

**28TH MBI LAKE ARROWHEAD
RESEARCH CONFERENCE
& ANNUAL RETREAT**



OCTOBER 27-29, 2006

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

28TH MBI LAKE ARROWHEAD RESEARCH CONFERENCE
OCTOBER 27-29, 2006

Friday

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

6:30 - 7:45	DINNER
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8:00 Welcome

Session I: Proteins Gone Wild

(Chairs: Dev Majumdar & Minghua Nie)

- 8:10-8:25 **Frederic Halgand (Pasarow Mass Spec Lab, Visiting scientist/CNRS)**
Top down identification and characterization of intact human saliva proteins
- 8:25-8:40 **Adam Lunceford (Clarke C lab, C&MB trainee, Chem&Biochem grad)**
Renal disease associated with coenzyme Q deficiency
- 8:40-8:55 **Sehat Nauli (Bowie lab, Chem&Biochem Postdoc)**
Using the TELSAM domain as a crystallization module for recalcitrant soluble and membrane proteins
- 8:55-9:10 **Jason DeChancie (Houk lab, CBI trainee, Chem&Biochem grad)**
How similar are enzyme active site geometries derived from quantum mechanical theozymes and crystal structures?
- 9:10-9:25 **Mohamad Abbani (Clubb lab, Chem&Biochem postdoc)**
Structure of the cooperative Excisionase (Xis)-DNA complex reveals a micronucleoprotein filament that regulates phage lambda intasome assembly
- 9:25-9:55 **Parag Mallick (Cedars-Sinai/UCLA Chem&Biochem faculty)**
Discovering disease markers and mechanisms using quantitative proteomics



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: V for Virology: Host-Cell Interactions

(Chairs: Linda Chan and Tamar Tomassian)

- 9:00-9:30 **Benhur Lee (MIMG faculty)**
Virological vendetta: The pathobiology of Nipah virus entry
- 9:35-9:50 **Jennifer Woo (Berk lab, MBIDP grad)**
Adenovirus E1B-55K oncoprotein is a novel E3 SUMO ligase
- 9:50-10:05 **Eric Pietras (Cheng lab, C&MB trainee, MIMG grad)**
Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif
- 10:05-10:20 **Qi Wang (Lee lab, MBIDP grad)**
Distinguishing functional amino acid covariation from background linkage disequilibrium in HIV protease and reverse transcriptase

Session III: Breaking the Code: Epigenetics, Chromatin Remodeling & Re-interpreting Mendel

(Chairs: Mike Balamotis and Rosemarie Tsoa)

- 10:25-10:55 **Siavash Kurdistani (Biological Chem faculty)**
Biological information is embedded in diversity of histone modifications
- 11:00-11:15 **Jian Xu (Smale lab, MBIDP grad)**
Windows of unmethylated CpG dinucleotides mark tissue-specific enhancers in embryonic stem cells
- 11:15-11:30 **Steve Knowles (Tobin lab, MCDB grad)**
Testing whether CCA1 is a central oscillator component in plants
- 11:30-11:45 **Yibin Wang (Anesthesiology, Physiology & Medicine faculty)**
Novel protein phosphatases in stress-regulation

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



- 4:30 - 6:30 **Poster Session** (*All posters must be up by 4:30 for award consideration*)
and **Social Hour** with hors d'oeuvres

6:30-7:45	DINNER
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7:50 Dessert with MBI Dissertation Year Awardees

8:00-8:15 Edward Chow (Cheng lab, MBIDP grad)

Type I IFN dependent and independent mechanisms of gene regulation by innate anti-viral immune response

8:20-8:35 Parthive Patel (Tamanoi lab, MBIDP grad)

Growing up and getting old: Regulation of cell growth and stress response by Rheb/TOR signaling

Session IV: Can You Hear Me Now?

Communication throughout the Biosphere

(Chairs: Peter DeHoff and Jennifer Woo)

8:40 - 9:10 Ann Hirsch (MCDB faculty)

Signals and responses: Choreographing the complex interaction between legumes and rhizobia

9:15-9:30 Craig Herbold (Lake lab, GA&I trainee, MBIDP grad)

Evidence of horizontal transfer among archaeal eocyte small subunit ribosomal RNA genes

9:30-9:45 Kaori Shimazaki (Braun lab, MP trainee, MBIDP grad)

Epithelial membrane protein-2 (EMP-2): A candidate host receptor in Chlamydia trachomatis infection

10:00-MIDNIGHT	SOCIAL TIME (POKER - MUSIC - CONVERSATION - ETC.)
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2:00 AM

Set your clocks back one hour...!!!



Sunday

Did you remember to set your clocks back last night?

8:00 - 8:45	BREAKFAST
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8:50

Awards Presentation

Paul D. Boyer MBIDP Outstanding Teaching Awards
MBI Retreat Poster Awards

Session V: Fantastic Three: Genetics, Gene Expression and Genomics

(Chairs: Craig Herbold and Jennifer Murphy)

9:10-9:40

Stan Nelson (Human Genetics faculty)

Simplicity from complexity: Interpreting whole genome SNP and large scale gene expression data

9:45-10:00

Sam Hasson (Koehler lab, CBI trainee, Chem&Biochem grad)

Developing chemical genetic strategies to understand the targeting and import of proteins into the mitochondrion

10:00-10:15

Ozlem Equils (Cedars-Sinai faculty)

NF- κ B activation leads to functional progesterone withdrawal in silico

10:15-10:30

Rick Hayes (Johnson lab, MBIDP grad)

TrichoDB: Trichomonas vaginalis genome annotation validation via experiment and community collaboration

10:30-10:45

Ken Bradley (MIMG faculty)

Genetic screening using mammalian cells

10:45-11:00

Liz Massey Gendel (Bowie lab, C&MB trainee, Chem&Biochem grad)

Improving recombinant membrane protein overexpression

11:00-11:05

Closing Remarks

11:15-1:00	CHECK OUT - LUNCH
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Session Abstracts

(Listed Alphabetically by Speaker's Last Name)

Structure of the cooperative Excisionase (Xis)-DNA complex reveals a micronucleoprotein filament that regulates phage lambda intasome assembly

Mohamad A. Abbani, Christie V. Papagiannis, My D. Sam, Duilio Cascio, Reid C. Johnson and Robert T. Clubb

UCLA-DOE Laboratory of Structural Biology, Department of Chemistry & Biochemistry, University of California, Los Angeles

The Excisionase (Xis) DNA architectural factor regulates the construction of higher-order nucleoprotein intasomes that integrate and excise the genome of phage lambda from the *E. coli* chromosome. Xis modulates the directionality of site-specific recombination by stimulating phage excision 10⁶ fold, while simultaneously inhibiting phage reintegration. Control is exerted by cooperatively assembling onto a ~35 base pair DNA regulatory element, which it distorts to preferentially stabilize an excisive intasome. Here we report the 2.6 Å crystal structure of the complex between three cooperatively bound Xis proteins and a 33 base pair DNA containing the regulatory element. Xis binds DNA in a head-to-tail orientation to generate a micronucleoprotein filament. Although each protomer is anchored to the duplex by a similar set of non-base specific contacts, malleable protein-DNA interactions enable binding to sites that dramatically differ in nucleotide sequence. Proteins at the ends of the duplex sequence specifically recognize similar binding sites, and participate in cooperative binding via protein-protein interactions with a bridging Xis protomer that is non-specifically bound. Formation of this polymer introduces over 75° of curvature into the DNA with slight positive writhe, which functions to connect disparate segments of DNA bridged by Integrase within the excisive intasome. Like Xis, other DNA architectural factors form DNA polymers that regulate assembly of multicomponent complexes that transcribe, replicate, and recombine DNA, but this is the first filamentous nucleoprotein structure, to our knowledge, that has been captured by X-ray crystallography at high resolution.

Genetic screening using mammalian cells

Ken Bradley

Department of Microbiology, Immunology & Molecular Genetics, Molecular Biology Institute, CNSI, UCLA

Despite the success of genome sequencing projects, identification of gene function remains a major challenge. Phenotype-based (forward) genetic screens are powerful tools to address this problem, but are less well developed for mammalian systems. In order to improve this process, we have developed a novel forward genetic system that allows for conditional repression of gene transcription in host cells based on random retroviral integration. In addition, we employ cutting edge technologies such as chemical genetics and genome-wide siRNA screens available at UCLA through the Molecular Screening Shared Resource. The focus of our screens is to identify of host factors that influence cellular susceptibility to bacterial toxins. However, this systems-based approach is adaptable to the study of a wide range of biological functions.

