

# 18th Annual Life Sciences Undergraduate Research Symposium

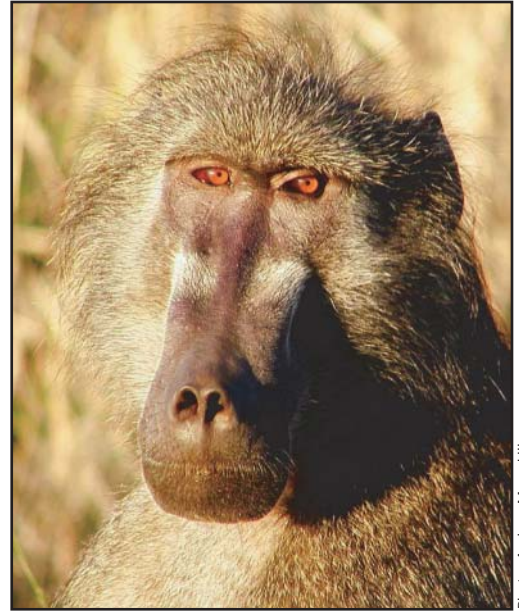


Photo: Andrew Macaskill

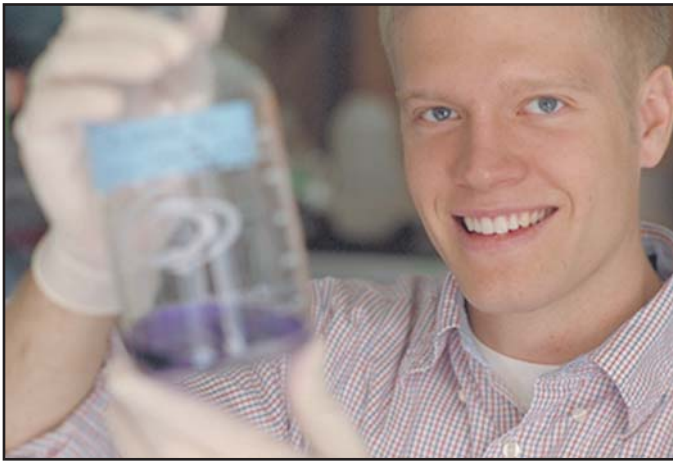


Photo: Richard Anderson

**University of Minnesota  
Minneapolis Campus  
Coffman Memorial Union  
Great Hall  
April 28, 2004**

## ***Oral Presentations***

1:30-2:00

## ***Poster Session***

2:00-4:30

## ***Reception & Awards***

4:30-6:00

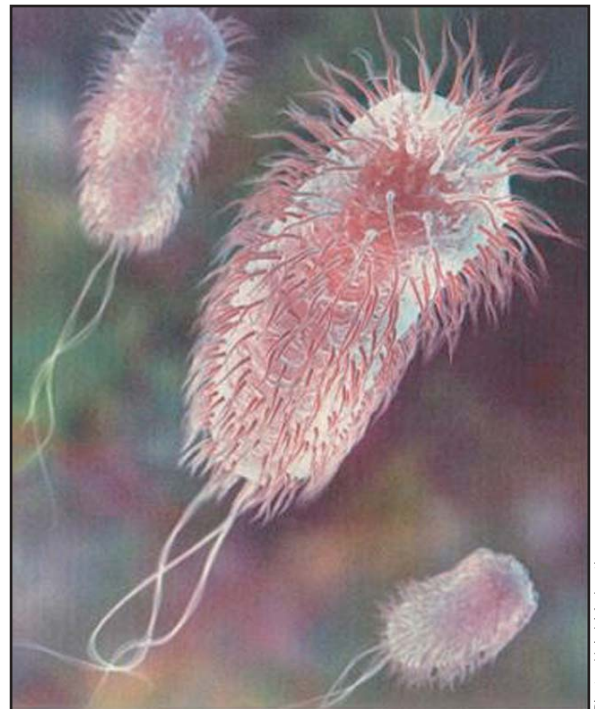


Photo: Hybrid Animations

**COLLEGE OF** ● **Biological**  
● **Sciences**  
● UNIVERSITY OF MINNESOTA

Hosted by the CBS Honors Program

## CONTENTS

	page
Poster Index (1 - 86) .....	1
Presenters (A - Z) .....	2
Abstracts .....	11
Mentor Kudos .....	53

## POSTER INDEX

- |                      |                                |
|----------------------|--------------------------------|
| 1. Elizabeth Brodeen | 19. Amy Kullas                 |
| 2. Lillian Magidow   | 20. Ryan Laux                  |
| 3. Holly MacCormick  | 21. Kathryn Brown              |
| 4. Ozge Goktepe      | 22. Caleb Bates                |
| 5. Michael Bush      | 23. Kim Ha                     |
|                      | 24. Stephen Hinkin             |
|                      | 25. Benji Mathews              |
| 6. Kelsey Dahl       |                                |
| 7. Charissa Lewis    | 26. Nicole Ali                 |
| 8. Lisa Lenarz-Wyatt | 27. Katherine Harrison         |
| 9. Todd Knutson      | 28. Cassandra Kistler-Anderson |
| 10. Erin Bequette    | 29. Harmony Tyner              |
| 11. Krsna Rangarajan | 30. Nicholas Winning           |
|                      | 31. Tate Winter                |
| 12. Sarah Malmquist  |                                |
| 13. Paul Lobitz      | 32. Remy Wong                  |
| 14. Xiaosong Liu     | 33. Mohamed Abdihalim          |
| 15. Fausta Ditah     | 34. Rebecca Long               |
| 16. Mohamed Moussa   | 35. Ryan Sunderman             |
| 17. Irene Dorweiler  | 35. Jill Grandt                |
| 18. Abraham Gol      | 36. Senit Debesai              |
|                      | 37. John Andersen              |

38. Ann Neumann  
39. Meenal Kapoor  
40. Carolyn Presley  
41. Andrea Zins  
42. Benjamin Miller  
43. Jamie Jones  
44. Nima Estharabadi

45. Molly Welle  
46. Shruthi Ravimohan  
47. Ashley Lawson  
48. Forum Kamdar  
49. Jennifer Gravelle  
50. Esther Kao  
51. Scott Perkinson

52. Cheuk-Man Wong  
53. Abhinav Arneja  
54. Kellie Leinen  
55. Ta-Chun Hang  
56. Teodora Platikanova  
57. Hong-Yiou (David) Lin

58. Wade Schulz  
59. Lindsey Thompson  
60. Katie Lee  
61. Meghan Richardson  
62. Brenda Saxton  
63. Jessie Kerns

64. Miranda Bernhardt  
65. Kristin Berg  
66. Jason Motl  
67. Kathryn Goeden  
68. Amanda Helvig  
69. Rania Habib

70. Matthew Schaefers  
71. Julie Kuruc  
72. Aili Salo  
73. Joshua Wilson-Grady  
74. Anu Elayaperumal  
75. Eileen Kerkhoven

76. David Atkinson  
77. Amber Martell  
78. Kristyn VanderWaal  
79. John Capen  
80. Catherine Pham

81. Christopher Erickson  
82. Jayna DeVore  
83. Richard Osness  
84. Philip Jensen  
85. Matthew Reeves

## ORAL PRESENTATIONS (1:30 – 2:00)

**Forum D. Kamdar**, Biology, Physiology (POSTER #48)

*Sponsor: Bernhard Hering, Brett Levay-Young, Surgery*

IN VIVO DIFFERENTIAL GENE EXPRESSION IN PANCREATIC ISLET CELLS

**Kristin M. Berg**, Biochemistry (POSTER #65)

*Sponsor: Deborah Ferrington, Ophthalmology*

CATALOG OF PROTEIN CHANGES PERTAINING TO AGE-RELATED MACULAR DEGENERATION IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS

## POSTER PRESENTATIONS (A to Z) (2:00 – 4:30) (Poster #)

**Mohamed M. Abdihalim**, Biochemistry (#33)

*Sponsor: Anja-Katrin Bielinsky, BMBB*

THE ROLE OF S PHASE CHECKPOINT PROTEINS IN REGULATING ORIGIN ACTIVATION

**Nicole Ali**, St. Paul Academy and Summit School (#26)

*Sponsor: Catherine Verfaillie, Medicine, Stem Cell Institute*

BONE MORPHOGENETIC PROTEIN ANTAGONISTS TWISTED GASTRULATION AND CHORIND CAN AID PRIMITIVE HUMAN HEMATOPOIETIC CELL EXPANSION EX VIVO

**John D. Andersen**, GCD (#37)

*Sponsor: Wufan Tao, Medicine, Stem Cell Institute*

PPXY(PY) MOTIF-MEDIATED hLATS2/SAV1 INTERACTION

**Abhinav Arneja**, Biomedical Engineering (#53)

*Sponsor: David Odde, Biomedical Engineering*

CELL SPRAYING TECHNIQUES FOR 3D TISSUE ENGINEERING

**David M. Atkinson**, Neuroscience, Psychology (#76)

*Sponsor: Jonathan Gewirtz, Psychology*

ROLE OF CORICOTROPIN-RELEASING FACTOR IN ACUTE OPIATE DEPENDENCE

**Caleb A. Bates**, Biochemistry, History (#22)

*Sponsor: Carston Wagner, Patrick Hanna, Medicinal Chemistry*

PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN ARYLAMINE N-ACETYLTRANSFERASE 1

