



**The University of Texas at San Antonio**  
**MBRS-RISE & MARC-U\*STAR**

SPRING 2006

RESEARCH SYMPOSIUM

**Friday, March 10, 2006**

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## Agenda

- 10:00 a.m. OPENING REMARKS  
**Dr. Andrew O. Martinez**  
MBRS-RISE & MARC-U\*STAR Program Director  
Associate Dean for Minority Programs  
Professor, Department of Biology, UTSA
- 10:10 a.m. KEYNOTE ADDRESS  
**“What does it feel like to be a scientist?”**  
**Dr. Charles Wilson**  
Professor, Department of Biology, UTSA
- 10:30 a.m. ORAL PRESENTATIONS (15 minutes each)  
**Moderator**  
**Dr. Gail P. Taylor**  
MBRS-RISE Assistant Program Director
- “Contrasting Mnemonic Processes in Schizophrenia: Neuropsychological and Functional Neuroimaging Evidence for Disrupted Memory Control”  
**Adir Abramoff, MARC-U\*STAR Undergraduate Senior**
- “Elucidation of Amino Acids Necessary for Dimerization of the Virulence Regulatory Protein ToxT of *Vibrio cholerae*”  
**Melissa Castaneda, MARC-U\*STAR Undergraduate Senior**
- “Secretory IgA Influences *Vibrio cholerae* O139 Biofilm Formation and Colonization”  
**Ty Troutman, MBRS-RISE Undergraduate Senior**
- “The Effects of Paraquat Induced Oxidative Stress on Young and Middle Aged Fischer-344 Rats in Trace Fear Conditioning”  
**Stephanie Perez, MBRS-RISE Master’s Student**
- 11:30 a.m. POSTER PRESENTATIONS  
*Isabel Desgagne-Penix*  
*Rander Draper*  
*Nicholas Fernandez*  
*Michou Kelley*  
*Cristal Lindell*  
*Erik Medellin*  
*Adriana Mejia*  
*Eric Mejia*  
*Jamie Parra*  
*James Perez*  
*Mary Jane Puente*  
*Natividad Ybarra*
- 12:30 p.m. AWARDS PRESENTATIONS AND CLOSING REMARKS  
**Dr. Andrew O. Martinez**  
MBRS-RISE & MARC-U\*STAR Program Director  
Associate Dean for Minority Programs  
Professor, Department of Biology, UTSA

## About the MBRS-RISE and MARC-U\*STAR Programs

The MBRS-RISE and MARC-U\*STAR Programs are funded by the National Institutes of Health/National Institute of General Medical Sciences. Both programs are designed to ensure talented and motivated minority students interested in developing professional careers as research scientists, have the opportunity to completely immerse themselves in a laboratory environment. Rather than using race as a selective factor at UTSA, we use a student's "disadvantaged" status as a selection factor for appointments.

MBRS-RISE offers support for both undergraduate and graduate student training. Student participants are either pursuing graduate education in the biological sciences, will do so in the future (Jr./Sr. and M.S.), or are strongly considering doing so (Fr./Soph. level participants).

The MARC-U\*STAR program at UTSA provides research training opportunities for outstanding undergraduate biology, chemistry, physics, mathematics, statistics, computer science or engineering majors who are interested in pursuing doctoral education (Ph.D. or combined professional/Ph.D.) and professional careers as research scientists.

Both programs equip participants with the knowledge and skills that they need for a successful career in the biological sciences. All participants perform original scientific research and present their findings at scientific meetings. They take courses and participate in activities designed to enhance their qualifications at their particular level of career development. They attend an off-campus summer research program. They are introduced to a broad range of scientific disciplines and are provided with networking opportunities with representatives of some of the nation's top research universities. It is the goal and the hope of program staff that all of our students obtain doctoral school admission, successfully complete their degrees, and pursue satisfying careers in the research sciences.