Type I IFN dependent and independent mechanisms of gene regulation by innate anti-viral immune response

Edward K. Chow¹, Antonio Castrillo^{2,3}, Arash Shahangian^{4,7}, Liming Pei^{2,3}, Ryan M. O'Connell⁴, Stephen Schilling⁸, Xiao-Fan Wang⁸, Xin-Yuan Fu⁹, Robert L Modlin^{4,5}, Peter Tontonoz^{2,3}, and Genhong Cheng^{1,4,6}

¹Molecular Biology Institute, ²Howard Hughes Medical Institute, ³Department of Pathology and Laboratory Medicine, ⁴Dept. of Microbiology, Immunology and Molecular Genetics, ⁵Division of Dermatology, ⁶Jonsson Comprehensive Cancer Center, ⁷Medical Scientist Training Program, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095,

⁸Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, USA, ⁹ Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, USA

The immune system protects the host from pathogens through the activation and repression of genes. We have found multiple mechanisms of gene repression by innate anti-viral immune responses that affect a wide variety of diseases and biological systems, from autoimmune disorders to metabolic systems and hepatotoxicity associated with drug metabolism during a viral infection. The mechanisms of repression involve anti-viral activation of Type I IFN signaling pathways as well as Type I IFN independent mechanisms. Through our work, we have demonstrated that mechanisms of repression are important to the regulation of biological systems and that crosstalk between seemingly disparate systems can have significant pathogenic effects.

How similar are enzyme active site geometries derived from quantum mechanical theozymes and crystal structures?

Jason DeChancie, Adam Smith, Fernando Clemente, Hakan Gunaydin, K.N. Houk, and David Baker

Department of Chemistry & Biochemistry, UCLA

Quantum mechanical optimizations of theoretical enzymes (theozymes), which are predicted catalytic array of biological functionalities stabilizing a particular transition state, have been carried out for a set of 10 diverse enzyme active sites. For each theozyme, the rate determining TS plus the catalytic amino acid side chains are optimized using B3LYP/6-31G(d). To determine if the theozyme can reproduce the evolutionary catalytic geometry in nature, the positions of optimized catalytic atoms (covalent, partial covalent, or stabilizing interactions with TS atoms) are compared to the positions of the crystal structure. Using RMSD criteria of $\leq 1 \text{ \AA}$ between theozyme and crystal structure catalytic atoms successful recapitulation is achieved in 9 of 10 enzymes. The comparisons between theoretically predicted catalytic sites and those present in enzymes are discussed.

NF- κ B activation leads to functional progesterone withdrawal *in silico*

Rajgopalan P[#], Equils O^{*}, Vali S[#].

*Cedars-Sinai Medical Center, UCLA School of Medicine and [#]Cell Works Group, Inc. San Jose CA

Introduction: Premature delivery is one of the most important maternal-fetal health problems in the US and costs more than \$4 billion /year. Currently there are no effective preventive measures against preterm delivery and the mechanisms are not clearly known. Although inflammation and functional progesterone withdrawal have been suggested to play a role in the initiation of normal and premature labor, the interactions between NF- κ B activation and progesterone expression are not clearly known. Progesterone treatment has recently been recommended for prevention of preterm delivery by the American Association of Obstetrics and Gynecology; however, for unclear reasons, it is not effective in all women at risk.

Hypothesis: We assessed the effect of inflammation (NF- κ B activation) on estrogen receptor alpha (ER), progesterone receptor A (PRA; suppressive) and progesterone receptor B (PRB, stimulatory) mRNA and protein levels *in silico*.

Methods: We constructed a computer model using previously published data and assessed steady state kinetics.

Results: We observed that at higher levels of NF- κ B activation, PRB expression was lower and that PRA/PRB ratio was increased. Increased PRA/PRB ratio has been previously shown to reflect functional progesterone withdrawal. Increasing estrogen levels did not modulate steady state levels of ER or PRA mRNA and protein expression, however, during inflammation (higher NF- κ B activation) estrogen-induced PRA levels were 4 fold higher.

Conclusion: Although our *in silico* experiment is based on many assumptions, it closely approximates results of the published data. It suggests that only those women with low levels of NF- κ B activation have conserved PRB expression and may benefit from progesterone administration. In women with high levels of NF- κ B activation, progesterone receptor B may not be expressed.

Top down identification and characterization of intact human saliva proteins

Julian P. Whitelegge, Frederic Halgand, Sara Bassilian, Jennifer Zhang, Vlad Zabrouskov, Thomas C. McClure, Joseph A. Loo, Kym F. Faull and David T. Wong.

The Pasarow Mass Spectrometry Laboratory, The Jane & Terry Semel Institute for Neuroscience and Human Behavior, The Molecular Biology Institute and The Brain Research Institute, University of California, Los Angeles.

Complementary to bottom-up approaches, top-down proteomics is being applied as a method to provide complete characterization of protein primary structure, including post-translational modifications. This goal fits into the general rubric of systems biology in the sense that the attempt is to apply these methods to comprehensively profile entire suites of proteins.

We are applying this approach to several different experimental paradigms including the human salivary proteome project and cancer biomarker profiling. In this presentation select aspects of the salivary proteome project will be described. As a result, it appears that description of protein isoforms, and intra- and inter-individual polymorphisms would provide a better understanding of structure to function relationship of proteins and the discovery of disease biomarkers.

Whole saliva and stratified parotid gland secretions collected from human subjects were analyzed by LC-MS⁺. Collected fractions were then subjected to tandem mass spectrometry using both quadrupole time-of-flight (QSTAR XL) and hybrid linear ion-trap Fourier-transform ion cyclotron resonance (LTQ-FT) mass spectrometers. Peak lists from intact protein tandem mass spectra were data mined (Prosight PTM), firstly to identify parent open reading frames, and secondly to assign primary structure, including post-translational modifications. Candidate sequences were manually processed and matched to peak list data using Single Protein Mode. In this way several saliva proteins were clearly identified by Top Down MSMS experiments. These proteins include peptide C, protein IB-8c which contains peptide F, proteins IB-6, IB-9, II-2, IB-1, PRP-3, PRP-1, Db-f, Db-s and cystatin SA1. These experiments revealed new post translational modifications in protein II-2 and cystatin SA1. Unexpectedly, isomeric sequence changes were found for peptide C and PRP-3.

Salivary proteins present a significant challenge for protein identification via top-down strategies because they are often proteolytically processed at both N- and C-termini and contain a high rate of sequence repeat unit, such that automatic matching of peak lists to unmodified translations of open reading frames are unsatisfactory. Furthermore, the diversity of potential sequence polymorphisms and post-translational modifications precluded generation of (prejudiced) shotgun-annotated databases. The conclusion of this study highlights the ultimate requirement for unprejudiced interpretation of tandem mass spectral data with the view to discover new biomarkers of human pathologies in saliva.



Developing chemical genetic strategies to understand the targeting and import of proteins into the mitochondrion

Sam Hasson and Carla M. Koehler

Department of Chemistry & Biochemistry, UCLA

The mitochondrion functions at the center of cellular respiration, oxidative metabolism, and lipid biosynthesis. Even though the mitochondrion contains its own small genome that codes for a handful of proteins, most of the mitochondrial proteome is coded in the nuclear genome and imported from the cytosol. Therefore, precursors bound for the mitochondrion contain targeting and sorting information, whereas the mitochondrion has developed an elaborate set of translocons to mediate import and assembly of the mitochondrial proteome. Our lab has previously characterized mitochondrial biogenesis using classical yeast genetics and biochemical assays with purified mitochondria. However, my research project focuses on developing new strategies to elucidate the mechanisms of protein translocation and mitochondrial assembly using chemical genetics and *in vivo* approaches with the long-term goal of extending our studies in yeast to zebrafish, cultured cells, and mouse. To this end, I am utilizing a chemical-genetic approach in yeast to extend the genetic studies and identify novel compounds that modulate protein translocation. The mitochondrion contains the TIM23 translocation system for precursors with a typical N-terminal targeting sequence and the TIM22 translocation machinery for inner membrane proteins. Currently, I have identified a collection of molecules that are synthetic lethal with *tim10* mutants and am characterizing the specificity of these compounds in the TIM22 pathway. Long-term, my plan is to use these small molecules in zebrafish and cell culture models to determine if they alter mitochondrial biogenesis. To facilitate these studies, I am also developing an *in vivo* system with reconstituted GFP that can be used to monitor protein translocation in real-time. In yeast, the reconstituted GFP has been optimized to monitor protein import into the mitochondria matrix. By coupling the chemical-genetic approach with the *in vivo* import assay, our goal is to identify a set of molecules that can be used to elucidate mechanisms of mitochondrial biogenesis in a wide array of systems.

TrichoDB: *Trichomonas vaginalis* genome annotation validation via experiment and community collaboration

Hayes, Richard D.¹, Xie, Yongming², Yang, Yanan², Carlton, Jane M.^{3,4}, Loo, Joseph², Johnson, Patricia J.⁵

¹Molecular Biology Institute, University of California - Los Angeles, Los Angeles, CA, USA, ²Chemistry and Biochemistry, University of California - Los Angeles, Los Angeles, CA, USA, ³Department of Medical Parasitology, New York University School of Medicine, New York, NY, USA, ⁴The Institute for Genomic Research, Rockville, MD, USA, ⁵Department of Microbiology, Immunology, and Molecular Genetics, University of California - Los Angeles, Los Angeles, CA, USA

Trichomonas vaginalis is one of the most prevalent non-viral sexually transmitted infectious parasitic protists in the world, with over 200 million new cases of infection every year. Sequencing of a laboratory strain of *T. vaginalis* was completed in April 2005 by The Institute for Genomic Research. Genome curation and the development of an accurate and complete annotation is an important next step in the study of this protist. To facilitate involvement of the *T. vaginalis* research community in this ongoing process, a web-based database application has been developed (available at <http://www.trichodb.org/>). A password protected system of individual researcher accounts houses individual contributions to the genome annotation from a wide range of potential experimental sources in a secure environment, simultaneously providing an easy method for transition of annotation data to the public database upon publication.