**Erin L. Bequette**, Animal Science (#10)

*Sponsor: Jim Mickelson, Michelle Wagner, Veterinary Pathobiology*

COMPARATIVE GENOME MAPPING OF HUMAN CHROMOSOME THREE TO EQUINE CHROMOSOME SIXTEEN

**Kristin M. Berg**, Biochemistry (#65)

*Sponsor: Deborah Ferrington, Ophthalmology*

CATALOG OF PROTEIN CHANGES PERTAINING TO AGE-RELATED MACULAR DEGENERATION IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS

**Miranda L. Bernhardt**, GCD (#64)

*Sponsor: Ken Roberts, Urologic Surgery*

INVESTIGATION OF THE EGG-BINDING PROPERTY OF RAT CRISP-1 SPERM PROTEIN

**Elizabeth C. Brodeen, EEB (#1)**

*Sponsor: Jennifer King, EEB, Soil, Water, and Climate*

EFFECTS OF LAND MANAGEMENT SYSTEMS ON SOIL GREENHOUSE GAS EMISSIONS

**Kathryn M. Brown, Biochemistry, Microbiology (#21)**

*Sponsor: Sharon Murphy, BMBB, Cancer Center*

THE CONTRIBUTION OF HUMAN LUNG CYTOCHROME P450S IN NICOTINE METABOLISM

**Michael R. Bush, Fisheries (#5)**

*Sponsor: Andrew Simons, Fisheries, Wildlife, and Conservation Biology*

CRANIAL MYOLOGY OF NORTH AMERICAN PERCIDAE

**John B. Capen, GCD (#79)**

*Sponsor: W. Gibson Wood, Pharmacology*

BRAIN GENE EXPRESSION WITH STATIN DRUG TREATMENTS

**Kelsey J. Dahl, Wildlife Biology (#6)**

*Sponsor: Jim Perry, Mark Williams, Fisheries, Wildlife, and Conservation Biology, Biotechnology Institute*

VIRAL EXCITOTOXIC NEUROTRANSMISSION OF THE GONADOTROPIN RELEASING HORMONE INDUCING STERILITY: CAN IT WORK?

**Senit R. Debesai, Microbiology (#36)**

*Sponsor: Ronald Jemmerson, Microbiology*

INVESTIGATION OF THE BASIS FOR ACTIN-MITOCHONDRIA INTERACTIONS

**Jayna L. DeVore, Biology (#82)**

*Sponsor: Frank Barnwell, EEB*

TIME AND TIDE WAIT FOR NO CRAB: MANIPULATING CIRCATIDAL CLOCKS IN AN INTERTIDAL FIDDLER CRAB

**Fausta A. Ditah, Biochemistry (#15)**

*Sponsor: Howard Towle, BMBB*

CIRCADIAN LIPOGENIC GENE EXPRESSION

**Irene J. Dorweiler, GCD (#17)**

*Sponsor: Mary Porter, GCD*

IDENTIFYING THE PF2 MUTATION IN *CHLAMYDOMONAS REINHARDTII* STRAIN SUP-PF3

**Anu Elayaperumal, Biology, Physiology (#74)**

*Sponsor: William C. Engeland, Surgery*

PITUITARY SUPPRESSION BY DEXAMETHASONE ATTENUATES THE COMPENSATORY ADRENAL GROWTH RESPONSE

**Christopher M. Erickson, Biology (#81)**

*Sponsor: Chester B. Whitley, Pediatrics*

MONITORING URINARY GLYCOSAMINOGLYCAN EXCRETION IN EXPERIMENTAL MICE USING AN AUTOMATED METHOD

**Nima Estharabadi, Biochemistry (#44)**

*Sponsor: Craig Henke, Medicine*

REGULATION OF A  $\beta 1$  INTEGRIN P13K/AKT VIABILITY PATHWAY BY PTEN

**Kathryn R. Goeden**, Microbiology (#67)

*Sponsor: Gary Dunny, Microbiology*

DETERMINING THE SPECIFICITY OF cCF10 PEPTIDE FOR *ENTEROCOCCUS FAECALIS* PHEROMONE BINDING PROTEINS, PRGZ AND PRX.

**Ozge Goktepe**, GCD (#4)

*Sponsor: Anne Kapuscinski, Fisheries, Wildlife, and Conservation Biology*

MEASURING MATING SUCCESS OF TRANSGENIC VERSUS WILD-TYPE FISH TO HELP ESTIMATE THE RISK OF GENE FLOW TO WILD POPULATIONS

**Abraham K. Gol**, GCD (#18)

*Sponsor: Michael Simmons, GCD*

TRANSPOSASE ACTIVITY OF MODIFIED P ELEMENTS IN *DROSOPHILA MELANOGASTER*

**Jill M. Grandt**, Biology (#35)

*Sponsor: Pamela Skinner, Veterinary and Biomedical Sciences*

EVALUATION OF PERFORIN IN SIV SPECIFIC CD8+ T CELLS

**Jennifer A. Gravelle**, GCD (#49)

*Sponsor: Catherine Verfaillie, Medicine*

THE SEARCH FOR CANINE MAPC AND THEIR USE IN GENE THERAPY FOR DEVELOPING A CURE TO HEMOPHILIA

**Kim N. Ha**, Chemistry (#23)

*Sponsor: Gianluigi Veglia, Chemistry*

EXPRESSION, PURIFICATION, AND MUTAGENESIS STUDIES OF THE INTEGRAL MEMBRANE PROTEIN PHOSPHOLAMBAN

**Rania A. Habib**, Microbiology (#69)

*Sponsor: David L. Dunn, Karen R. Wasiluk, Surgery*

THE INVADER: A NOVEL mRNA ASSAY

**Ta-Chun Hang**, Biomedical Engineering, Biology (#55)

*Sponsor: Robert Tranquillo, Biomedical Engineering*

SCHWANN CELL-MEDIATED CONTACT GUIDANCE OF AXONS IN ALIGNED FIBRIN GELS

**Katherine Harrison**, Microbiology (#27)

*Sponsor: Robin Wright, GCD*

REGULATION OF NUCLEAR ARCHITECTURE

**Amanda J. Helvig**, Microbiology (#68)

*Sponsor: David Brown, Lucy Vulchanova-Hart, Veterinary Pathobiology*

EXPRESSION AND FUNCTION OF NITRIC OXIDE SYNTHASE (NOS) IN *SALMONELLA ENTERICA* SEROVAR *TYPHIMURIUM*

**Stephen M. Hinkin**, Biology (#24)

*Sponsor: Carston R. Wagner, Medicinal Chemistry*

THE IMPACT OF THE PURINE NUCLEOSIDE PHOSPHORAMIDASE (hHINT-1) DIMER INTERFACE ON CATALYSIS

**Philip A. Jensen**, GCD (#84)

*Sponsor: Michael Simmons, GCD*

GENETIC REGULATION OF TRANSPOSABLE P ELEMENTS IN *DROSOPHILA*

**Jamie M. Jones, GCD (#43)**

*Sponsor: David Largaespada, GCD*

A MODEL OF NRAS-DRIVEN MAST CELL DISEASE IN THE MOUSE

**Forum D. Kamdar, Biology, Physiology (#48)**

*Sponsor: Bernhard Hering, Brett Levay-Young, Surgery*

IN VIVO DIFFERENTIAL GENE EXPRESSION IN PANCREATIC ISLET CELLS

**Esther E. Kao, GCD (#50)**

*Sponsor: Jennifer Hall, Cardiology*

THE ROLE OF REF-1 IN VASCULAR SMOOTH MUSCLE CELL APOPTOSIS

**Meenal Kapoor, GCD (#39)**

*Sponsor: Kathleen Conklin, GCD*

UTILIZATION OF A YEAST TWO HYBRID SCREEN TO IDENTIFY PROTEINS THAT INTERACT WITH HS7, A CANDIDATE ONCOGENE

**Eileen F. Kerkhoven, Science in Agriculture, Animal Science (#75)**

*Sponsor: Douglas Foster, Animal Science*

ISOLATION OF PUTATIVE CHICKEN STEM CELLS

**Jessie L. Kerns, Biology (#63)**

*Sponsor: Carston Wagner, Jonathan Carlson, Medicinal Chemistry*

SURFACE INTERFACE EFFECTS IN INDUCED PROTEIN DIMERIZATION

**Cassandra Kistler-Anderson, Biology (#28)**

*Sponsor: Cheryl Gale, Pediatrics*

THE ROLE OF BUD1 IN *CANDIDA ALBICANS*

**Todd P. Knutson, GCD, Neuroscience (#9)**

*Sponsor: Kent Reed, Veterinary and Biomedical Sciences*

APPLICATION OF CHICKEN MICROARRAYS FOR COMPARATIVE STUDIES OF GENE EXPRESSION IN TURKEY

**Amy L. Kullas, Microbiology, GCD (#19)**

*Sponsor: Robert Elde, Steve Yussen, College of Biological Sciences, College of Education and Human Development*