# Oral Presentations

## **Contrasting Mnemonic Processes in Schizophrenia: Neuropsychological and Functional Neuroimaging Evidence for Disrupted Memory Control.**

JL Ritch<sup>1,2</sup>, DC Glahn<sup>2,3</sup>, J Barrett<sup>2</sup>, DI Velligan<sup>2</sup>, C Franklin<sup>3</sup>, **Adir Abramoff<sup>4</sup>**, PT Fox<sup>3</sup>

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Memory impairment is closely linked to schizophrenia, yet which memory related processes is disrupted in schizophrenia remains unclear. Here, we contrast the ability to generate novel memory representations (linked to medial temporal cortex) with the ability to control existent representations (associated with prefrontal executive circuits). This distinction is closely related to the putative specialization of the medial temporal and lateral prefrontal lobes. We propose that the ability to control items in memory is relatively more impaired in schizophrenia than the ability to create new internal representations. This differential effect will be evidenced by poorer performance and reduced prefrontal activity on a task requiring controlling memoranda compared to a task requiring creation of new representations. In the present study we use 2 experiments, one behavioral the other functional MRI, designed to contrast memory control and the generation of new memoranda using a novel a delayed non-match to sample task modeled after those used in electrophysiological studies with nonhuman primates. (Supported in part by NIGMS MARC USTAR GM 07717.)

## Elucidation of Amino Acids Necessary for Dimerization of the Virulence Regulatory Protein ToxT of *Vibrio cholerae*

**Melissa M. Castaneda**<sup>1</sup>, B. M. Childers<sup>1</sup>, G. Weber<sup>1</sup>, M. G. Prouty<sup>1</sup>, K. E. Klose<sup>1,2</sup>;  
<sup>1</sup>University of Texas at San Antonio, San Antonio, TX, <sup>2</sup>South Texas Center for Emerging Infectious Diseases, San Antonio, TX.

*Vibrio cholerae*, is a motile, gram-negative, rod-shaped bacterium and is the etiologic agent responsible for the diarrheal disease cholera. Research into the pathogenesis of *V. cholerae*, which afflicts thousands of people worldwide each year, is critical for the development of novel vaccines and therapeutics. ToxT is a key regulatory protein in *V. cholerae*, which directly activates the transcription of the two major virulence gene clusters, *ctx* and *tcp*, which encode cholera toxin (CT) and the toxin-co-regulated pilus (TCP), respectively. Previous data demonstrated that the N-terminus of ToxT (aa1-165) functions in dimerization of the protein, whereas the C-terminus (aa166-276) functions in DNA binding. We are interested in determining which amino acids of ToxT are necessary for dimerization. Scanning alanine mutagenesis studies have shown that there are approximately 22 N-terminal (aa1-165) ToxT alanine substitution mutants defective for transcriptional activation. To test the effects of the individual N-terminal alanine substitutions on dimerization, the mutations were reintroduced into a plasmid that expresses a ToxT<sup>N</sup>-LexA<sup>N</sup> protein fusion. These plasmids were transformed into a *sulAp-lacZ* reporter strain (JL1436) and then assayed for  $\beta$ -galactosidase activity. We expect that alanine substitutions that cause defects in dimerization will exhibit greater than 50%  $\beta$ -galactosidase activity in comparison to wild-type (ToxT<sup>N</sup>-LexA<sup>N</sup>) due to their inability to dimerize and repress *sulA* transcription. Preliminary data suggests that of eight N-terminal alanine substitution mutations known to affect ToxT transcriptional activation, four affect ToxT dimerization. Elucidation of the amino acids that are necessary for ToxT dimerization will help to further characterize the virulence regulator ToxT. This work was supported in part by NIGMS MARC USTAR GM 07717.