In parallel to the genome website development, a proteomics approach to cataloging the genes in the *T. vaginalis* cytoskeleton, thereby validating their genome annotation, has been initiated using the MudPIT mass spectrometry workflow. Preliminary results of this study, focused on cellular fractionations enriching for anterior flagella, have been promising. The full dataset collected to date highlights the first-draft nature of the current genome annotation, as close to half of proteins identified are annotated only as "hypothetical."

Evidence of horizontal transfer among archaeal eocyte small subunit ribosomal RNA genes

Craig W. Herbold^{1,4}; Maria C. Rivera^{2,4}; Jacqueline A. Servin^{1,4}; Ryan G. Skophammer^{2,4}; James A. Lake^{1,2,3,4}

¹Molecular Biology Institute, University of California, Los Angeles 90095, USA, ²Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles 90095, USA, ³Human Genetics, University of California, Los Angeles 90095, USA, ⁴Astrobiology Institute, University of California, Los Angeles 90095, USA

The gene encoding the RNA portion of the small subunit of the ribosome evolves slowly, making it ideal for inferring ancient relationships. To date, there have been few studies that provide evidence for horizontal transfer of ribosomal RNA, therefore transfer of this gene is assumed to be rare or nonexistent. Here, an analysis was conducted of a set of ten archaeal eocyte small-subunit ribosomal RNA genes. A reference tree representing the overall relationship among the ten chosen taxa was determined using traditional phylogenetic methods. Using this tree as a reference, simulations of all possible horizontal transfer events were carried out to determine which four-taxon subsets would be informative of particular horizontal transfer events. Segmented horizontal transfer would result in topological breakpoints in an alignment of the small-subunit ribosomal RNA gene. The existence of one or more topological breakpoints in an alignment of four-taxon subsets was assessed using the dual multiple change-point model of Suchard et al. The observed patterns of four-taxon subsets containing strong evidence for or against segmented horizontal transfer was compared to simulated patterns of horizontal transfer. No single transfer event could have produced the observed patterns of horizontal transfer detection in this set of ten archaeal eocytes. Simulations suggest that a minimum of four horizontal transfer events must have occurred in order to produce the observed patterns of segmented horizontal transfer. Future work will be focused on describing and testing the multiple transfer scenarios that are implicated by the current results.

Signals and responses: Choreographing the complex interaction between legumes and rhizobia

Ann Hirsch

Molecular, Cell & Developmental Biology, UCLA

The nitrogen-fixing symbiosis between bacteria in the family Rhizobiaceae and members of the legume family (Fabaceae) has been well studied, particularly from the perspective of the early signaling and recognition events. Recent studies of non-nodulating legume mutants have resulted in the identification of a number of genes encoding proteins that are responsive to signal molecules from the bacteria. The reciprocal signaling and further communication between legume root and rhizobia results in the formation of a nitrogen-fixing nodule, a novel structure on the root where the bacteria convert atmospheric nitrogen into ammonia. For nodules to develop, bacteria produce a substituted chitooligosaccharide molecule, known as Nod factor, which is synthesized by the action of the products of their *nod* genes. However, not all legumes that establish a nitrogen-fixing symbiosis with rhizobia nodulate, and moreover, a second group of nodule-forming bacteria, completely unrelated to the Rhizobiaceae, which are alpha-Proteobacteria, has been discovered. These bacteria belong to the beta-Proteobacteria and have been designated beta-rhizobia to distinguish them from the better-known alpha-rhizobia. We have been studying the non-nodulating legume *Gleditsia triacanthos*, which is a basal legume in the caesalpinoid legume sub-family. *Gleditsia* establishes a symbiosis both with an alpha-rhizobial strain, TAL1145 and a beta-rhizobium, *Burkholderia*PVA5, which has nitrogen fixation (*nif*) genes, but appears to lack canonical *nod* genes. Because the beta-rhizobia have been reported to be even more efficient nitrogen fixers than the alpha-rhizobia, and because sources of fixed nitrogen in addition to the energy-intensive Haber-Bosch process are required for sustainable food production, we have been studying these bacteria and their interactions with legumes. Details on the interaction between the two symbiotic partners and the various genes involved in this agronomically and ecologically important pas de deux will be described.

Testing whether CCA1 is a central oscillator component in plants

Steve Knowles and Elaine Tobin

Department of Molecular, Cell & Developmental Biology, UCLA

Circadian rhythms are a universal way for eukaryotic and some prokaryotic organisms to maintain coordination with the daily changes of light and temperature. In plants, many biological processes ranging from leaf movements to the rhythmic expression of many genes display circadian oscillations. The established model for circadian rhythms consists of a central oscillator, inputs, and outputs. The central oscillator generates and maintains an endogenous rhythm of about 24 hours even in the absence of external cues such as day/night changes.

In the plant *Arabidopsis*, the central oscillator has been proposed to function via a negative feedback loop consisting of two similar MYB-related transcription factors, CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), as well as the pseudo-response regulator TIMING OF CAB EXPRESSION1 (TOC1). Although CCA1, LHY and TOC1 are necessary for normal clock function, it is unclear whether they truly constitute the central oscillator itself. Central oscillator components should satisfy the following criteria: 1) the protein is expressed with circadian rhythm, 2) it represses its own expression, 3) constitutive expression stops all rhythms, 4) null mutation stops all rhythms, and 5) an abrupt transient increase in its expression resets all

rhythms. The first four criteria have already been shown to be substantially fulfilled by *CCA1* and *LHY*. To test whether *CCA1* fulfills the fifth criterion, we have utilized an ethanol-inducible system to generate plants (*Alc-CCA1*) that transiently express *CCA1* protein after a short exposure to ethanol vapor. While rhythms in wild-type plants are unaffected by ethanol vapor, rhythms in the *Alc-CCA1* line are reset to the time of induction, showing *CCA1* is indeed a component of the oscillator. In addition, RNA levels of many clock-controlled genes decrease after *CCA1* induction, consistent with previous data implying that *CCA1* acts as a transcriptional repressor (a negative element) in the circadian system. Together these results indicate *CCA1* plays an important role as a negative element within the central oscillator.

Biological information is embedded in diversity of histone modifications

Siavash K. Kurdistani

Department of Biological Chemistry, UCLA

In eukaryotes, DNA associates tightly with histone proteins to generate chromatin, the physiologically relevant form of the genome. Through a wide range of histone modifications, chromatin not only regulates all cellular processes that are based on DNA, but also serves to increase the capacity of the genome to store and utilize biological information beyond the DNA sequence. The occurrence of various histone modifications differs at the level of single genes, large chromosomal domains and individual cells and may be regulated temporally. Such spatial and temporal differences generate molecular and cellular epigenetic diversity in the patterns of histone modifications. We provide evidence that the epigenetic diversity is not stochastic but may occur in stereotypical patterns from which important biological information can be extracted.

Virological vendetta: The pathobiology of Nipah virus entry

Benhur Lee

Department of Microbiology, Immunology & Molecular Genetics, UCLA

Emerging viral pathogens present a critical threat to global health and economy. Nipah (NiV) and Hendra (HeV) viruses are members of the newly defined Henipavirus genus of the Paramyxoviridae. Nipah virus (NiV) is an emergent paramyxovirus that causes fatal encephalitis in up to 70% of infected patients, and there is increasing evidence of human-to-human transmission. NiV is designated a priority pathogen in the NIAID Biodefense Research Agenda, and could be a devastating agent of agrobioterrorism if used against the pig farming industry.

Endothelial syncytia are a pathognomonic feature of NiV infections, and are mediated by the fusion (F) and attachment (G) envelope glycoproteins. We recently identified the receptor for Nipah virus entry as ephrinB2. EphrinB2 is the membrane bound ligand for the ephB class of receptor tyrosine kinases (RTKs), and plays critical roles in vascular and neuronal development. EphrinB2's expression on endothelial cells and neurons is consistent with the known cellular tropism for NiV. More recently, we also show that ephrinB3, a related protein, can serve as an alternative receptor. EphrinB2 and B3 expression is overlapping but distinct, and differential usage of ephrinB2 versus B3 may explain the variant pathogenic profiles observed between NiV and HeV. Identifying the NiV receptor will not only spur the development of effective vaccines and therapeutics, but the extraordinarily high affinity of the Nipah envelope for ephrinB2 allows its use as a reagent to explore the interesting biology of ephrinB2 itself.