TAKING BIOLOGY TO THE CLASSROOM: A TEACHING INTERNSHIP

**Julie A. Kuruc, Microbiology (#71)**

*Sponsor: Franciso Diez-Gonzalez, Food Science and Nutrition*

USE OF CARBONATE TO REDUCE *ESCHERICHIA COLI* 0157:H7 IN WATER

**Ryan A. Laux, Biology (#20)**

*Sponsor: Kathryn Hanna, College of Biological Sciences*

NEW PLASMA DONOR VALUES

**Ashley E. Lawson, Biology (#47)**

*Sponsor: William Frey, Tinna Ross, Department of Pharmaceutics, Alzheimer's Research Center*

HOW ALZHEIMER'S DISEASE IS LINKED TO FREE RADICAL DAMAGE

**Katie N. Lee, Biochemistry (#60)**

*Sponsor: Keli Hippen, Timothy Behrens, Medicine*

A NOVEL MODEL FOR ADDRESSING B CELL SOMATIC HYPERMUTATION

**Kellie M. Leinen, Chemical Engineering (#54)**

*Sponsor: Ted Labuza, Food Science and Nutrition*

RAFFINOSE INCREASES THE STABILITY OF SUCROSE COTTON CANDY

**Lisa M. Lenarz-Wyatt, Biochemistry (#8)**

*Sponsor: Claudia Schmidt-Dannert, Kevin Watts, BMBB*

THE NADPH-CYTOCHROME P450 REDUCTASE ATR2 ALLEVIATES BLOCKAGE OF THE FLAVONOID PATHWAY IN METABOLICALLY ENGINEERED *ESCHERICHIA COLI*

**Charissa J. Lewis, GCD (#7)**

*Sponsor: Paul T. Magee, GCD*

THE PHYSICAL MAP OF CHROMOSOME 3 OF *CANDIDA ALBICANS*

**Hong-Yiou (David) Lin, GCD, Biochemistry (#57)**

*Sponsor: Peter Bitterman, Vitaly A. Polunovsky, Medicine*

GENE THERAPY FOR LUNG FIBROSIS

**Xiaosong Liu, Biochemistry, Chemistry (#14)**

*Sponsor: Howard Towle, BMBB*

THE ROLE OF CHREBP IN GLUCOSE-REGULATED GENE TRANSCRIPTION

**Paul D. Lobitz, GCD (#13)**

*Sponsor: David Largaespada, GCD*

MOUSE GENE ANNOTATION USING THE *SLEEPING BEAUTY* TRANSPOSON: STUDY PROGRESS AND MECHANISMS OF TRANSPOSITION

**Rebecca Long, GCD (#34)**

*Sponsor: Robin Wright, GCD*

YEAST ON DRUGS: ANALYZING THE EFFECTS OF ALTERED STEROL PRODUCTION ON DRUG SENSITIVITY

**Holly A. MacCormick, EEB (#3)**

*Sponsor: Craig Packer, EEB*

BABOON AGGRESSION

**Lillian C. Magidow, Science in Agriculture (#2)**

*Sponsor: Paul Porter, Agronomy and Plant Genetics*

FARMER PERSPECTIVES ON MAIZE, GLOBALIZATION, AND CHANGE IN CHIAPAS, MEXICO

**Sarah J. Malmquist, Biochemistry, GCD (#12)**

*Sponsor: Jeffrey Simon, GCD*

MUTATIONAL ANALYSIS OF A *DROSOPHILA* TRANSCRIPTIONAL REPRESSOR COMPLEX

**Amber L. Martell, Neuroscience (#77)**

*Sponsor: Cathy Kotz, Kevin Silverstein, Food Science and Nutrition, Plant Biology*

OREXIN A-INDUCED FEEDING AND ACTIVITY: ROLE OF THE LOCUS COERULEUS

**Benji K. Mathews, Chemistry (#25)**

*Sponsor: Mark Distefano, Chemistry*

PREPARATION AND EVALUATION OF ISOPRENOID DIPHOSPHATE ANALOGUES THAT INCORPORATE AZIDE FUNCTIONAL GROUPS: TOOLS FOR UNDERSTANDING THE ANTICANCER PROPERTIES OF FARNESYLTRANSFERASE INHIBITORS

**Benjamin T. Miller, Biology (#42)**

*Sponsor: Deanna Koepp, GCD*

LOCATE AND ANNIHILATE: REGULATED PROTEIN DEGRADATION

**Jason A. Motl, Biochemistry, GCD (#66)**

*Sponsor: Janet Schottel, BMBB*

EFFECTS OF DESICCATION ON RNA AND PROTEIN EXPRESSION IN *E. COLI*

**Mohamed E. Moussa, GCD (#16)**

*Sponsor: Howard Towle, BMBB*

THE EFFECT OF GLUCOSE ON THE CIRCADIAN RHYTHM IN PRIMARY CULTURED HEPATOCYTES

**Ann N. Neumann, GCD (#38)**

*Sponsor: Jamie Lohr, Pediatrics*

THE ROLE OF NEURAL CREST IN XENOPUS LEFT-RIGHT GENE EXPRESSION AND HEART DEVELOPMENT

**Richard E. Osness, Biochemistry (#83)**

*Sponsor: Alexander Khoruts, Medicine*

DTA-1: AN ANTIBODY AGAINST GLUCOCORTICOID-INDUCED TNF RECEPTOR INCREASES CLONAL EXPANSION INDEPENDENTLY OF CD28-MEDIATED CO-STIMULATION AND REGULATORY CD25+CD4 T CELLS

**Scott G. Perkinson, GCD (#51)**

*Sponsor: David Largaespada, GCD*

LONG-TERM GENE TRANSFER AND EXPRESSION IN HUMAN GLIOBLASTOMA USING THE *SLEEPING BEAUTY* TRANSPOSON SYSTEM

**Catherine L. Pham, Biochemistry (#80)**

*Sponsor: Sharon Murphy, Mary Dempsey, BMBB, Cancer Center*

THE 2A6 GENE AND NICOTINE METABOLISM

**Teodora N. Platikanova, Biology (#56)**

*Sponsor: Cheryl Neudauer, James B. McCarthy, Laboratory Medicine and Pathology*

DETERMINATION OF PROTEIN KINASE C EXPRESSION IN CANCER CELL LINES ISOLATED FROM VARIOUS STAGES OF MELANOMA PROGRESSION

**Carolyn J. Presley, GCD (#40)**

*Sponsor: Clifford Steer, Medicine*

PURIFICATION OF THE VIRE2 NUCLEAR TRANSPORT PROTEIN AND ITS INVOLVEMENT IN GENE REPAIR

**Krsna V. Rangarajan, GCD (#11)**

*Sponsor: Andrew Simons, Tony Gamble, Fisheries, Wildlife, and Conservation Biology*

EVALUATING THE PHYLOGENETIC UTILITY OF SEVERAL NUCLEAR GENES IN GECKO LIZARDS

**Shruthi Ravimohan, GCD (#46)**

*Sponsor: David Largaespada, GCD*

THE *SLEEPING BEAUTY* TRANSPOSON FOR CANCER THERAPY: REGULATED TRANSPOSASE AND ANTI-CANCER TRANSPOSON GENES

**Matthew D. Reeves, Biochemistry, GCD, Physiology, Chemistry (#85)**

*Sponsor: Mark Distefano, Cheng-MinTann, Chemistry*

CATALYSIS OF ALDOL REACTIONS WITH SEMISYNTHETIC ENZYMES

**Meghan A. Richardson, Science in Agriculture (#61)**

*Sponsor: Douglas Foster, Animal Science*

ANALYSIS OF CELL CYCLE GENES IN PRIMARY AND IMMORTAL CHICKEN CELLS

**Aili V. Salo, Science in Agriculture (#72)**

*Sponsor: Scott Fahrenkrug, Byung-Whi Kong, Animal Science*

GREEN FLUORESCENCE PROTEIN TRANSFECTION IN PRIMARY PIG FETAL FIBROBLAST AND IMMORTAL PIG ENDOMETRIAL GLANDULAR EPITHELIAL CELLS