## Secretory IgA Influences *Vibrio cholerae* O139 Biofilm Formation and Colonization

Ty D Troutman, Karl E Klose, PhD, M Neal Guentzel, PhD, Bernard P Arulanandam, PhD  
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*Vibrio cholerae* is a bacterium that colonizes the human small intestine and causes cholera, a disease characterized by severe diarrhea and a high mortality rate if left untreated. In the marine environment, during inter-epidemic periods, *V. cholerae* is capable of forming an acid and chlorine resistant biofilm, thus enabling the establishment of a reservoir for the reinfection of humans. In murine *V. cholerae* infections, anti-cholera toxin (CT) immunoglobulin (Ig) A and anti-bacterial secretory IgA (sIgA) play a protective role by neutralizing CT and aggregating the bacteria. However, the role of non-specific sIgA in *V. cholerae* intestinal colonization and/or biofilm formation has yet to be elucidated. We have examined the contribution of sIgA on *V. cholerae* biofilm formation and the ability to colonize the infant mouse small intestine. Biofilm formation by *V. cholerae* O139 strain MO10 in the presence of human sIgA was analyzed using crystal violet staining and spectrophotometry. The contribution of sIgA to infection was analyzed by oral-intragastric inoculation into seven day old IgA<sup>-/-</sup> and IgA<sup>+/+</sup> mice. Levels of *V. cholerae* in the infant mouse small-intestines were determined by homogenization and dilution-plate counting. *V. cholerae* cultures grown under defined biofilm inducing conditions (LB, 30°C, 24 h) in the presence of non-specific human sIgA produced significantly less biofilm in a dose dependent manner when compared to cultures grown in the presence of non-specific human serum IgA, human serum IgG, human serum IgM, or bovine serum albumin. Inhibition of *V. cholerae* biofilm formation by non-specific human sIgA was shown to take place at or before 6 hours in a time-course analysis. Additionally, the ability of *V. cholerae* to colonize the seven-day old infant mouse small intestine was significantly greater in IgA<sup>+/+</sup> mice than in IgA<sup>-/-</sup> mice. These results suggest that non-specific human sIgA may be an environmental signal to differentially modulate *V. cholerae* biofilm formation and may influence the intestinal colonization of these biofilm-associated bacteria upon entry into the gastrointestinal tract. (Supported by National Institutes of Health grant NIGMS/MBRS-RISE GM 60655)

## **The Effects of Paraquat Induced Oxidative Stress on Young and Middle Aged Fischer-344 Rat in Trace Fear Conditioning**

**Stephanie Perez** and Edwin Barea-Rodriguez. Department of Biology, University of Texas at San Antonio, San Antonio, Texas, 78249.

Previous studies have found that aged animals display memory deficits especially in tasks that are associated with the hippocampus. Aged animals also show an increase in oxidative stress in the hippocampus, which then leads to the damage of DNA, lipids, and proteins. Thus, it is possible that age-related deficits in learning and memory occur as a result oxidative damage. Oxidative damage can be induced by the herbicide paraquat. Paraquat causes dose and time-dependent deficits both physically and behaviorally. We hypothesized that paraquat treated young and middle aged rats would show age related memory deficits due to oxidative stress. In order to study such effects, young and middle aged Fisher 344 rats (3-6 months; 12 months) were injected with 25 mg/kg of paraquat intraperitoneally. Three hours following injection, rats received 10 trials of trace fear conditioning and were then tested 24 hours later for fear to the tone. Results showed that there were no significant differences found between the control and paraquat treated rats, despite the apparent increase in carbonyl groups, a marker of oxidative stress, which was observed in the paraquat treated rats. This data suggests that the amount of oxidative damage induced by our treatment may not be enough to cause memory impairments. (Supported by NIGMS/MBRS-RISE GM 60655)

# Poster Presentations

**Effects of Altered Growth on the Levels of the Plant Hormones Auxin and Gibberellin  
in *Arabidopsis thaliana***

**Isabel Desgagné-Penix** and Valerie Sponsel.

Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249.

Hormone homeostasis and interactions are critical for normal growth and development. Growth is defined as an irreversible change in the amount of living material, which leads to an increase in cell size and cell division. In plants, the two hormones auxins and gibberellins (GAs) promote germination, increase growth, induce flowering and fruit development and can be used commercially to affect the yield of crop plants, flowering time or fruit size. Recently, we demonstrated that seedlings of the model organism *Arabidopsis thaliana*, treated with paclobutrazol (an inhibitor of GA biosynthesis) or naphthylphthalamic acid (an auxin transport inhibitor, ATI), showed an up-regulation of the stem-expressed isoform of the GA biosynthetic enzyme GA 20-oxidase (GA20ox1). Since both of these treatments caused stunted growth, we proposed to examine the effect of altering growth on the levels of auxin and GA20ox1 to define if the up-regulation of genes in the GA biosynthetic pathway is a direct consequence of altered auxin polar transport OR if it is a global consequence of reduced growth. We first determined different media composition that gave us reduced growth. We then examined the expression of two GUS reporter gene constructs: one for the GA20ox1 and the other for a synthetic auxin response element, which gives an indication of auxin levels and distribution. We showed that neither GA20ox1 or auxin levels were increased by treatments that stunted growth. Therefore, we conclude that the up-regulation of the GA20ox1 following treatment with ATIs is specific to an altered auxin transport and not due to a global effect on growth. The mechanism(s) by which auxin status affects transcriptional regulation of GA biosynthetic genes are still under investigation. It is clear that most of these genes have orthologues in crop plants and some of the GA signaling components in plants have mammalian homologues, extending the significance of this work beyond the model species. This work is supported by the NIGMS MBRS-RISE GM60655 and the NSF 0080934.

