cullin 5 (NTD Cul5) in hopes of trapping substrates before they are ubiquitinated and sent to degradation. The cul5 Δ Nedd8 and NTD Cul5 are unable recruit the E2 ubiquitin conjugating enzyme, but still able to bind to substrates. Since they cannot recruit the E2, substrates will not be ubiquitinated and sent to degradation. These approaches will hopefully help identify other potential substrates of the E4orf6/E1B55K ubiquitin ligase, and thus help explain how E4orf6/E1B55K plays multiple roles in adenovirus infection.

Biochemical characterization of the Arabidopsis PINOID kinase using mass spectrometry and site-directed mutagenesis

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The *Arabidopsis* PINOID (PID) kinase was shown to be a critical component of auxin signal transduction. In order to investigate the PID kinase function, we used recombinant PID-GST protein in kinase assays combined with mass spectrometry. We have identified a number of factors that influence PID activity including cations concentration, autophosphorylation and auxin. Using MALDI-MS, we identified 4 autophosphorylation sites (3 serines and 1 threonine). To investigate the role of these phosphorylated residues in PID function, we created mutagenized versions of PID protein where these sites were changed from serines or threonine to Alanine and their activities in vitro and in planta are being analyzed.