**Brenda J. Saxton, Neuroscience, GCD (#62)**

*Sponsor: Michael Murtaugh, Craig R. Johnson, Veterinary Pathobiology*

DIRECT PHYSICAL CHARACTERIZATION OF PRRSV VIRION PROTEINS

**Matthew M. Schaefers, Microbiology (#70)**

*Sponsor: Patrick Cleary, Haesun Park, Microbiology*

BACTERIOPHAGE TRANSDUCTION OF BIOLUMINESCENT GROUP A STREPTOCOCCUS

**Wade L. Schulz, GCD (#58)**

*Sponsor: Patricia Tam, Medicine*

DETERMINATION OF RNA SECONDARY STRUCTURE IN THE 5' NONCODING REGION OF COXSACKIEVIRUS B1

**Ryan M. Sunderman, Microbiology (#35)**

*Sponsor: Pamela Skinner, Veterinary and Biomedical Sciences*

EVALUATION OF PERFORIN IN SIV SPECIFIC CD8+ T CELLS

**Lindsey R. Thompson, Biology (#59)**

*Sponsor: Robin Wright, GCD*

SARS-RELATED VIRAL ASSEMBLY: AN EXPERIMENTAL STUDY IN YEAST

**Harmony L. Tyner, Microbiology (#29)**

*Sponsor: Ronald Jemmerson, Microbiology*

IN SEARCH OF THE CYTOCHROME C TRANSLOCATION CHANNEL IN THE MITOCHONDRIA OUTER MEMBRANE

**Kristyn E. VanderWaal, GCD (#78)**

*Sponsor: Lorene Lanier, Neuroscience,*

THE ROLE OF N-WASP IN CELL MOTILITY

**Molly M. Welle, Biology (#45)**

*Sponsor: Erin Malone, Eli Hendrickson, Clinical and Population Sciences*

THE USE OF B-MODE ULTRASOUND TO IDENTIFY RECOVERY PATTERNS OF COLIC IN HORSES FOLLOWING SURGERY OR MEDICAL MANAGEMENT

**Joshua T. Wilson-Grady**, Biochemistry (#73)

*Sponsor: David Bernlohr, Sandra Lobo, BMBB*

EXPRESSION OF GROWTH AND DIFFERENTIATION FACTORS IN TYPE II DIABETES

**Nicholas H. Winning**, Biology (#30)

*Sponsor: Cheryl Gale, Pediatrics*

CANDIDA ALBICANS INTIP LOCALIZATION DEPENDS ON A REGION BETWEEN AMINO ACIDS 1229 AND 1382

**Tate N. Winter**, Neuroscience (#31)

*Sponsor: William Elmquist, Pharmaceuticals*

IS NICOTINE ACTIVELY TRANSPORTED AT IMPORTANT BIOLOGICAL BARRIERS? ROLE OF P-GLYCOPROTEIN AND BCRP TRANSPORT PROTEINS

**Cheuk-Man Wong**, Kinesiology (#52)

*Sponsor: Jurgen Konczak, Kinesiology*

THE EFFECT OF AMES TRAPEZOID WINDOW ILLUSION ON AIMING ACTION

**Remy E. Wong**, Microbiology, GCD (#32)

*Sponsor: Deanna Koepf, GCD*

CELL CYCLE DEPENDENT EXPRESSION OF F-BOX PROTEINS

**Andrea M. Zins**, Biochemistry (#41)

*Sponsor: Ross Johnson, GCD*

COMMUNICATION BREAKDOWN: DRUG EFFECTS ON CONNEXIN-43 TRAFFICKING

## ABSTRACTS

### 01

#### EFFECTS OF LAND MANGEMENT SYSTEMS ON SOIL GREENHOUSE GAS EMISSIONS

Elizabeth C. Brodeen, (Jennifer Y. King)

Department of Ecology, Evolution, and Behavior, University of Minnesota

Global climate change is inextricably linked to the increase of greenhouse gases in the atmosphere, due primarily to anthropogenic sources such as fossil fuel burning and land use changes. Few researchers have quantified the emissions of greenhouse gases from soils of different land management systems. This study sought to determine if there were differences between three land management systems and soil emissions of the greenhouse gases carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O). Soils were sampled from conventional management agriculture, organic agriculture, and prairie. Soils were incubated, and emitted gas was analyzed for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, using gas chromatography. Three trials were performed: soils incubated with no manipulations (field soil trial), soils incubated with the addition of water to simulate rainfall (wet soil trial), and soils incubated with the addition of fertilizer (fertilized soil trial). In the field and wet soil trials, rates of CH<sub>4</sub> production were significantly lower in prairie soils compared to conventional management soils (repeated measures ANOVA,  $p = 0.023$  and  $p = 0.001$ , respectively). In the field and wet soil trials, rates of CO<sub>2</sub> production were significantly lower in the conventional management soils compared to the prairie soils (repeated measures ANOVA,  $p = 0.003$  and  $p = 0.006$ , respectively). No significant differences between systems were found for the field soil trial for N<sub>2</sub>O (repeated measures ANOVA,  $p = 0.134$ ). No wet soil data for N<sub>2</sub>O were collected. Data from the fertilized soil trial are still being collected and analyzed; initial data suggests that conventional management soils emit a greater amount of N<sub>2</sub>O than organic and prairie soils. The results of the field and wet soil trials suggest that rates of organic matter decomposition are higher in prairie and organic soils, as compared to conventional management soils. These considerations are crucial to the farming industry as it aims to find more sustainable agricultural methods.

### 02

#### FARMER PERSPECTIVES ON MAIZE, GLOBALIZATION, AND CHANGE IN CHIAPAS, MEXICO

Lillian C. Magidow and (Paul Porter)

Department of Agronomy, University of Minnesota

The free trade policies of the North American Free Trade Agreement (NAFTA) have had significant impacts on the trade and prices of commodities in Mexico, including maize, [*Zea mays* (L.)]. Domesticated in Southern Mexico, maize still serves as the primary subsistence crop for millions of indigenous Mexicans in this region. This project examined what effects, if any, NAFTA has had among indigenous maize farmers in the Los Altos highlands of Chiapas, Mexico. Farmers in three communities were interviewed about the cultural importance of maize, its cultivation and change thereof in the recent past, and if they have perceived effects of NAFTA. Of the seventeen farmers interviewed none produced maize for sale and all grew less than 3 hectares, with an average cultivated area of 0.7 ha and median of 1 ha. Of those cultivating maize (88 percent), 76 percent were unable to grow enough to meet their family's needs in at least some years. In two of the communities the area of maize cultivation stayed constant or declined over the past 30 years. In the other community an increase was due to local easing of land clearing restrictions. Among the farmers who felt affected by NAFTA, key concerns were raised. These concerns included contamination by transgenic maize, loss of land races, low prices for maize, economic disparities, land tenure, employment, self-sufficiency, and land degradation. A number of these issues can be related to the policies of NAFTA, but this lone trade agreement is only one part of the changes that have been affecting small-scale Mexican farmers. Though these hardships are often attributed to NAFTA alone, it is the larger trend of globalization that includes trade liberalization and governmental decisions benefiting large corporations rather than small farmers, which maintain the economic marginalization of indigenous peoples in the Chiapas highlands.

### 03

#### **BABOON AGGRESSION**

Holly A. MacCormick and (Craig Packer)

Department of Ecology, Evolution, and Behavior, University of Minnesota

The importance of dominance hierarchies in structuring primate societies has been well documented over the past fifty years. But while numerous studies have explored the role of aggression in the formation and maintenance of hierarchies, none have directly measured the risk of injury to individuals of different rank or whether high ranking individuals are better able to survive wounding. Furthermore, no studies have explored how the sex of an individual can influence the relationship between dominance rank and the risk/consequences of injury. This is of particular interest because male social rank is determined through direct competition so social status is expected to be highly correlated with fighting ability and genetic “quality.” In contrast, females inherit their mothers’ rank through coalition formation and nepotistic behavior, thus female rank may be a poor predictor of “quality.” To address the relationships between dominance and injury in primates, my research incorporates 26 years of data on olive baboons (*Papio anubis*) in Gombe National Park in Tanzania, Africa. Like many primates, olive baboons live in groups structured by linear dominance hierarchies. Thus, the findings of my studies will have broad implications for other primates. I predict that higher ranking male olive baboons will receive fewer injuries, and they will also have a better chance of survival after being wounded than males of lower rank. I also expect that there will be little to no correlation between rank and injury for female olive baboons. I am currently translating these field notes from Swahili to English to obtain information on baboon survival and injury. The results of my study will be presented in a poster at the 2004 Life Sciences Undergraduate Research Symposium.

### 04

#### **MEASURING MATING SUCCESS OF TRANSGENIC VERSUS WILD-TYPE FISH TO HELP ESTIMATE THE RISK OF GENE FLOW TO WILD POPULATIONS**

Ozge Goktepe (Anne R. Kapuscinski)

Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota

Genetically engineered fish may become the first transgenic animal approved for commercial use. Transgenic fish escaping from fish farms could interbreed with wild relatives. This raises the need to assess whether their transgenes would spread or be purged by natural selection. A net fitness model can be used to estimate the fate of a transgene<sup>1</sup>. I examined mating advantage, one of six net fitness traits in the model, and tested the hypothesis that growth-enhanced transgenic male medaka have a mating advantage over wild-type males. I used one line of medaka (*Oryzias latipes*) bearing a salmon growth hormone gene construct that enhances growth rate and final size. I randomly selected five pairs of wild-type (avg. 0.3499 g) and transgenic males (avg. 0.4205 g) competing for five wild-type females and recorded which male attained the first successful mating in each of five trials, of five copulations each. After every trial, the males were moved to compete with a different, previously unencountered male and female. Statistical analyses of these results indicate that there is no difference ( $p \geq 0.30$ ) between the mating advantage of transgenic and wild-type males in this line, and reinforce the need to test each transgenic line.

1. Muir WM and Howard RD (2001) Fitness components and ecological risk of transgenic fish release: a model using Japanese Medaka (*Oryzias latipes*). *Am Natur* **158**:1-16.