Renal disease associated with coenzyme Q deficiency

A. L. Lunceford, R. Saiki, J. Pachuski, D. L. Gasser, and C. F. Clarke

Department of Chemistry & Biochemistry, UCLA

The genetic information preserved within an organism's DNA often indicates the predisposition of that organism to develop certain diseases. Such is the case in the *kd/kd* mouse model in which a particular mutation leads to the development of kidney disease early in development. This mutation has been mapped to the *PDSS2* gene, which is also known as *PLMP* or *mDLP1* (1). The *PDSS2* gene product is required to generate the isoprenyl tail of coenzyme Q (2), an important component of the respiratory chain and a potent lipid soluble antioxidant. Mutations in the human homolog of *PDSS2* increase the probability of developing renal disease by five fold. We find that after about 75 days of development *kd/kd* mice have coenzyme Q levels that are significantly lower than age matched wild type mice. Livers taken from the *kd/kd* mice also appear to have this coenzyme Q deficiency that manifests itself later in development. Our current work focuses on the characterization of the wild type and mutant *PDSS2* gene products expressed in *Escherichia coli* and on the coenzyme Q levels in the spleen as well as in plasma from mutant and wild type mice. The study of this mutation in mice will provide greater insights into the mechanisms behind this form of renal disease as well as lead to the development of possible treatments for the disease.

1. Peng, M., Jarett, L., Meade, R., Madaio, M. P., Hancock, W. W., George, A. L., Jr., Neilson, E. G., and Gasser, D. L. (2004) *Kidney Int.* **66**, 20-28.
2. Saiki, R., Nagata, A., Kainou, T., Matsuda, H., and Kawamukai, M. (2005) *Febs J.* **272**, 5606-5622.

Discovering disease markers and mechanisms using quantitative proteomics

Parag Mallick

Center for Applied Proteomics, Cedars-Sinai Medical Center, Department of Chemistry & Biochemistry, UCLA

Over the last few years a number of mass spectrometry-based quantitative proteomics methods have been developed that attempt to quantitatively and comprehensively identify the proteins present at different quantities in the samples compared. Such differences, in turn have been used to identify cellular functions and pathways affected by perturbations and disease, identify new components and changes in the composition of protein complexes and organelles and have led to the detection of putative disease biomarkers. We will specifically discuss the use of proteomics to identify putative markers in a murine skin cancer model, the discovery of cellular networks indicative of AR stimulation in prostate cancer and the discovery of SNPs and alternative splice forms for the annotation of the human genome.

Improving recombinant membrane protein expression

Elizabeth Massey^{1,2}, Brian Aspell², Gabriella Boulting², Hye-Yeon Kim², Robert K. Nakamoto³ James U. Bowie²

¹Cell & Molecular Biology Training Grant, NIGMS, ²Department of Chemistry & Biochemistry, UCLA, ³Molecular Physiology & Biological Physics, University of Virginia

Despite the fact 25% of all reading frames are membrane proteins, they constitute less than 1% of all known structures. One major reason for this is that protein production by recombinant overexpression is a major bottleneck. In an effort to improve the overexpression of membrane proteins, I have developed a selection system to isolate *E. coli* cell lines and to identify genes that improve expression. The system utilizes constructs in which the protein of interest is fused to a C-terminal selectable marker, so that the survival of cells on selecting media will indicate expression of the target protein. The selected cell lines can then be analyzed to determine the cause of the improvement of expression.

Two methods have been used in this study to introduce changes to an *E. coli* host cell line that may potentially improve expression. The first is mutagenesis. In these experiments, randomly mutagenized cells that have gained the ability to express the TB membrane target Rv1337 have been selected. Some mutant cell lines improve expression by approximately ten-fold, and one of these selected cell lines has been cured and, as a result, is free of all plasmids. After curing, this mutant cell line retained its ability to express Rv1337 at levels higher than the wild type; however, it did not perform as well as it did before undergoing the curing procedure. The mutant cell line was then applied to other TB membrane protein targets, and 2 out of 5 targets demonstrated a clear improvement when expressed in the mutant. As well, 3 out of 7 homologues of the intramembrane protease rhomboid showed a definite improvement due to the mutant. For 2 of these rhomboid targets, expression was increased to Western-detectable levels, whereas before they were not detectable.

The second method used to introduce changes to the host cell line is the overexpression of *E. coli* genes, and this is accomplished by co-overexpression of a genomic library along with the target protein/selectable marker fusion. An *E. coli* K12 strain genomic library was constructed and genes that improve expression of various TB membrane proteins were selected. Selected library plasmids were sequenced, and recovered genes included those involved in the heat shock response (σ^{32} , chaperones), amino acid biosynthesis, central intermediary metabolism, fatty acid biosynthesis, transcription (RNA polymerase beta subunit), and targeting (4.5S RNA, an SRP component).

I've demonstrated that some of these library plasmids improve expression of target protein by approximately 2-fold and up to 6-fold in some cases.

Using the TELSAM domain as a crystallization module for recalcitrant soluble and membrane proteins

Sehat Nauli¹, Cynthia Lee^{2*}, Saman Farr^{2*}, and James Bowie^{1,2}

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Transmembrane proteins are the gatekeepers of the cell and as such play a very important role in information relay between the intra- and extra-cellular environments. A major stumbling block in understanding how membrane proteins function is the lack of three dimensional structures. Structure determination of detergent-solubilized membrane proteins using X-ray crystallography suffers an intrinsic problem; the dynamic nature of the detergent molecules and the small polar surface area of membrane proteins prohibit the formation of well-ordered crystals. These problems have been overcome recently by co-crystallization of several detergent-solubilized ion channels with antibody (Ab) molecules that specifically recognize these channels. The Ab makes most or all the crystal packing interactions, thereby acting as a crystallization module for these channels. The difficulty associated with obtaining a specific Ab and the possibility that Ab-protein interaction might result in a non-native protein conformation prevent this method from being widely applicable. Here, we use the unique property of the TEL Sterile Alpha Motif (SAM domain), which crystallizes by forming helical polymers, to drive the crystallization of soluble target proteins between 50 to 200 residues, by merely fusing TEL-SAM to target proteins. Out of twelve soluble proteins we tried, eleven successfully crystallized. The structures of these fusion proteins reveal that SAM polymer interactions dominate crystal packing interactions. Three of these target proteins are of unknown structures and have resisted conventional crystallization attempts. The ability of the TELSAM module to drive the crystallization of various passenger proteins makes this system a potentially powerful approach in the crystallization of membrane proteins. We are currently applying this method to a set of membrane proteins of known and unknown

structures and have obtained microcrystals of the TELSAM module fused to glycoporphin A, a transmembrane protein in red blood cells.

Simplicity from complexity: Interpreting whole genome SNP and large scale gene expression data

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I will show two examples of how to use large complex datasets to extract simple meaning. The first example is the use of human genetic diversity to identify genetic linkage using dense SNP typing information. This approach permits the direct mapping of ancestral identity-by-descent intervals using high density SNP data between individuals. The approach identifies long continuous intervals of identity between genomes which provides an efficient linkage mapping and association mapping approach to human diseases. The second example is the use of genome-scale expression data to dissect and understand gliomas. In addition to identifying novel characteristics of the cancers from the expression data, the relative importance of individual genes can be determined using informatics approaches. These data can be placed in perspective and additional insights gained using data amalgamation permitted by the ease and ubiquitous implementation of genome-scale experimentation in the scientific community.

Regulation of cell growth and stress response by Rheb/TOR signaling

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Insulin/IGF signaling plays an important role in the growth (the increase in mass) of metazoan embryos. In adults, while insulin/IGF signaling is important for maintaining homeostasis, it also contributes to the senescence (or aging) of an individual. The nutrient responsive TOR signaling pathway promotes cell growth and protein synthesis and receives inputs from insulin/IGF signaling. We identified a small GTPase Rheb that promotes growth during *Drosophila* embryogenesis and interacts with the TOR signaling pathway. In adults, we show that Rheb/TOR signaling also contributes to the senescence of the adult and antagonizes the stress response. These studies reveal a pathway by which insulin/IGF signaling and diet can influence both development and disease. During embryogenesis, we find that Rheb is required for cell and tissue growth. Clonal loss of *Rheb* function in the eye results in reduced eye and head tissue due to decreased cell size (and likely cell number). In contrast, overexpression of *Rheb* in mitotic tissues (eye and wing imaginal discs) as well as post-mitotic larval tissues (fat body and hindgut) results in dramatic tissue overgrowth. This increase in tissue size is due to an increase in cell size rather than cell number. Furthermore, we find that Rheb stimulates TOR signaling to promote cell growth. In *Drosophila* adults, we find that Rheb/TOR signaling antagonizes the stress response. We find that adult flies with increased Rheb/TOR signaling are sensitive to starvation and oxidative stress and display an early senescence of locomotor activity with age. Decreasing TOR or S6K (an effector of TOR) signaling can rescue the sensitivity to stress as well as the early senescence of locomotor activity observed in flies with increased Rheb/TOR signaling. These data suggest that S6K is an important effector of Rheb/TOR signaling that promotes sensitivity to stress and senescence. Interestingly, we also find that increasing Rheb/TOR signaling in muscle (but not in neurons or fat bodies) can increase the sensitivity of adults to oxidative stress, possibly explaining the decrease in locomotor activity observed with age in flies with increased Rheb/TOR signaling.

Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif

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As viruses are intracellular pathogens, it has been hypothesized that host recognition of viral infection may occur via a cytoplasmic detection pathway which then activates type I interferons (IFNs) to impede viral replication and spread of infection. Recent work has identified some components of this pathway, including the intracellular dsRNA receptors RIG-I and Helicard. Upon processing of viral dsRNA, these have been shown to recruit a newly identified adaptor protein termed Cardif/MAVS/VISA/IPS-1, which in turn induces type I interferon-mediated antiviral responses through an unknown downstream mechanism.