## **Targeting Vector Expressed Vaccine Antigens to Antigen Presenting Cells Using Protein L**

**Rander E. Draper**, Jacqueline C. Williams, Jennifer S. Lee and Hans W. Heidner.  
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Sindbis virus is an alphavirus that can be used as an expression vector due to its ability to incorporate and express a foreign gene. Based on these properties, alphaviruses have been designed as vaccine vectors. One strategy for enhancing the efficacy of a vaccine vector is to target it to professional antigen presenting cells such as dendritic cells. The objective of this project was to construct and evaluate the Sindbis vectors that express a foreign antigen fused to protein L. Protein L (Ppl) is a bacteria-derived protein that binds the variable light (VL) chain regions of immunoglobulins of many mammalian species. Sindbis strain E2S1 which contained a previously cloned VP7 region downstream of the 26S promoter was engineered to link protein L by a 15 amino acid linker directly to the VP7 sequence. Of the two protein L viral prototypes made, one contained a fully functional protein L sequence while the other contained 3 mutations which blocked its immunoglobulin binding activity. Growth curve results of both functional and knockout protein L vectors verify their ability to replicate almost as well as the wild type strain of E2S1 in cultured cells. Immunoprecipitation results using normal mouse serum confirmed that PPL-VP7 fusion proteins are being expressed inside of cells and that PPL does in fact bind antibody. Future studies will be undertaken to analyze the serum of Balb/c mice which have been vaccinated with PPL expressing Sindbis virus vectors to determine if the presence of a functional version of protein L enhances the humoral immune response to VP7. Supported in part by NIGMS MARC USTAR GM 07717.

## **pH-dependent Photodamage of $\beta$ -lactoglobulin Mediated by Hydrophobic and Hydrophilic Porphyrins**

**Nicholas Fernandez**, Fang Tian, and Lorenzo Brancalion

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Certain dyes like the hydrophobic Protoporphyrin IX (PPIX) and hydrophilic m-Tetraphenylporphine sulfonato (TSPP) can bind proteins via non-covalent interactions. The dyes' affinity for binding  $\beta$ -lactoglobulin is pH dependent and their irradiation when non-covalently bound can generate photochemical events that may alter the conformation of the protein. The targeted damage of proteins could be important for both medical applications (cancer phototherapy) and basic research (proteomics). We investigated how the irradiation of these non-covalent complexes, at different pH, contributed to altering the structure of the protein. Our investigation used a combination of optical spectroscopic techniques that probe changes in the secondary and tertiary conformation of polypeptides. We observed changes in the integrated fluorescence intensity, peak and lifetime of the protein after the dyes were irradiated that lead us to believe that conformational changes may have been induced on the protein. These changes were most significant above pH 7 where  $\beta$ -lactoglobulin undergoes a conformational change that makes the binding site more accessible to the dyes. Above pH 7, irradiation of both PPIX and TSPP produces a slight shift (1 nm) in the emission maximum of the protein which does not occur at lower pH values. The effect of irradiation on the emission lifetime of  $\beta$ -lactoglobulin is even more dramatic as it lengthen the average lifetime of the protein's fluorescence from 1.68 to 1.95 (for PPIX), from 1.53 to 1.98 (for TSPP). The data suggest that at pH where they have access to the binding site of the protein, PPIX and TSPP have the chance of producing a photochemical reaction that modify the conformation and damage  $\beta$ -lactoglobulin. Supported in part by NIGMS MARC USTAR GM 07717.