## 05

### **CRANIAL MYOLOGY OF NORTH AMERICAN PERCIDAE.**

Michael R. Bush (Andrew M. Simons)

Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota

The North American darters are a group of approximately 140 species native to eastern North America. These fishes are in the family Percidae and are related to the perch and walleye. The darters are divided into several groups and several of these groups have marked differences in head shape. These differences in cranial shape are a function of the shape of the various bones that make up the skulls of these fishes. Although the anatomy of the skulls is relatively well known, no work had been done before on the anatomy of the musculature, particularly the muscles associated with the jaws. A knowledge of the anatomy of the jaw muscles will help with understanding the functional morphology of the jaws. The purpose of the research project was to discern morphological differences in the musculature of the jaws of these fishes. The study incorporated fishes that are relatively “derived” and those further down the phylogenetic tree. It includes 17 species. Specimens were selected from the Bell Museum’s Ichthyological Collection and were stained in an Alizarin red solution, which dyes the calcified areas of the fish, such as bone and scales, making the muscles easy to see. The specimens are then dissected under a dissecting scope and were drawn using a camera lucida, which features a tracing option. The drawings are then inked and results are determined from the drawings. Several differences have been found in the shape of the musculature, the muscles and their proximity to the eye, and the attachment of the maxillary ligament. Six groups have been identified within the four genera to have distinct musculature shapes and different attachment points for the maxillary ligament.

## 06

### **VIRAL EXCITOTOXIC NEUROTRANSMISSION OF THE GONADOTROPIN RELEASING HORMONE INDUCING STERILITY: CAN IT WORK?**

Kelsey J. Dahl, (Jim A. Perry, Mark D. Williams)

Fisheries, Wildlife, and Conservation Biology, University of Minnesota

European rabbits in Australia represent one of the world’s most dramatic examples of the effects of an exotic species. Such a rampant and damaging pest species needs population control. The best method of control appears to be through an anthropogenic virus. A virus can diminish the invasive rabbit species’ population by infecting a gonadotropin-releasing hormone (GnRH) neural tract, crucial to reproduction. In our work, we propose an excitotoxic molecule that acts as a neurotransmitter. We will induce domoic acid formation to cause neuronal death leading to sterility. In order for this to be successful, our viral model would require auto-production of domoic acid, which would then infect the GnRH neural tract – leading to infertility/sterility. This project has many intriguing facets to consider (e.g., species specificity, molecular endocrinology, virology) that will take many years of research to complete. Our long-term research goals include field release, preceded by the usage of an arthropod vector (probably mosquitoes), and developing the GMO virus. Our immediate goal, to be reported in this symposium is dose response of the GnRH neuron as affected by domoic acid.

## 07

### THE PHYSICAL MAP OF CHROMOSOME 3 OF *CANDIDA ALBICANS*

Charissa J. Lewis (Paul T. Magee)

Department of Genetics, Cell Biology, and Development, University of Minnesota

With the increase of immunocompromised patients worldwide *Candida albicans*, normally a commensal organism, has emerged as a leading fungal pathogen. Currently 93% of the genome has been sequenced to a level of 10.9x coverage and has been made public through Assembly 19<sup>1</sup> from Stanford University. Sequence assembly is not able to determine the chromosomal order of the sequence contigs and their direction relative to one another. In addition, repeated regions of the sequence larger than approximately 600 bp cannot be correctly assigned. To complete *Candida albicans* genome characterization we are preparing a sequence-tagged site (STS) map of the *Candida albicans* genome using a 3840 member genomic library in a vector that allows cloning of tandemly repeated DNA. The information gained from the STS map will allow sequence projects to complete an accurate, aligned, and ordered sequence of the genome. This on-going project has previously completed chromosome 7, and is nearing completion of chromosomes 5 and 6. The published data of the STS map of chromosome 7 allowed for the completion of the chromosome's sequence<sup>2</sup>. Chromosome 3 is the fourth largest chromosome of the genome and the only chromosome of *Candida albicans* without Repeat Sequence 1 (RPS1) or Major Repeat Sequence (MRS). The specific goal of this project is to build upon the STS map of chromosome 3, concentrating specifically on regions near the telomeres, the centromere, and a few weak spots. We have successfully assigned 24 probes to the data of the map, connecting the 3P telomere and increasing the overall probe data. When completed, the map will facilitate finishing the sequence of chromosome 3.

<sup>1</sup> <http://www-sequence.stanford.edu/group/candida/index.html>

<sup>2</sup> H. Chibana, *et al.* 2004. Sequence Gap Closing for *Candida albicans* Chromosome 7, (poster abstract). ASM Conference Candida and Candidiasis (7<sup>th</sup>). Austin, TX, March 18-22.

## 08

### THE NADPH-CYTOCHROME P450 REDUCTASE ATR2 ALLEVIATES BLOCKAGE OF THE FLAVONOID PATHWAY IN METABOLICALLY ENGINEERED *ESCHERICHIA COLI*

Lisa M. Lenarz-Wyatt, Kevin T. Watts, and (Claudia Schmidt-Dannert)

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota

Flavonoids are a class of plant compounds synthesized via the general phenylpropanoid pathway. Their roles in plant function include induction of nodulation, pigmentation of fruits and flowers, defense against pathogens, and protection from UV-radiation. Flavonoids are present in foods such as fruits, vegetables, soy, red wine and green tea. Studies have demonstrated their effectiveness in the prevention and treatment of coronary heart disease and cancer. These positive health implications, and the availability of well-defined genes, make flavonoid biosynthesis an excellent choice for metabolic engineering in *Escherichia coli*. Previous work involved the cloning of phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), and chalcone synthase (CHS), from *Arabidopsis thaliana*. Together they comprise the minimal set of enzymes required for flavonoid biosynthesis. Simultaneous expression of all four proteins resulted in blockage after the first enzymatic step due to a non functional cinnamate-4-hydroxylase. C4H is one of over 270 cytochrome P450 monooxygenase genes found in the *Arabidopsis* genome. The key to plant P450 function is an association with an electron donating reductase, which *E. coli* does not provide. In the present study, the *A. thaliana* NADPH-cytochrome P450 reductase, ATR2, was cloned and coexpressed with PAL and C4H. Results show that the addition of ATR2 alleviates the blockage at C4H, as is indicated by an accumulation of p-coumaric acid. This is the first successful demonstration of plant cytochrome P450 activity *in vivo* in *E. coli*. Subsequent attempts were made to improve the production of p-coumaric acid by truncating a membrane-targeting sequence of the ATR2 gene. In addition, studies were initiated to evaluate promoter strength on ATR2 expression. In the future, we hope to coexpress ATR2 with other plant cytochrome P450s in metabolically engineered *E. coli*. This will provide an opportunity for further functional analysis of this important class of enzymes.

## **09**

### **APPLICATION OF CHICKEN MICROARRAYS FOR COMPARATIVE STUDIES OF GENE EXPRESSION IN TURKEY**

Todd P. Knutson, Lee D. Chaves, Mitchell Abrahamsen, and (Kent M. Reed)

Department of Veterinary and Biomedical Sciences, University of Minnesota

The relatedness between the turkey and chicken at the genomic level is remarkable. The completion of the chicken genome sequencing project and current research in turkey genomics has allowed researchers to directly compare DNA sequences of these two species. These studies have demonstrated the extensive conservation in both coding and non-coding DNA sequences of these avian species. Therefore, it is reasonable to ask whether turkeys and chickens express the same gene products. This study is designed to test the efficacy of an immune system-specific chicken microarray containing 1,200 oligonucleotides developed from expressed sequences for functional genomics applications in the turkey. Specifically, we wish to examine differences in expression between turkeys infected with avian pneumovirus (APV) and healthy (control) birds. Gene expression in two tissues (spleen and liver) will be analyzed by extracting total RNA, creating cDNA, and fluorescently labeling products for hybridization to microarrays. This research may provide another tool for the comparative genomic studies between turkeys and chickens.

## **10**

### **COMPARATIVE GENOME MAPPING OF HUMAN CHROMOSOME THREE TO EQUINE CHROMOSOME SIXTEEN**

Erin L Bequette (Jim Mickelson and Michelle Wagner)

Department of Veterinary Pathobiology, University of Minnesota

There are numerous equine diseases that have been identified as genetic in origin, including Polysaccharide Storage Myopathy, Severe Combined Immune Deficiency, and Recurrent Exertional Rhabdomyolysis. Furthermore, there are many similar genetic diseases in other species such as pigs and humans. Understanding correlations between similar diseases found in differing species allows more rapid development of therapies for these diseases. In this research, primers from human chromosome three were used to replicate equine DNA by using polymerase chain reactions. The equine DNA that was generated was used in turn to design equine specific primers. The products generated from the equine specific primers were then typed on the 5000 rad International Equine Whole Genome RH panel to add density to the human-equine comparative map. Fourteen statistically significant type I markers were added to the map of equine chromosome sixteen, more than doubling the number of type I markers previously mapped by Bhanu Chowdary at Texas A&M University and increasing total loci density by 43%. The results of this research will not only allow researchers to locate target genes more quickly, but will also enable studies of the genetics of evolution as well.