We recently demonstrated that TRAF3, like Cardif, is required to transduce this antiviral signal in response to intracellular dsRNA. In the current study, we sought to identify how TRAF3 interacts with other components of the intracellular viral recognition pathway. We show that Cardif-mediated induction of type I IFN but not NF- κ B proceeds through a direct interaction between the TRAF domain of TRAF3 and a TRAF-interaction motif (TIM) within the proline-rich region of Cardif. Furthermore, we examined the specificity of TRAF3 versus its close homologue TRAF5 in the activation of the IFN response. Interestingly, while the RING, zinc finger and isoleucine zipper domains of TRAF3 were functionally interchangeable with those of TRAF5, the TRAF domain of TRAF5 was not. We show that this functional distinction may be due to the fact that the TIM of Cardif can bind the TRAF domain of TRAF3 but not that of TRAF5. Furthermore, we identify key residues in both Cardif and TRAF3 required for this interaction. Thus, we suggest that the mechanism by which TRAF3 participates in the antiviral signal flow involves a direct and specific interaction with a TIM in Cardif, which in turn provides insight into further downstream antiviral signaling components and mechanisms.

Epithelial membrane protein-2 (EMP-2): A candidate host receptor in *Chlamydia trachomatis* infection

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Purpose: Epithelial membrane protein-2 (EMP-2), a member of GAS3/PMP22 tetraspan protein family, is known to localize to lipid rafts and also to regulate trafficking of cell surface proteins such as integrins, GPI-linked proteins and MHC I molecules. EMP-2 is highly expressed in the eye, lung, and genitourinary tracts, sites of *Chlamydia* infection. In this study, we hypothesize that EMP-2 is a candidate host receptor involved in *Chlamydia* attachment and infection.

Methods: The human endometrial adenocarcinoma cell line HEC1A, human corneal limbal cell line HCLE, and human conjunctival cell line HCJE were used in these studies. In order to modulate native expression of EMP-2, the following stably transfected HEC1A cell lines were constructed: transfection controls (HEC1A-GFP), increased expression (HEC1A-hEMP2), or decreased expression (HEC1A-hRZ2). EMP-2 expression levels in each cell line were determined by RT-PCR and Western Blot. Cells were infected with *Chlamydia muridarum* (MoPn), and inclusions were detected using an anti-*Chlamydia* LPS. Blockade of EMP-2 was performed in infectivity studies using a specific antibody against the EMP-2 2nd extracellular loop.

Results: EMP-2 was highly expressed in HEC1A, HCLE, and HCJE cell lines. Infectivity of *Chlamydia* correlated significantly with levels of EMP-2 with the HEC1A cell lines. Compared to HEC1A-GFP controls, EMP-2 over-expressing HEC1A-hEMP2 cells showed a greater susceptibility to *Chlamydia* infection. Concordantly the HEC1A-hRZ2 cells showed a decrease in infectivity. In all tested cell lines, antibody against EMP-2, but not a control antibody, suppressed *Chlamydia* infectivity.

Conclusions: *Chlamydia* infectivity positively correlated with EMP-2 expression levels, and the infectivity was successfully blocked with anti-EMP-2 antibody in multiple cell lines. These data support a role for EMP-2 as a candidate host receptor important for host-*Chlamydia* interaction and ultimate infection. Further studies on the EMP-2-*Chlamydia* interactions and identification of other associated molecules involved in the infection may lead to new strategies for disease prevention.

Distinguishing functional amino acid covariation from background linkage disequilibrium in HIV protease and reverse transcriptase

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Correlated amino acid mutation analysis has been widely used to infer functional interactions between different sites in a protein. However, this analysis can be confounded by important phylogenetic effects broadly classifiable as linkage disequilibrium (LD). We have systematically separated the covariation induced by selective interactions between amino acids from that induced by linkage disequilibrium, using synonymous (S) mutations *versus* amino acid (A) mutations. Covariation between amino acid mutation vs. amino acid mutation (AA) pairs can be affected by selective interactions between amino acids, whereas covariation between AS pairs or SS pairs cannot. Our analysis of the *pol* gene in HIV reveals that AA covariation levels are enormously higher than either AS or SS covariation levels, and thus cannot be attributed to background LD. The magnitude of these effects suggests that a large portion of AA covariation in HIV *pol* results from selective interactions. Inspection of the most prominent AA interactions in HIV reverse transcriptase (RT) showed that they are known sites of independently identified drug resistance mutations, and physically cluster around the drug binding site. Moreover, the specific set of AA interaction pairs was reproducible in different drug treatment studies, and vanished in untreated HIV samples. The AS and SS covariation curves reproducibly measured a low but detectable level of background LD in HIV.

Novel protein phosphatases in stress-regulation

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Cellular signal transduction requires both positive and negative regulatory mechanisms to achieve delicate and specific outcome. Using genomic and proteomic approaches combined with targeted genetic modification in animal models, we have identified a number of uncharacterized negative regulators in stress signaling via different mechanisms. 1). We establish that TAB-1 is a negative regulator of stress-activated protein kinase p38 in inflammatory responses. It functions as a novel scaffold protein to modulate p38 sub-cellular localization and protein complex formation. 2). We identify Sprouty/Spreed family members are potent negative regulators of Ras mediated MAP kinase pathway in adult hearts. They function to dissociate activated Ras from downstream target molecules. 3). We discovered two new members of the PP2C family of protein phosphatases. One is exclusively localized in mitochondria matrix and regulates cell survival and death via mitochondrial permeability transition pore regulation. The other one is exclusively localized on ER

membrane and regulates ER stress signaling and calcium cycling. From both in vitro and in vivo studies, our findings demonstrate that these novel negative regulators functioning in diverse mechanisms are important components of cell signaling regulation and play important roles in development and diseases.

Adenovirus E1B-55K oncoprotein is a novel E3 SUMO ligase

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Adenovirus E1B-55K oncoprotein binds to host cell p53, stabilizing it and converting it from a regulated activator to a constitutive repressor. Previous studies showed that E1B-55K must be sumoylated to repress p53 activation function. However, the mechanism of E1B-55K to repress p53 is unclear. Here we show that E1B-55K oncoprotein functions as an E3 sumo-p53 ligase that stimulates p53 sumoylation both in vitro and in vivo. Sumoylation of p53 by E1B-55K may contribute to E1B-55K's ability to repress activation by p53 because mutation of the p53 sumoylation site, p53 K386 to R, transactivates luciferase expression from a construct with a p53-responsive p21 promoter to a greater extent than wild type p53, and E1B-55K represses p53 K386R less than wild type p53. We also found that sumoylation of E1B-55K is required for E1B-55K to stimulate p53 sumoylation in vivo, but not in vitro. This may be because sumoylation of E1B-55K determines its nuclear localization in vivo. E1B-55K can only stimulate p53 sumoylation when it is localized to the nucleus. Previous studies have shown that the sumo E3 ligase PIAS co-localizes with PML nuclear bodies (NBs) and re-localizes its substrates such as p53 and LEF1 to the PML NBs. We found that E1B-55K also co-localizes with p53 in PML NBs. PML has at least seven isoforms due to alternative splicing. We found that among PML I through V, E1B-55K associates with PML IV. Coexpression of PML IV and E1B-55K in transiently transfected cells led to complete tethering of p53 and E1B-55K in the nuclear PML body. However, co-expression of PML IV and E1B-55K mutated at the E1B-55K site of sumoylation (K104R) or the E1B-55K R443in mutant, that is defective for repression of p53 activation, did not lead to co-localization of E1B and p53 in PML NBs. These results indicate that PML IV co-localizes with sumoylated E1B-55K and p53 in PML nuclear bodies, repressing p53 transcriptional activation function. To date, only three unrelated proteins have been suggested to have SUMO E3-protein ligase activity: RanBP2, the PIAS proteins, and the polycomb group protein Pc2. Our results indicate that adenovirus E1B-55K is a fourth E3 sumo-protein ligase and strongly support the hypothesis that p53 sumoylation at K386 inhibits p53 function as a transcriptional activator.

Windows of unmethylated CpG dinucleotides mark tissue-specific enhancers in embryonic stem cells

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Recent studies have suggested that, in embryonic stem cells (ESC), chromatin is less compact than in differentiated tissues and a large number of genes encoding regulators of early development are associated with bivalent histone modification domains, suggestive of a chromatin structure poised for activation. However, non-regulatory tissue-specific genes were not generally associated with these bivalent domains. Here, we show that, in ESC, enhancers for typical tissue-specific genes contain windows of selectively unmethylated CpG dinucleotides, suggesting that the enhancers are already associated with DNA-binding proteins in pluripotent cells. The unmethylated windows, found in close proximity to binding sites for key transcription factors, expand in cells that express the gene and can contract, disappear, or remain unchanged in non-expressing tissues. However, they do not always coincide with common histone modifications. Following integration of pre-methylated constructs into ESC, the unmethylated windows readily appear, demonstrating that ESC actively express transcription factors that bind the enhancers. These findings suggest that, in ESC, enhancers for typical tissue-specific genes are already marked by association with DNA-binding proteins, and that DNA methylation analyses may be a preferred strategy for identifying these marked regions. We speculate that these protein-DNA interactions may be critical for maintaining the pluripotent state, for marking the locations of tissue-specific genes, and/or for creating a chromatin environment permissive to subsequent gene activation.