**Glow discharge gas plasma and alkaline hydrolysis surface treatments improve wettability on poly (D, L-lactide) film**

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Cell adhesion on biomaterials is dependent on the surface properties, including charge, surface energy, roughness, topography, and the presence or absence of proteins. Surface wettability of poly D L-lactic acid (PLA) films plays a pertinent role in cell adhesion. We studied the degree of wettability of PLA films after various surface treatments, by measuring the contact angle. Treatments included sodium hydroxide (1N NaOH) hydrolysis (5 minutes, 10 minutes, and 1, 6, 12, 24 hours) or oxygenation by glow discharge gas plasma (GDGP, 30 second exposure). We hypothesized both GDGP and NaOH (24hr) film would exhibit a decrease in contact angle compared to non-treated PLA films. We found that contact angles were significantly reduced in GDGP and NaOH (24hr) treated films (SNK  $p < 0.05$ ). There was no significant difference between NaOH(24hr) and GDGP films (SNK  $p < 0.05$ ). These findings demonstrate PLA film wettability, and thus hydrophilicity, is increased by GDGP and NaOH (24hr) surface treatments. These findings suggest that these treatments may improve biocompatibility of PLA films. This work was supported in part by NIGMS MARC USTAR GM 07717.

## **Raman Spectroscopy: A Novel Approach for Studying Extremophiles in Hydrothermal Microbial Communities**

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For the first time, Raman spectroscopy is being used as an optical probe to delineate and investigate biological-geological interfaces under stressed environmental conditions in the Yellowstone NP hydrothermal systems. The current picture of geobiology/geochemistry is based on an integration of results from microbial techniques, analytical methods, and spectroscopic probes of structure and characterization. Raman techniques are being implemented to provide useful data due to the sensitivity of vibrational frequencies to molecular structure and because of the electronic selectivity conferred by resonance enhancement. This selectivity makes it possible to do small molecule spectroscopy inside larger molecules, and to extract structural details for key molecular fragments in biologically relevant species. Raman spectroscopy is particularly suited to the detection of biosignatures, which are defined as macromolecules that are directly linked to biogenic metabolism or other cellular functions. The data generated is being used to build the first comprehensive spectral database determined by selective resonance enhancement of the extant microbial biomolecules from these potentially analogous primitive microbes. This library will contain spectral features that can be extended to elucidate and characterize minerals, biochemical processes, and biochemical products (photosynthetic pigments, hopanoids, RNA, and DNA) present in hydrothermal systems using 'hot' sampling techniques in the field. Supported in part by NIGMS MBRS RISE GM60655.

## Identification and Stability of a Disulfide-Linked Homodimer in Ovine Prolactin

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Ovine prolactin (oPRL) is a heterogeneous mixture of protein isoforms that differ in charge and molecular weight. The **purpose** of this research was to analyze biochemical aspects of mercaptoethanol-resistant (MER) 46-kDa oPRL. The **hypothesis** tested in this work is that the 46-kDa oPRL is a stable disulfide-linked dimer of the 23-kDa monomer of oPRL. The **methods** used included an initial separation of a mixture of oPRL, which was partially purified, in a 7% preparative acrylamide gel by SDS-PAGE. Separated proteins were then electroeluted (pH=9.4), and the sample containing the 46-kD oPRL was separated by analytical SDS-PAGE to verify its purity. This fraction was then incubated at 95°C for 3, 30, 60, 300, and 410 minutes in 10% 2-mercaptoethanol. The products of the reaction were separated by analytical SDS-PAGE and stained. Next, they were quantified with a densitometer and their respective densities were compared. **Results** showed that as the reaction proceeded, 23-kDa oPRL formed while the amount of 46-kDa oPRL diminished as time increased. Disassociation of the dimer was dependent on time and temperature. Western blot analysis confirmed that the fragments were prolactin isoforms. In **conclusion**, the 46-kDa oPRL variant was broken down into 23-kDa. The original dimer of 23-kDa oPRL monomers was connected by stable interchain disulfide bonds. Isolation of the disulfide-linked dimer of oPRL in its native state will assist comprehension of its biological significance. (This work was supported by NSF Louis Stokes AMPUTLSAMP0217691, NIH MBRS-RISE GM60655, NIH MARC-U\*STAR GM07717, NIH MBRS-SCORE GM08194, and the Sloan Foundation)