## 11

### **EVALUATING THE PHYLOGENETIC UTILITY OF SEVERAL NUCLEAR GENES IN GECKO LIZARDS**

Krsna V. Rangarajan and Tony Gamble (Andrew M. Simons)

Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota

DNA sequence data are widely used to determine phylogenetic relationships among species. Mitochondrial genes have been found to be particularly useful and are perhaps the most commonly used genes for this purpose. Because of lineage sorting, a gene tree may not be congruent with the underlying species tree and often several independent genes are needed to resolve a species' phylogeny. Since the mitochondrial genome is inherited as a single unit, without recombination, trees based on several mitochondrial genes are not independent estimates of phylogeny. To resolve this limitation of the mitochondrial genome, nuclear genes are becoming more widely used to provide independent estimates of phylogeny. Individual genes provide different levels of phylogenetic resolution and some initial screening must be done to determine which genes will work best at the level of divergence in question. The principle focus of this project was to determine the phylogenetic utility of twelve nuclear genes in gecko lizards. We extracted DNA from ten species of gecko and used PCR to amplify the selected genes. PCR product was sequenced at Advanced Genetic Analysis Center and sequences were assembled using Sequencher software. Sequences were aligned by eye and phylogenetic analyses, including parsimony and maximum likelihood, was performed using the software PAUP\*. The phylogenetic utility of these genes was assessed by comparing the overall corrected and uncorrected sequence divergences between taxa for each gene. Genes with high levels of sequence divergence between taxa were better at resolving more recent divergences but not older divergences. Genes with low levels of sequence divergence were better able to resolve older divergences but were less informative for more recent diversification. In large groups, like the geckos, using multiple genes helped resolve these different regions of the phylogenetic tree.

## 12

### **MUTATIONAL ANALYSIS OF A *DROSOPHILA* TRANSCRIPTIONAL REPRESSOR COMPLEX**

Sarah J. Malmquist (Jeffrey A. Simon)

Department of Genetics, Cell Biology and Development, University of Minnesota

The Hox gene family of transcription factors are expressed in the developing *Drosophila* embryo in strict anterioposterior domains. Hox expression is established early in embryogenesis by maternal factors and the gap and pair-rule genes, but these disappear after only a few hours. After this, the expression of genes in the correct location is maintained by the Polycomb group of proteins, which act to repress gene expression, and the Trithorax group, which activate expression. We study several members of the Polycomb group. 15 proteins in the Polycomb group have been identified and characterized, and 4 of these have been found to function in a multimeric complex, the Esc-E(z) complex. This complex, made up of E(z), Esc, Su(z)12 and NURF55, has histone methyltransferase (HMTase) activity. The methylation of Lys27 of histone H3, which is catalyzed by the E(z) protein, is associated with gene repression. Although E(z) alone has HMTase activity, assembly into the complex increases its activity greater than 1000-fold. To understand the functions of the non-catalytic subunits, we are using the baculovirus expression system to purify and study recombinant complexes. Several point mutations in esc functional domains have been made to mimic mutant esc alleles and these mutant proteins have been expressed using the baculovirus system. They are being tested for both assembly and catalytic activity. A mutant esc protein containing a deletion of the N-terminal tail has also been made in the baculovirus system, and this tailless esc gene has been inserted into a P-element vector for use in germline transformation experiments. These experiments will lead to a greater understanding of how esc functions within the Esc-E(z) chromatin complex to maintain control of gene expression.

## 13

### MOUSE GENE ANNOTATION USING THE *SLEEPING BEAUTY* TRANSPOSON: STUDY PROGRESS AND MECHANISMS OF TRANSDUCTION

Paul D. Lobitz, Aron M. Geurts and (David A. Largaespada)

Department of Genetics, Cell Biology and Development, University of Minnesota

*Sleeping Beauty*, SB, is a cut-and-paste vertebrate transposon system consisting of a transposon and a transposase that were reconstructed from defunct fish sequences and is active in the laboratory mouse<sup>1, 2</sup>. We use SB as a tool for transgenesis and insertional mutagenesis in the mouse germline<sup>2, 3</sup>. Using a linker-mediated PCR protocol to clone transposon/genomic DNA junction sequences, we can easily map transposon insertions. Online databases from Celera and Ensembl make available draft mouse genome sequences that allow for assignment of a genomic position to each insertion. Transposons that land in genes can disrupt them and cause a phenotype<sup>3</sup>, allowing us to functionally annotate genes in the mouse genome. To date, I have mapped 29 insertions in a strain of transgenic mice carrying a gene-trap transposon. Twelve of these insertions landed in genes, and we are currently characterizing these transposon-induced mutations. While SB is highly active in the mouse germline, the molecular mechanisms that might limit transposition are incompletely known. Two questions I am addressing are whether or not an excised transposon can transpose into itself, and whether a transposon can “skip” along a target sequence, leaving several footprints before coming to rest at its final location. Experiments in cultured cells are in progress to address these questions. Preliminary data indicate that transposons can integrate into themselves, but the frequency at which this occurs has yet to be determined. A method to detect transposon “skipping” has been developed and is currently being tested.

1. Ivics, Z., Hackett, P. B., Plasterk, R. H. & Izsvak, Z. (1997). *Cell* 91, 501-10.

2. Dupuy, A. J., Fritz, S. & Largaespada, D. A. (2001). *Genesis* 30, 82-8.

3. Carlson, C. M., Dupuy, A. J., Fritz, S., Roberg-Perez, K. J., Fletcher, C. F. & Largaespada, D. A. (2003). *Genetics* 165, 243-56.

## 14

### THE ROLE OF ChREBP IN GLUCOSE-REGULATED GENE TRANSCRIPTION

Xiaosong Liu, Lin Ma, and (Howard C. Towle)

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Lipogenic enzymes catalyze conversion of carbohydrate into triglycerides, the primary energy storage form in mammals. Feeding of a high-carbohydrate diet can up-regulate transcription of lipogenic enzyme genes in liver. Genes that respond to the carbohydrate diet contain a regulatory DNA sequence called the carbohydrate response element (ChoRE). It has been proposed that a liver-specific transcription factor ChREBP (carbohydrate response element binding protein) mediates the carbohydrate induction. ChREBP is a member of the basic helix-loop-helix leucine zipper (bHLHZip) family of transcription factors and requires a dimer partner to bind to DNA. We have recently identified Mlx, another bHLHZip protein, as an interaction partner of ChREBP by yeast two-hybrid screen. The aim of this study was to test whether ChREBP is a component of the glucose-regulated transcription factor that regulates lipogenic enzyme genes. Binding of ChREBP to the ChoRE, as assessed by electrophoretic mobility shift assays, only occurred in the presence of Mlx. In addition, luciferase reporter assay showed that co-transfection of ChREBP and Mlx into 293 cells led to increased ChoRE promoter activity while neither factor can activate the ChoRE promoter alone. Two dominant negative mutations of Mlx were generated using site-directed mutagenesis PCR. The dominant negative forms of Mlx were expected to heterodimerize with ChREBP, but block its ability to bind to DNA. Using electrophoretic mobility shift assays, we found that the dominant negative forms of Mlx inhibited ChREBP binding to DNA. Co-transfection of dominant negative forms of Mlx into primary rat hepatocytes with a ChoRE-containing luciferase reporter construct showed that dominant negative Mlx dramatically blocked glucose-response in hepatocytes. Together these data indicate that ChREBP is a component of the glucose-regulated transcription factor that regulates lipogenic enzyme genes and functions as a heterodimer with Mlx. The role of ChREBP in regulating lipogenic enzyme genes will be further studied in regard to its possible involvement of the glucose-regulated gene transcription factor in diabetes or obesity-related complications.

## 15

### **CIRCADIAN LIPOGENIC GENE EXPRESSION**

Fausta A. Ditah (Howard C. Towle)

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota

Circadian rhythms are a ubiquitous adaptation of all organisms to the most predictable of environmental challenges. Circadian describes biological phenomena that oscillate with an approximately 24-hour cycle. Circadian clocks control daily rhythms in physiology and behavior including body temperature, hormone levels, sleep-wake cycle and protein levels. These are important for the anticipation of daily variations in environmental conditions. Lipogenesis is the process by which carbohydrates are converted to triglycerides for energy storage. Some lipogenic genes including pyruvate kinase, fatty acid synthase and S14 are circadianly regulated with increasing levels just prior to normal feeding in rats. Following a high carbohydrate diet, the levels of these lipogenic enzymes are altered to balance energy needs and glucose homeostasis. The regulatory region responsible for the upregulation has been mapped and identified as the carbohydrate response element (ChoRE). The ChoRE element contains two E-box motifs, the DNA binding site for bHLH proteins. CLOCK and BMAL1 are bHLH transcription factors that bind to E-box elements as heterodimers to regulate gene expression in a circadian fashion. Preliminary evidence from the lab suggested that CLOCK and BMAL1 bind to the ChoRE element. To investigate the binding of CLOCK and BMAL1 to the ChoRE element, I used the techniques of sub cloning, transfections, and electrophoretic mobility shift assays. Here, I present data suggesting the binding of CLOCK and BMAL1 to the ChoRE of the S14 gene. Furthermore, I provide evidence for the specificity of this interaction using competition techniques. Thus in addition to the carbohydrate regulation of the expression levels of S14, we suggest CLOCK and BMAL1 also interact with the ChoREs to regulate its circadian rhythm. This research will be useful to the clinical world because obesity and diabetes are fast becoming mainstays in our community. Thus, understanding the intake and utilization of carbohydrates on a 24-hour cycle would be invaluable.