Poster Abstracts

(Listed Alphabetically by Retreat Participant's Last Name)

Small molecule enhancers of mTOR inhibition

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The TOR (target of rapamycin) protein kinase pathway is activated by growth factors and is fundamental to cell growth control in eukaryotes. Aberrant activation of the pathway underlies the loss of cell growth control in many diseased states, including cancer. With the TOR pathway hyperactivated in cancer cells, cell intrinsic differences between cancer and normal cells allow for high selectivity of drugs that target the TOR pathway. As a result, rapamycin or other drugs that can effectively modulate this pathway have great potential as anti-cancer drugs. Through a chemical genomics approach, a variety of novel small molecules that enhance mTOR inhibition (SMERs) have been identified in yeast and cell culture systems. These SMERs have been further tested in cell lines with and without rapamycin to investigate their effects on cell morphology, cell viability, cell growth, and cell signaling patterns. We will present our preliminary studies of the mechanisms of these molecules in the context of mTOR signaling and cell growth control. Through the study of these small molecules, we aim to better understand the molecular regulation of the TOR pathway as well as to potentially develop novel therapeutics in this area.

Towards understanding the atomic basis of prion transmission and strain

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An aberrant protein-protein interaction between prion molecules is hypothesized to be the cause of transmissible neurodegenerative diseases such as Creutzfeldt-Jakob in humans and Bovine Spongiform Encephalopathy in cows. The self seeding property of this interaction has a resemblance to amyloid formation and suggests how prion disease can propagate among individuals and across species without the need for a nucleic acid component. To gain an understanding of the atomic nature of this interaction we used a computationally based 3D profile method to identify peptide segments of prion that contribute to the formation of amyloid. These identified six and seven amino acid segments readily form needle-like crystals in which the molecules adopt cross-beta structures as seen by X-ray diffraction. These likely represent the interfaces between prion molecules leading to its aggregation and species specific transmissibility.

A Mechanism for Coordinating Chromatin Remodeling and PIC Assembly

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Transcription of eukaryotic genes within a chromatin environment requires the sequential recruitment of histone modification enzymes and the general transcription factors (GTFs) by activators. However, it is unknown how preinitiation complex assembly is coordinated with chromatin modification. Here we show that the model activator GAL4-VP16 directs the ordered assembly of Mediator, histone acetyltransferases (HATs) and general transcription factors (GTFs) onto immobilized chromatin and naked DNA templates in vitro.

Using purified proteins we found that the Mediator regulates this assembly process by binding to p300 and TFIID. Both the purified acetyl-coA-dependent catalytic switch causes p300 to acetylate chromatin and then dissociate. Dissociation of p300 enhances TFIID binding and active transcription. The dissociation is caused by an autoacetylation-induced conformational change in the catalytic domain of p300. As we conclude that autoacetylation-induced dissociation of p300 acts as a catalytic switch, which allows TFIID binding and subsequent preinitiation complex assembly.

Polyhedral bacterial organelles

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Bacterial microcompartments were initially discovered and described over 40 years ago, but have only recently attracted significant attention for focused investigation. Microcompartments consist of a self-assembling protein shell that encapsulates a particular enzyme, or group of related enzymes inside bacterial cells. Unlike typical cellular organelles, microcompartments are protein bound, and not surrounded by a lipid bilayer. Under TEM, they appear as approximately 1000Å diameter, electron dense intracellular polyhedra, and might be mistaken for viruses. Unlike viruses, however, their morphology tends to vary somewhat within each species of microcompartment. The best studied of the microcompartments, the so-called "carboxysome" found in the cyanobacteria, encloses the CO₂-fixing enzyme Rubisco. Recently, our laboratory has determined the atomic structure

of the major carboxysome structural subunits CcmK2 and CcmK4 from the species *Synechococcus* 6803. These structures are the first characterized representatives of a broad class of homologous genes believed to be structural elements of microcompartments in many prokaryotes. The gene products are referred to as BMC (bacterial microcompartment) proteins and are characterized by a single conserved protein domain. Structures of both the CcmK2 and -K4 subunits contained hexamers in the asymmetric unit of the crystal. Other studies show that CcmK2 may also form pentamers *in vivo*, a structural arrangement allowing the formation of a closed shell. The crystal structure also reveals a cationic 7 Å pore at the sixfold axis created by the close arrangement of six arginine residues, which we suggest may play a functional role in regulating uptake of bicarbonate. Future projects in the lab will focus on comparative structural studies with other bacterial microcompartments, including the propanediol utilizing (pdu) microcompartment structure from *Salmonella enterica*, which converts 1,2 propanediol into propenediol and propionyl-CoA, and is critical for virulence. Our collaborator's work has demonstrated that the required catalytic enzymes are contained together with the BMC-like structural genes *pduA* and *-J* in the propanediol utilizing (*pdu*) operon. This project will focus on structural determination of these, and other relevant structural microcompartment proteins. Ultimately, these structural studies of the bacterial microcompartments will broaden our understanding of microcompartment assembly, the mechanism of enzyme compartmentalization, and the structural basis for regulation of microenvironments in prokaryotic cells.

p27 regulates the transition of beta cells from quiescence to proliferation

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Diabetes results from an inadequate mass of functional beta cells. Such inadequacy could result from loss of beta cells due to an immune assault or the inability to compensate for insulin resistance. Thus, mechanisms that regulate the number of beta cells will be key to understanding both the pathogenesis of diabetes and for developing therapies. In this study we show that the cell cycle regulator p27, plays a crucial role in establishing the number of beta cells formed before birth. We show that p27 accumulates in terminally differentiated beta cells during embryogenesis. Disabling p27 allows newly differentiated beta cells that are normally quiescent during embryogenesis to reenter the cell cycle and proliferate. As a consequence, excess beta cells are generated in the p27^{-/-} mice, doubling their beta cell mass at birth. The early postnatal expansion of beta cell mass was unaffected in p27^{-/-} mice, indicating that the main function of p27 is to maintain the quiescent state of newly differentiated beta cells generated during embryogenesis. The expanded beta cell mass was accompanied by increased insulin secretion, however, the p27^{-/-} mice were glucose intolerant as these mice were insulin

insensitive. To assess the role of p27 to affect regeneration of beta cells in models of diabetes, p27^{-/-} mice were injected with streptozocin. In contrast to control mice that displayed elevated blood glucose levels, p27^{-/-} mice showed decreased susceptibility to develop streptozocin-induced diabetes. Furthermore, beta cells retained the ability to reenter the cell cycle at a far greater frequency in p27^{-/-} mice after develop streptozocin-induced diabetes compared to wild-type littermates. This data indicates that p27 is a key regulator in establishing beta cell mass and an important target for facilitating beta cell regeneration in therapies for diabetes.

Chemical genetic screen for small molecule inhibitors of lethal toxin induced macrophage death

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The successful use of weaponized *B.anthraxis* spores against humans in the United States exposed the dire need for more effective therapeutics. The licensed vaccine is burdened by cumbersome booster shot scheduling and has so far been limited to at-risk populations. Exposed individuals can be treated with antibiotics to clear systemic bacilli, but this does not typically alter the outcome of the disease once symptoms appear. Experimental animal models have implicated secreted anthrax lethal toxin (LT), which consists of a binding moiety (protective antigen, PA) and a catalytic moiety (lethal factor, LF), as contributing to this lethality. In order to identify compounds that might be used in conjunction with antibiotic therapy, we screened 30,000 molecules for the ability to protect murine macrophages from LT-induced death in a well-established intoxication model. We have successfully identified a compound that provides near complete protection to cells (>90% of positive controls) with an IC50 of 2.5uM. We have also identified a class of compounds patented as integrin antagonists that strongly protected cells in our screen. Based on structural similarities between cellular anthrax toxin receptors and integrins, we hypothesized that these inhibitors interrupt the interaction of the receptors and protective antigen. Flow cytometric analysis using AlexaFluor-labelled PA provides evidence that these compounds are indeed inhibiting the binding of protective antigen to the cell surface. Our finding of small molecule inhibitors of protective antigen binding is a departure from much recent research aimed at identifying small-molecule inhibitors of lethal factor.



Preparation of Protein-Polymer Conjugates: Cysteine Reactive Polymers and Polymerization from Protein Macroinitiators

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Proteins have naturally evolved for highly specialized and specific biological functions and are ideal signaling molecules for applications in medicine and biotechnology. Physical properties of proteins, such as stability, solubility, and biocompatibility, are known to be improved by the covalent attachment of synthetic polymers. Well-defined conjugates are advantageous for applications in these areas, and their preparation generally involves the attachment of a preformed polymer to a specific residue of the protein. Recently, we have shown that bioconjugates can be synthesized by first modifying the protein with initiation sites to form a protein-macroinitiator and then polymerizing directly from the protein to form the conjugate in-situ. The research described herein focuses on employing these two approaches to prepare protein-polymer conjugates.