## **Biofilm Formation in *Vibrio Cholerae* Is Affected by GGDEF Domain in VCA0697**

**Adriana Mejia**, Khalid Ali Syed, and Karl E. Klose,  
Department of Biology, University of Texas at San Antonio, San Antonio, TX

*Vibrio cholerae* causes the diarrheal disease cholerae by colonizing the human intestine and expressing various virulence factors, including cholera toxin. Cholera pandemics are initiated by the continued reintroduction of the organism into the human population through contaminated food and water, due to the persistence of the bacterium in both freshwater and saltwater environments. In the aquatic environment the bacteria are presumed to be found in multi-cellular surface adhered structures called biofilms. *V.cholerae* biofilms are more resistant to antibiotics, chlorine, and other environmental stressors compared to planktonic cells, and this likely leads to enhanced environmental persistence. Flagellar synthesis is known to influence *V.cholerae* biofilm formation. Whole genome transcription profiling of *V.cholerae* flagellar mutants utilizing microarrays revealed a number of genes positively regulated by flagellar genes. One gene of interest was VCA0697, a putative transmembrane protein with a GGDEF domain. GGDEF proteins are cyclic diguanylate synthases which have been shown to regulate virulence and biofilm expression in *V.cholerae*. Thus the apparent flagellar regulation of VCA0697 implicates a mechanism whereby flagellar synthesis influences virulence and biofilm formation. We created *V.cholerae* strains with mutations in VCA0697, and assessed their ability to form biofilms. Interestingly, the VCA0697 mutation led to an increased biofilm formation in strain 0395, the same strain in which microarray experiments were performed. It is known that as *V.cholera* develop into biofilms, flagellar synthesis is decreased. Our results suggest that the decrease in flagellar synthesis may lead to decreased levels of VCA0697, which in turn induces biofilm formation. Thus VCA0697 may play an important role signaling events involved in *V.cholerae* biofilm development. This research was supported by MBRS-RISE GM60655.

## **Determination of Banned Sudan Dyes (I, II, III and IV) in Chili Powder by Capillary Chromatography-UV Detection**

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There is a controversial level of risk associated with the potential carcinogenicity of Sudan dyes, a class of synthetic organic colorants characterized by a chromophoric azo-group, that are used in many industrial applications. Despite this, they have been banned in the United Kingdom and the rest of the European Union as an additive in products intended for human consumption. The recent contamination of hot chilli and derived products originating from India and marketed in the European Union, has demanded the development of reliable and accurate analytical methods for the fast identification and quantification of Sudan dyes in foodstuffs. We have developed a simple and fast method for the determination of Sudan dyes (I, II, III, and IV) in chili-powder products. The dyes are analyzed by micellar electrokinetic chromatography (MEKC) in combination with UV detection. The reversed phase chromatographic separation is carried out inside of a capillary, where surfactant micelles act as a pseudo-stationary phase, and an electrolytic buffer solution as a mobile phase. The effects of buffer concentration and pH, surfactant concentration, temperature, addition of organic modifiers, and the applied separation potential were studied. The separation of a standard mixture of the four dyes was achieved using optimized conditions and the method was subsequently applied to the screening of numerous chili-powder samples for the presence of the four Sudan dyes. Supported by NIGMS MBRS-RISE GM60655.

## **Western Blot Method Validation For A SREBP-1 Antibody to Study Cultured Rat Renal Mesangial Cells**

**Jamie Parra\***, Clyde F. Phelix\*, Moshe Levi, Rosa E. Villanueva\* and Nayeli Gaona\*.  
Department of Medicine, The University of Colorado Health Science Center and VA Medical Center, Denver, Colorado, \*Department of Biology, The University of Texas at San Antonio, San Antonio, Texas.