## 16

### **THE EFFECT OF GLUCOSE ON THE CIRCADIAN RHYTHM IN PRIMARY CULTURED HEPATOCYTES**

Mohamed E. Moussa (Howard C. Towle)

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota

Living organisms function according to a circadian rhythm. The expression of a set of genes in mammals in the suprachiasmatic nucleus and other tissues regulates this daily cycle. Although an organism's response to light and dark plays a substantial role in the circadian rhythm, recent research is beginning to reveal that nutrition and metabolism, especially at certain times of the day, also play a role in the expression of genes controlling the circadian rhythm. In an effort to further understanding of the mechanisms involved the breakdown of glucose and lipids in the liver, the effects of metabolism on circadian gene expression and its regulation is studied. Therefore, the objectives of this study were 1) to determine whether or not circadian regulation of gene expression is maintained in cultured primary hepatocytes and 2) to test the influence of glucose on circadian transcription factors. This was accomplished by monitoring mRNA levels for the circadian-regulated genes *Dbp* and *Per1* by Reverse Transcriptase PCR. The results of this study indicate that circadian genes do indeed maintain their expression in cultured hepatocytes, but this expression may vary significantly by changing glucose concentrations in the cell.

## 17

### **IDENTIFYING A NOVEL PF2 MUTATION IN THE *CHLAMYDOMONAS REINHARDTII* STRAIN SUP-PF3**

Irene Dorweiler (Dr. Mary Porter)

Department of Genetics, Cell Biology and Development, Medical School, University of Minnesota

Cilia and flagella play important roles in fertility and development, and defects in ciliary and flagellar motility have been associated with a number of diseases in vertebrates. Motility is generated by the activity of the dynein ATPases, which must be precisely regulated by components of the dynein regulatory complex, or DRC. The dynein regulatory complex (DRC) is a structural component of cilia and flagella that is composed of at least seven polypeptides and is located between the base of the radial spokes and the inner dynein arms. Several mutant strains with defects in the assembly of the DRC have been identified in the unicellular alga, *Chlamydomonas reinhardtii*. It is thought that these strains represent mutations in different genes encoding different subunits of the DRC. We have shown that *pf2* mutants fail to produce DRC subunit #4 resulting in lack of DRC assembly. PF2 is a homologue of the mammalian growth arrest-specific genes Gas8/Gas11. Our findings suggest that *sup-pf3* maybe another type of PF2 mutant. Western blots of *wild-type* and *sup-pf3* flagella indicate the presence of a modified PF2 protein. Southern blots of *wild-type* and *sup-pf3* DNA hybridized with a probe for the PF2 gene indicate that there has been a rearrangement of the PF2 gene in *sup-pf3*. We believe that an altered PF2 protein is produced in *sup-pf3* that prevents the assembly of the other DRC components. Our findings show a large (~6) kb insertion in *sup-pf3* in the PF2 gene region. We believe that this insertion may be a transposable element. We are currently subcloning and sequencing this insertion to learn more about the nature of the *sup-pf3* mutation and how it affects the PF2 protein.

## 18

### **TRANSPOSASE ACTIVITY OF MODIFIED P ELEMENTS IN *DROSOPHILA MELANOGASTER***

Abe K Gol (Michael Simmons)

Department of Genetics, Cell Biology, and Development, University of Minnesota

Transposable elements are found in both prokaryotic and eukaryotic cells. According to the International Human Genome Sequencing Consortium nearly 45% of the human genome consists of transposable elements or sequences derived from them. P transposable elements, which are found in the genome of the fruit fly *Drosophila*, carry a gene for a protein, the P transposase, which catalyzes the transposition of P elements. Previous work has shown that P transposase activity can be transmitted maternally through the *Drosophila* egg independently of the P element itself. This transmission appears to depend on the presence of the last intron in the transposase gene. I tested this idea using a modified P element that lacks the last intron and that is driven by an constitutive promoter. The test results show that transposase activity is transmitted through the egg even when the last intron has been deleted. These results therefore require that the apparent dependence of maternal transmission of transposase activity on the last intron be re-evaluated.

## 19

### **TAKING BIOLOGY TO THE CLASSROOM: A TEACHING INTERNSHIP**

Amy L. Kullas, Christine L. Chan, Holly A. Koslowski, Elizabeth A. Paulson, Nguyen T. Hoang, Andrew E. Buttler (Robert P. Elde, Steve Yussen)

College of Biological Sciences, College of Education and Human Development

What sparked your interest in science? Was it a particular teacher when you were in high school that has led you to your particular career path? Would you like to help others realize just how fun science can be? This new internship is a collaborative between CBS and CEHD partially funded by Howard Hughes Medical Foundation allowed us to experience what it is like to be a teacher by working side by side with a mentor teacher in a community in greater Minnesota. This program is called Science Education Partnership in Greater Minnesota (SEPGM). We applied for this internship in January of 2003 and got notice of our acceptance in February of 2003. Our first career exploration was in a research lab where we conducted 3-credits of directed research during the summer months. This allowed us to work with brilliant principal investigators and we would be able to take some of this knowledge to our classrooms in the fall. We also got to spend a week at beautiful Lake Itasca during the summer, where we got to meet our teacher mentor and work with them at a summer institute. We also had periodic meetings during the semester at Lake Itasca, where we would discuss things that occurred in the classroom. This program gave us the unique perspective of what it feels like to be on the other side of the classroom; being the teacher, instead of the student. We slowly worked our way into the classroom. At first being more of an observer and then by the end we felt comfortable enough to construct lesson plans, teach the lesson, and then evaluate the students at the end of the lesson. This was a wonderful way to explore teaching as a career before making a full commitment to preparing for that career.

## 20

### **NEW PLASMA DONOR VALUES**

Ryan A Laux (Kathryn Hanna)

College of Biological Sciences, University of Minnesota

One of the major problems in the plasma donation industry is the retention of new donors so they become regular donors. To better understand what motivates new donors, surveys were conducted, and new donors were interviewed to find out what aspects of the donation process could be improved. Written surveys along with in-person and telephone interviews of new donors revealed several themes that indicated why new donors return to donate plasma. The most common reason people cite for initially donating is money. Additional reasons beyond money are highly varied. The most cited complaint of the donation process for new donors is a problem with their venipuncture, with over 30 percent of donors reporting a problem at some point since their first donation. The success rate for venipunctures should be over 90 percent. The data suggest improving qualifications, training, and oversight of phlebotomists who perform venipunctures on new donors will improve new donor satisfaction.

## 21

### THE CONTRIBUTION OF HUMAN LUNG CYTOCHROME P450S IN NICOTINE METABOLISM

Kathryn M Brown (Sharon E Murphy)

Cancer Center, Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota

As the major contributing factor to addiction in tobacco users, the biochemistry of nicotine metabolism must be understood if one wishes to understand its role in addiction. Cytochrome P450 enzymes are predominantly located in the liver, but are also present in many extrahepatic areas including the lung and esophagus. In the lung, it is suspected that P450 2B6 plays a significant role in nicotine metabolism. Additionally, the contributions by other P450s such as 2A13, 2F1, and 4B1 may also be important. As the lung is a primary site of contact with tobacco smoke, it is essential to know the extent of nicotine metabolism in this tissue. The relative abundance and activity of these P450 enzymes in the lung is unknown and the extent of their contribution needs to be investigated. Research goal: To determine the contribution of lung cytochrome P450s to the metabolism of nicotine in humans. Specifically, to determine the P450 kinetic parameters ( $V_{\max}$  and  $K_m$ ) for 2B6, 2A13, and 2F1 for nicotine metabolism in the lung using purified P450 enzyme assays. Metabolites are quantified using HPLC analysis. Findings: Lung P450s, 2B6 and 2A13, metabolize nicotine at different rates. P450 2B6 appears to have  $V_{\max}$  and  $K_m$  values greater than those, which were previously published. P450 2A13 metabolizes nicotine at a significant, but slower rate than P450 2B6. Preliminary P450 2F1 assays have not yielded product and require more investigation.