A pyridyl disulfide functionalized initiator for atom transfer radical polymerization (ATRP) was synthesized and employed for the polymerization of 2-hydroxyethyl methacrylate (HEMA). The polydispersity index (PDI) of the resultant polyHEMA was found to be as low 1.20, and inspection by ¹H NMR indicated that greater than 85% of the pyridyl disulfide group remained on the polymer. This activated disulfide allowed for direct conjugation to BSA, and the BSA-polyHEMA conjugate was confirmed by gel electrophoresis.

In the alternate approach to form bioconjugates, a BSA macroinitiator was synthesized through a reversible disulfide bond by modifying Cys-34 of BSA with an initiator for polymerization. Polymerization of *N*-isopropylacrylamide (NIPAAm) from the macroinitiator resulted in BSA-polyNIPAAm conjugates in greater than 65% yield, and the conjugates were readily isolated by preparative size exclusion chromatography. Cleavage of the polyNIPAAm from the conjugate was performed under disulfide reducing conditions, and the isolated polymer had a PDI as low as 1.34. To determine applicability of this methodology to prepare biologically active conjugates, a mutant T4 lysozyme (T4L) bearing one cysteine was recombinantly expressed. Cys-131 of T4L was modified through a reversible disulfide and through an irreversible thioether bond. The lysozyme-polyNIPAAm conjugates from both macroinitiators were formed in greater than 65% yield. Bioactivities of the resultant T4L conjugates were analyzed

and compared to that of the unmodified enzyme; no statistical difference in bioactivity was observed. These results show that the lysozyme survives the polymerization reaction and the resulting polymer attachment, suggesting that this new method is viable to produce bioactive polymer conjugates.

Notch expression patterns in the retina: An eye on receptor-ligand distribution during angiogenesis

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The critical contribution of the Notch signaling pathway to vascular morphogenesis has been underscored by loss-of-function studies in mouse and zebrafish. Nonetheless, a comprehensive understanding as to how this signaling system influences the formation of blood vessels at the cellular and molecular level is far from reached. Here, we provide a detailed analysis of the distribution of active Notch1 in relation to its DSL (Delta, Serrate, Lag2) ligands, Jagged1, Delta-like1, and Delta-like4 during progressive stages of vascular morphogenesis and maturation. Important differences in the cellular distribution of Notch ligands were found. Jagged1 (Jag1) was detected in "stalk cells" of the leading vasculature and at arterial branch points, a site where Delta-like4 (Dll4) was clearly absent. Dll4 was the only ligand expressed in "tip cells" at the end of the growing vascular sprouts. It was also present in stalk cells, capillaries, arterial endothelium, and in mural cells of mature arteries in a homogenous manner. Delta-like1 (Dll1) was observed in both arteries and veins of the developing network, but was also excluded from mature arterial branch points. These findings support alternative and distinct roles for Notch ligands during the angiogenic process.

Characterization of SOD1 complexes from transgenic mice spinal cords

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting motor neurons in which 10% of the cases are familial and of which about 20% of the familial cases are linked to a dominant mutation in the Cu,Zn Superoxide Dismutase protein (SOD1). Protein inclusions rich in SOD1 are a hallmark of pathology, and SOD1 aggregation, possibly with other proteins, is suspected to be involved in pathogenesis. In order to investigate the protein composition of these aggregates, high and low molecular weight SOD1 complexes were purified from the spinal cords of end stage ALS symptomatic hSOD1 transgenic mice through a two-step chromatographic method.

Two ALS-related mutants were used as well as the human wild type SOD1 in these experiments.

First, the presence and high abundance of SOD1 complexes varying in molecular weight was observed in diseased mice through western analysis. The complexes were then subjected to proteomic characterization by electrospray ionization mass spectrometry (ESI-MS) in order to identify the non-SOD1 components. The vast majority of the samples contained full length SOD1, along with low amounts of other proteins. Identification of these non-SOD1 proteins was confirmed by immuno-histochemical methods. In addition to analyzing the components, the SOD1 protein from the complexes is also now being examined for the presence of post-translational modifications using ESI-MS as well as matrix assisted laser desorption ionization (MALDI) mass spectrometry.



Single molecule studies on the conformational dynamics of lactose permease

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What little is known of the structural dynamics of membrane proteins comes mainly from the few available crystal structures, limiting our understanding of critical cellular processes. Here, we study conformational change in the *E. coli* lactose permease (LacY), which is found in an inward-facing conformation in a recent crystal structure. We probe changes in conformation of LacY using single-pair FRET, an approach to measure distance changes in single, diffusing molecules. The cytoplasmic and periplasmic face of LacY were labeled with fluorophores and probed for distance changes upon binding of the sugar substrate p-nitrophenyl alpha-D-galactopyranoside (NPG). We find sugar binding causes a contraction of the cytoplasmic helices of LacY and a commensurate dilation of the periplasmic face of LacY. The single-molecule data demonstrates the same K_D for NPG as earlier ensemble experiments and advances a potential two-state model for NPG binding in LacY. Thus, we provide direct evidence for large-scale structural changes in LacY and a new biophysical tool to probe conformational changes in membrane proteins.

Structural characterization of the yeast prion Sup35

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Molecular Imaging of Intestinal Inflammation with 2-deoxy-2-¹⁸F-fluoro-D-glucose (FDG) and Positron Emission Tomography (PET)

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FDG has emerged as a molecular biomarker for PET imaging of tumors and antigen-receptor-activated lymphocytes based on the up-expression of GLUT-1 glucose transport activity in these cells. In this study, we explored the possibility that FDG and PET could be used to image the pathogenic lymphocyte activity in mouse models of immune colitis. C3H/HeJ.IL-10-/- mice, which undergo spontaneous immune colitis, were i.p. injected with 250 µCi FDG, followed by one-hour uptake time, and finally scanned by PET and CT. The large intestine was identified by CT contrast agent, regions-of-interest (ROIs) throughout the colon were quantitated for FDG signal (%ID/g), and intestinal signal was normalized using heart and brain ROIs. Data was compiled longitudinally in C3H/HeJ.IL-10-/- and control mice and the resultant values were statistically analyzed by a one-tailed Mann-Whitney test. C3H/HeJ.IL-10-/- mice, compared to control C3H/HeJ, had significantly higher FDG signal in the large intestine (p<0.05) at both early and later periods of disease progression. In a parallel study, mice were injected i.p. with ³H-2-deoxyglucose; after an hour of uptake, mice were sacrificed, intestinal cells were fractionated, and quantified for ³H-2DG levels. Preliminary data demonstrated increased 2-DG transport selectively in intestinal lymphocytes rather than epithelial cells. These findings suggest the feasibility of the FDG/PET as a noninvasive diagnostic tool to detect and measure the severity of lymphocyte-mediated colonic inflammation. Further studies are underway to further define the cell types responsible for FDG/PET signal in immune and non-immune colitis. Supported by NIH CA86306 (JB), DK46763 (JB), GM07185 (MM), AI52031 (SB).

Studying membrane protein folding energetics using a steric wedge

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Compared to the wealth of information known about soluble protein folding, there is very little understood about the folding processes for membrane proteins. There is a huge discrepancy between the fact that membrane proteins are the target of half of all drugs, but comprise less than 1% of the known protein structures in the Protein Data Bank. In order to learn more about the mechanism of protein folding for membrane proteins, we are developing a technique of using avidin¹ to capture the unfolded state of a dually biotinylated target protein given that two avidin molecules can only bind to the second biotin group on the protein of interest when it is in an unfolded state due to sterics. Due to the difficulty of working with membrane proteins, we must first develop this method for a model, soluble protein system for which we have

chosen the well-characterized enzyme, dihydrofolate reductase (DHFR). DHFR catalyzes the reduction of DHF to THF, coupled to the oxidation of NADPH to NADP⁺. Therefore, the activity of DHFR is easily measured by monitoring loss of absorbance at 340 nm². Upon addition of increasing amounts of avidin, the DHFR should be doubly bound by avidin molecules, trapping the DHFR in an unfolded, and therefore, inactive state. The binding affinity of avidin for the second biotin site on the target protein will be correlated to the unfolding energy of the protein since binding can only occur when the protein is unfolded. We have shown that avidin binding can indeed render DHFR inactive at ratios greater than 1:1 of moles of binding sites on avidin to moles of DHFR. This inactivation in the presence of avidin is reversible. Application to a membrane protein has been successful in inactivating a transmembrane enzyme upon incubation with avidin, however reversibility remains a challenge. This method will ultimately be applied to membrane proteins in bilayers to study membrane protein folding in their physiological state.