Sterol Regulatory Element Binding Protein (SREBP) belongs to a family of transcriptional factors which has been found in three isoforms consisting of SREBP-1a, -1c and -2. SREBP-1a is transcription factor needed for the biosynthesis of lipids and might be important in nephropathy. This study focused on the methodological validation for using a Western Blot procedure to detect and quantify SREBP-1 extracted from cultured rat renal mesangial cells (CRL-2573), using a commercially available antibody. Methodological validation procedures included omission of primary antibody, replacement of primary by normal serum, dilution of primary antibody, use of avidin-biotin peroxidase or peroxidase labeled secondary antibody, and use of diaminobenzidine chromogen or chemiluminescence. Mesangial cells were grown in T-75 flasks to produce at least 10 million cells then exposed to either a low (100mg/dl) or high (450mg/dl) glucose medium for 24 hours. The cells were homogenized and separated into cytoplasmic and nuclear fractions, followed by a protein assay to determine total protein concentration; 20 µg of protein were loaded per well for the Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Subsequently the Western Blot procedure was used to assess protein expression. The SREBP-1 detection was measured using optical density of bands from the nitrocellulose membranes from samples of cells grown in the two glucose concentrations. Omission of primary antibody or replacement with normal serum showed no bands corresponding to the molecular weight of SREBP-1. Whenever the avidin-biotin procedure was used several other bands appeared on the membrane. In all cases where the primary antibody was used, two bands were detected that corresponded to the known molecular weights of SREBP-1. When the peroxidase labeled secondary was used, the additional bands were not detected. A 1:600 dilution of the primary antibody with 1:2000 secondary antibody provided the optimal results with less background, and the use of chemiluminescence provided the best visualization. Results demonstrate that SREBP-1 expression can be detected in renal mesangial cells when a minimum of 10 million cells are processed and differences in expression levels can be evaluated. Early results showed that SREBP-1 was detected at higher concentrations in nuclear extractions and lower concentration in cytoplasmic extractions for cells exposed to the higher glucose concentration. Supported by R25 GM63963-01, VHA Grant, and NIGMS MBRS-RISE GM60655.

## **Detecting Intruders on Computer Networks Using Field Programmable Gate Arrays**

**James R. Perez** and Parimal Patel, PhD

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Presently, computer networks implement intrusion detection using software that scans “header” and “trailer” information in incoming data packets. Thus intruders are determined solely based on the origin of the data packet. Network security is of especially high priority to homeland security and other “post-911” applications. Furthermore, it is also a concern to the general public because of its increasing security needs both on personal and commercial computer networks. Current intrusion software and detection methods used on computer networks are inefficient and intrinsically unreliable. This study addresses these inefficiencies and limitations. More specifically, we investigate two novel approaches to arrive at a hybrid solution. The first approach involves digital hardware called Field Programmable Gate Arrays, (FPGA’s). These FPGA devices implement the detection of network intruders on a silicon chip. This gives the technology portability, on-site programmability and also a breadth of application. Secondly, software is still needed to implement detection. The difference however is how the detection of intruders is executed. Intrusion detection by examining the content of a data packet instead of focusing on the “header” and “trailer” information is the novel solution developed by this research. These technologies, when merged, provide a more effective means of intruder detection than conventional software algorithms in existence. Supported in part by NIGMS MARC USTAR GM 07717.