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Synthesis and the reactivity of *trans*-dichlorocyclohexane derivatives using tungsten hexachloride as a route for the synthesis of dichlorolissoclimide

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Dichlorolissoclimide is an extremely potent cytotoxic substance [IC₅₀ 1 ng/mL (P₃₈₈); 14 µg/ml (human KB)] that was isolated from the ascidian *Lissoclinum voeltzkowi* Michaelson in 1988 on New Caledonia. Not only has this compound been shown to exhibit toxicity against mouse lymphocytic leukemia cells, but it has been found to be active against nasopharyngeal carcinoma (KB), non-small-cell bronchopulmonary carcinoma (NSCLC-N6) and doxorubicin resistant P₃₈₈ cell lines. In order to determine why dichlorolissoclimide has such strong activity, it is important to have a general synthetic route for its preparation as well as the preparation of structural analogues to test the boundaries for biological activity. Synthesis of this dihalogenated diterpene is a synthetic challenge due to the vicinal *trans*-diequatorial dichloride functionality. Addition of chlorine to a cyclohexene has been shown to give mainly the *trans*-diaxial dichloride. Our group has developed two different routes to prepare this novel structure. The first route is a thermal rearrangement from the diaxial to diequatorial dichloride; the second route uses tungsten hexachloride to induce the formation of diequatorial dichlorides from an epoxide. With the key step of our synthesis existing as one of the last steps

in our proposed synthetic route, we are exploring the effects of tungsten hexachloride on model systems containing complex functionality. Here, we describe our investigation into the stability of many different functional groups towards tungsten hexachloride reaction conditions to generate *trans*-diequatorial dichloride compounds.

In vivo dynamics of activator-mediator interactions

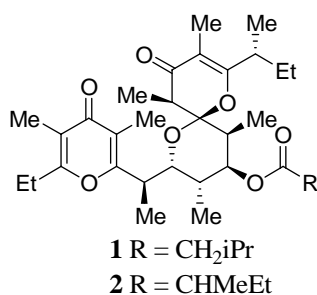
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Progress Towards the Synthesis of Auripyrones A and B

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Our synthetic targets, auripyrones A **1** and B **2**, constitute a class of polypropionates which are capped at one end by a 4-pyrone and at the other by a dihydropyrone moiety. Auripyrones A **1** and B **2**, isolated from the sea hare *Dolabella auricularia*, exhibit cytotoxicity against HeLa S₃ cells with IC₅₀ values of 260 and 480 ng/ml, respectively. We plan to synthesize both natural products via non-aldol chemistry, and investigate their structure-activity relationships. The mechanism for cytotoxicity of auripyrones A **1** and B **2** has not yet been elucidated.

Investigation of the role of the intrasubunit disulfide Bond in copper-zinc superoxide dismutase

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Copper-zinc superoxide dismutase (Sod1) is a 32 kD homodimer which protects the cell from superoxide (O₂⁻) stress by dismutation of superoxide into dioxygen (O₂) and hydrogen peroxide (H₂O₂). Sod1 is found in the nucleus, the intermembrane space of the mitochondrion, and the cytosol. Sod1 contains an intrasubunit disulfide bond, which is unusual in the reducing environment of the cytosol. Maturation of Sod1 includes oxidation of this C57-C146 intrasubunit disulfide bond and incorporation of one Cu and one Zn per monomer. However, the exact role of the disulfide bond in both Sod1 folding and in copper incorporation is unknown. Here we used C57S mutants of human and yeast Sod1 and a C146S mutant of yeast Sod1 to mimic the disulfide-reduced protein and investigate the role of the disulfide bond. Metal titration studies and native activity gels showed the disulfide bond is important for full metal binding and enzyme activity. In vivo studies showed partial rescue of several *sod1Δ* phenotypes by the yeast and human C57S mutants. In vivo studies also showed in the C57S and C146S mutants a difference between rescue of the lysine and leucine auxotrophies. These in vivo results suggest that inability to form the disulfide impacts the cytosol differently than the mitochondria and that interaction with the copper chaperone for Sod1 (Ccs1) may be impaired.

The Active-Site Geometry of Anti-Tumor Epothilones from Conformer-Activity Relationships

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The epothilones are a class of macrolactone natural products that are potent anti-neoplastic agents, with several compounds advancing through phases I and II of clinical trials. The identity of the active conformation of the epothilones has been the target of much study, yet has been controversial. Previous proposals about the active conformer were derived from transfer cross-correlated relaxation rate NMR (trCCR) on epothilone and tubulin in solution, or from electron crystallography (EC) of epothilone complexed with tubulin. These proposed conformations are very different, and there is no consensus about which, if either, is the active conformation. We report a third, previously unidentified, conformation that accounts for the major variation in potencies across 15 analogs, spanning a range of IC₅₀'s on the order of 10⁵ M. We have identified this conformation by a method named "conformational panning." This involves computation of the energies required to achieve either the trCCR or EC conformation in a series of epothilone analogs, and comparing to trends in experimental potencies. Neither structure gives a good correlation. The new structure found by conformational

panning provides a three dimensional template for the future design of active epothilone analogs or novel compounds that could act in the same way. As a technique that allows for the determination of an active conformation *a priori* of a macromolecular target, conformational panning will be relevant to future drug design and development, especially in cases with membrane protein receptors.

Caveolin-1 is a positive regulator of murine T cell function

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T cell receptor (TCR) antigen recognition and costimulation are required for proper T cell activation and induction of the formation of an immunologic synapse. Central to this process is the repositioning of surface receptors, signal transducers, cytoskeletal linkers and lipid rafts within the T cell:APC synapse. Caveolin-1 is a molecular scaffold involved in lipid raft organization in several cell types. It has been implicated in protein and membrane trafficking, cytoskeletal reorganization, and regulation of signal transduction. Despite initial reports suggesting that caveolin-1 is not present in lymphocytes, we have recently identified caveolin-1 expression in murine T cells through Western blot analyses of lysates from purified primary T cell populations. In addition, we show that caveolin-1 regulates TCR/CD28 induced CD8+ T cell activation. Indeed, CFSE-labeled CD8+ T cells from caveolin-1 deficient mice hypoproliferate and undergo fewer cell divisions in response to TCR/CD28 engagement relative to wildtype littermates. Proliferation assays measuring 3H thymidine uptake corroborate defective proliferation in caveolin-1 null CD8+ T cells in response to TCR/costimulation. In addition, CD8+ cells from caveolin-1 deficient mice have reduced cell survival when compared to those from wildtype mice after stimulation. In these experiments, CD8+ T cells from caveolin-1 deficient mice had a lower percentage of live cells after 48 and 72 hours of stimulation. CD8+ cells from caveolin-1 deficient mice stimulated with anti-CD3/28 for 48 and 72 hours also had a higher number of cells undergoing apoptosis, as measured by Annexin V. Experiments are underway to assess caveolin-1 subcellular localization during T cell synaptogenesis and elucidate the molecular basis of its activity. These data suggest an important role for caveolin-1 as a positive regulator of T cell activation and cell survival.

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The structure of carboxysome small shell protein CsoS1A from *Halothiobacillus neapolitanus*

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The carboxysome is a primitive organelle that exists in cyanobacteria and some chemoautotrophs. It is a microcompartment involved in the carbon concentrating mechanism. Many small proteins come together to form an enclosed shell containing carbonic anhydrase (CA), for the conversion of bicarbonate to carbon dioxide, and ribulose biphosphate carboxylase oxygenase (RuBisCO), responsible for carbon fixation utilizing carbon dioxide to form phosphoglyceric acid (3PGA). We have solved the structure of CsoS1A, one of the shell proteins in the carboxysome *Halothiobacillus neapolitanus*. In comparison to previously solved structures of carboxysome shell proteins from *Synechocystis* PCC 6803, CsoS1A has a larger contact surface between hexamers, which results in a tighter packing of CsoS1A hexamers in a layer. Through crystal soaking methods, we have observed the existence of sulfates bound to CsoS1A, which was not observed in previous structures. The pore of CsoS1A is compared to the pores of membrane channels. The thickness of CsoS1A is on the order of the thinnest virus shell.

Turnover of beta1 integrin in smooth muscle cells in vivo

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Beta1 integrin is a heterodimeric transmembrane protein that recognizes extracellular matrix (ECM) molecules and has been shown to play a role in numerous cellular functions including mechanotransduction and cell survival. Further, some experimental evaluation using animal models suggests that beta1 integrin may play a role in cancer metastasis and serve as a useful therapeutic target. Knowledge of its in vivo half-life will enable us to identify and evaluate more effectively transcription and translation targeted therapies. While in vitro studies indicate that beta1 integrin has an 8 hour half-life (Vekeman, 1993), studies in vivo were not conducted; therefore this question remains unanswered. To determine the in vivo half-life of beta1 integrin, we have crossed the sm22alpha cre inducible mouse (Kuhbandner, 2000) to a mouse carrying the floxed beta1 integrin gene (Potocnik, 2000) with a beta-galactosidase reporter. We chose to use a tamoxifen inducible cre-recombinase as it enables us to manipulate the timing and amount of gene deletion. The resulting transgenic mouse shows

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a high degree of cre activity in smooth muscle cells of the bladder with less penetration in the intestine and vascular smooth muscle. We tested a range of tamoxifen regimens and quantitated cre activity in bladder smooth muscle cells by counting the number of reporter positive cells. Our goals are to (1) determine percent over time of gene deletion through cell counts and quantitative PCR, (2) establish the rate of beta1 integrin RNA degradation and (3) evaluate time to complete protein depletion; thereby fully characterizing the temporal nature of beta1 integrin from gene to protein. Our preliminary data indicates that beta1 integrin protein is maintained for several days following in vivo gene deletion.

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