## **Response of *Arabidopsis* Serine/Threonine Kinase 11 (AKIN11) to Geminivirus Infection**

**Mary Jane Puente**, Surendranath Baliji, Janet Sunter, Garry Sunter  
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Plant DNA geminiviruses encode proteins that suppress host defense responses by inactivating an *Arabidopsis* serine/threonine kinase (AKIN11), which is homologous to mammalian AMP-activated kinase (AMPK). These kinases are highly conserved between animals and plants, and act as a metabolic sensor of cellular AMP and ATP levels. This suggests ties between metabolism and viral pathogenesis, leading to the hypothesis: "*Geminivirus infection alters expression of Arabidopsis AKIN11*". Our objectives are to identify sequences sufficient for AKIN11 expression and to test whether geminivirus infection alters expression of the *AKIN11* gene. The full promoter for the *Arabidopsis AKIN11* gene has been cloned, and a series of promoter deletions fused with the  $\beta$ -glucuronidase (GUS) reporter gene. Cloned DNAs were transfected into *Nicotiana benthamiana* protoplasts, and differences in GUS activity (Student's *t*-test,  $P < 0.05$ ) measured. Our results indicate that sequences important for AKIN11 expression lie between 375 and 475bp upstream of the transcription start site. Using electrophoretic mobility shift assays (EMSA) we demonstrated that this 100bp fragment was capable of specifically binding a protein(s) from *N.benthamiana* nuclear extracts. Using RT-PCR, we compared levels of AKIN11 mRNA in geminivirus-infected and mock-inoculated plants to assess changes in AKIN11 expression in response to infection. Our results indicate that steady state RNA levels are ~two-fold higher in infected plants relative to control plants. We have identified a minimal sequence sufficient for AKIN11 expression and our data suggests that the stress of viral infection increases expression of AKIN11. Future work will involve identifying the host factor(s) that bind the AKIN11 promoter. Supported by NIGMS MBRS-RISE GM60655.

## Spine densities on CA1 pyramidal neurons decrease in a lamina-specific fashion in aged mice

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While several studies suggest that spine densities on hippocampal CA1 pyramidal neurons decrease in aged rodents, it is not known if such decreases occur in all three dendritic laminae. This is a critical issue because the afferents to each layer are distinct. Here we quantified spine densities on CA1 pyramidal neurons in the three laminae in adult and aged male mice. We used transgenic mice in which some hippocampal neurons express green fluorescent protein (GFP; line GFP-O, Feng et al., 2000; also see Perez et al., 2005, Soc. Neurosci. Abstr.). Male mice at 6, 12, 18 and 24 months ( $n=4$  at each age) were anesthetized and perfused. Brains were coronally sectioned at  $300\ \mu\text{m}$  and spine densities quantified on CA1 pyramidal neuron dendrites in stratum lacunosum-moleculare (SL-M), stratum radiatum (SR), and stratum oriens (SO). In each layer, one dendritic segment per neuron ( $n \geq 10$  neurons/animal) was imaged from each animal using confocal microscopy. Segment lengths (average  $47.7 \pm 0.28\ \mu\text{m}$ ) were determined in 3-D, spines counted, and densities calculated. Results showed that spine densities decreased in the stratum radiatum and stratum oriens at 24 months, while a significant decrease was observed at 18 months in the stratum lacunosum-moleculare. There were no statistically significant differences in spine densities between the 6, 12, and 18-month-olds in the stratum oriens and stratum radiatum or between 6 and 12-month-olds and 18 and 24-month-olds in the stratum lacunosum-moleculare. At 18 and 24 months, spine densities in the SL-M averaged  $0.72 \pm 0.02$  and  $0.72 \pm 0.01$  spines/ $\mu\text{m}$  respectively, as compared to 6-month ( $0.88 \pm 0.02$ ,  $p=0.002$ ;  $p<0.001$ ) and 12-month-olds ( $0.86 \pm 0.03$ ,  $p=0.02$ ;  $p=0.007$ ). In the SR, densities at 24 months were  $1.47 \pm 0.02$  spines/ $\mu\text{m}$ , as compared to 6-month ( $1.63 \pm 0.05$ ,  $p=0.03$ ), 12-month ( $1.62 \pm 0.04$ ,  $p=0.01$ ) and 18-month-olds ( $1.61 \pm 0.04$ ,  $p=0.02$ ). At 24 months, densities in the SO were  $1.13 \pm 0.03$  as compared to  $1.26 \pm 0.03$ ,  $1.31 \pm 0.04$ , and  $1.32 \pm 0.05$  at 6, 12 and 18 months ( $p=0.03$ ,  $p=0.01$ ,  $p=0.02$ ). Thus, in the SR and SO, spine densities decreased by about 10%; these layers are both innervated by the Schaffer collaterals. In the SL-M, innervated by axons from the entorhinal cortex, densities decreased by 17%. These data suggest there are lamina-specific changes in connectivity patterns in the hippocampus of aged mice. Support Contributed By: *NIH GM08194 and MBRS RISE GM60655*

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