## 22

### PURIFICATION AND CHARACTERIZATION OF RECOMBINANT HUMAN ARYLAMINE N-ACETYLTRANSFERASE 1

Caleb A. Bates, Haiqing Wang, Gregory M. Vath, Akane Kawamura (Carston R. Wagner, Patrick E. Hanna)

Department of Medicinal Chemistry, University of Minnesota

Humans are exposed to carcinogenic arylamines from various sources including cigarette smoke, dietary intake, and from the environment. N-Acetyltransferases (NATs) catalyze acetyl coenzyme A-dependent N-acetylation of arylamines and O-acetylation of their N-hydroxylated metabolites. The latter pathway produces unstable N-acetoxyarylamines, which react with protein and DNA nucleophiles subsequent to conversion to arylnitrenium ions, resulting in tumorigenesis and carcinogenesis. Recombinant human arylamine N-Acetyltransferase 1 (NAT1) has been overexpressed in *E. coli* as mutant dihydrofolate reductase (DHFR) fusion protein with a thrombin sensitive linker. Milligram quantities of human rNAT1 were purified to homogeneity with this protocol. The purified human rNAT1 was further characterized for substrate specificity and the results were compared with hamster rNAT2. In both cases, the second order rate constants ( $k_{cat}/K_{mb}$ ) for *p*-aminobenzoic acid (PABA) and 2-aminofluorene (2-AF) are one thousand-fold higher than that for procainamide (PA), consistent with the expected values for the enzymes. However, *p*-aminosalicylic acid (PAS), previously reported as a human NAT1 and hamster NAT2 selective substrate, exhibits 20-fold higher specificity for hamster rNAT2 ( $k_{cat}/K_{mb}$  3410  $\mu\text{M}^{-1}\text{sec}^{-1}$ ) than for human rNAT1 ( $k_{cat}/K_{mb}$  169.4  $\mu\text{M}^{-1}\text{sec}^{-1}$ ). Detailed kinetic, mechanistic, and structural analysis of human NAT1 will provide insights into the molecular basis of the catalytic and bioactivation mechanisms, and its physiological roles. Further investigation will be greatly aided by the protocol for large-scale purification of the protein.

## 23

### **EXPRESSION, PURIFICATION, AND MUTAGENESIS STUDIES OF THE INTEGRAL MEMBRANE PROTEIN PHOSPHOLAMBAN**

Kim N Ha<sup>1</sup>, Jamillah Zamoon<sup>2</sup>, (Gianluigi Veglia<sup>1</sup>)

<sup>1</sup>Department of Chemistry, <sup>2</sup>Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Cardiac phospholamban (PLB) is a 52-amino acid membrane protein that is attributed to the regulation of the calcium pump in the sarcoplasmic reticulum. Single-site mutations of phospholamban are known to exhibit drastic changes in activity and alter its interactions with the Ca<sup>2+</sup>-ATPase. In these studies, the mutant constructs S16E, P21A, and R9C were cloned, expressed, purified, and underwent kinetic studies to see the effects of these mutations on the protein's ability to inhibit the calcium pump. In addition, monomeric PLBAFA, the synthetic super-inhibitor I40A, and the inactive phosphorylated form of phospholamban were also investigated for its activity. The enzyme assays conclude that while the S16E and P21A mutants inhibited the calcium pump similarly to PLBAFA, R9C, a mutant physiologically linked to dilated cardiomyopathy, did not inhibit the pump. Phosphorylated phospholamban also behaved similar to R9C, while the I40A mutant produced stronger inhibition of Ca<sup>2+</sup>-ATPase. Study of this elusive protein's structure and function are crucial in determining its contribution to normal heart function and in cardiac conditions such as cardiomyopathy, and mutagenesis studies of phospholamban are important tools in determining phospholamban's significance in cardiac activity.

## 24

### **THE IMPACT OF THE PURINE NUCLEOSIDE PHOSPHORAMIDASE (hHINT-1) DIMER INTERFACE ON CATALYSIS**

Steve Hinkin, Tsui-Fen Chou, (Carston R. Wagner)

Department of Medicinal Chemistry, University of Minnesota

Human histidine triad nucleotide binding protein 1 (hHint1) is a member of the histidine triad family of proteins so named for their conserved His-X-His-X-His-X-X sequence at the active site (where X is hydrophobic). hHint1 catalyzes the cleavage phosphoramidate bond and, while its function *in vivo* has yet to be determined. A putative phosphoramidase has been shown to be responsible for the activation of amino acid phosphoramidates. The role of hHint1 in the bioactivation of amino acid phosphoramidates needs to be addressed. hHint1 exists as a homodimer with apparent molecular weight of 27.4 kDa, however, the importance of the interface on enzyme activity is not known. Based on x-ray structural analysis, the monomer interface is not part of either active site. The monomers consist of five antiparallel beta-sheets and two alpha helices. Within that interface Val-97 appears to be strategically placed for hydrophobic stabilization. It was hypothesized that replacement on Val-97 with the larger and positively charged residue, Arg, would dramatically destabilize the dimer, allowing the importance of the interface on catalysis to be assessed. Mutant plasmid was prepared by site-directed mutagenesis and the protein was over-expressed and purified as a DHFR fusion protein by methotrexate affinity chromatography. The mutant protein was isolated after thrombin cleavage. Results from gel filtration to date have been unclear in showing whether or not the mutation has been successful in separating the dimer into its respective monomers, however, phosphoramidase activity, as monitored by fluorescence, has proved to be drastically reduced. These results suggest that the activity of hHINT-1 is highly dependent on the dimer interface. (Supported by NIH-NCI R01 CA89615, University of Minnesota Undergraduate Research Opportunities Program)

## 25

### **PREPARATION AND EVALUATION OF ISOPRENOID DIPHOSPHATE ANALOGUES THAT INCORPORATES AZIDE FUNCTIONAL GROUPS: TOOLS FOR UNDERSTANDING THE ANTICANCER PROPERTIES OF FARNESYLTRANSFERASE INHIBITORS**

Benji K. Mathews (Mark Distefano)

Department of Chemistry, University of Minnesota

Protein prenylation involves the post-translational lipid modification of specific protein-derived cysteine residues with farnesyl (C<sub>15</sub>) or geranylgeranyl (C<sub>20</sub>) isoprenoid groups through thioether linkages. In the last ten years, there has been intense interest in studying protein prenylation since oncogenic Ras protein is farnesylated and mutant forms have been detected in 30% of all human cancers including 90% of pancreatic, 50% of colon, and 30% of lung cancers. Since farnesylation is necessary for membrane association and cellular transformation of Ras oncoproteins, preventing this modification may eliminate the activity of oncogenic Ras and hence may serve as a possible cancer chemotherapy. Protein Farnesyl Transferase (PFTase) is the biological catalyst that attaches the farnesyl group to a corresponding protein. Isoprenoid analogues have proven to be versatile tools for probing the mechanism and structure of prenyltransferases. Azide groups have no counterpart in biology and possess unique reactivity. We are synthesizing analogues of geranyl diphosphate that incorporates azide groups. We plan to incorporate these analogues into prenylated proteins and use the azide reactivity to obtain and monitor the levels of prenylated proteins in cellular extracts. This will be done in the presence and absence of PFTase inhibitors and should allow the simultaneous monitoring of the extent of prenylation for multiple proteins. To date we have synthesized dihydrogeranylazidediphosphate and have shown that it is a substrate for PFTase. We are currently attempting to functionalize the resulting azide-containing product using an intramolecular Staudinger reaction. We plan to use this chemistry to selectively extract PFTase substrates that have not been prenylated due to treatment with PFTase inhibitors. Such experiments should enable us to better understand the mechanism of PFTase inhibitors in cancer treatment. The ability to introduce unique azide functional groups into proteins could have enormous applicability for the site-specific introduction of labels and incorporate groups into proteins

## 26

### **BONE MORPHOGENETIC PROTEIN ANTAGONISTS TWISTED GASTRULATION AND CHORDIN CAN AID PRIMITIVE HUMAN HEMATOPOIETIC CELL EXPANSION *EX VIVO***

Nicole Ali, Anskar Leung, Shannon Buckley (Catherine Verfaillie)

Stem Cell Institute, Department of Medicine, University of Minnesota

*Ex vivo* expansion of hematopoietic cells is needed not only for genetic modification and tumor purging, but also for amplification of hematopoietic stem cells (HSCs) from small grafts, such as umbilical cord blood (UCB) grafts. UCB is an alternative source of HSCs for transplantation that causes less incidence of graft-versus host disease than bone marrow. The small size of UCB grafts, however, limits their usefulness for adult transplant recipients. The specific aim of this research is to work towards developing an *ex vivo* culture system that expands UCB HSCs for transplantation. Previous studies have shown that bone morphogenetic proteins (BMPs), members of the transforming growth factor  $\beta$  superfamily, play a role in the formation of precursors for hematopoietic and endothelial cells. BMP-4 has been shown to play a major role in hematopoiesis during development. We hypothesize that blocking BMP-4 activity may provide a way to prevent differentiation of HSCs *ex vivo*. In this study, the effects were tested of BMP antagonists chordin (chd) and twisted gastrulation (tsg) on the proliferation and differentiation of primitive UCB cells in serum-free *ex vivo* cultures. We evaluated the effects of chd and tsg on the generation of committed progenitors after 1 or 2 weeks in culture, as well as on the maintenance/expansion of more primitive long-term culture initiating cells (LTC-IC) during this time period. We show here that tsg and chd significantly increase LTC-IC expansion, suggesting maintenance of HSCs. Even though LTC-IC assays detect cells that are closely related to repopulating HSCs, they do not directly measure the engraftment potential of progenitors. Therefore, studies are continuing to test whether human cells maintained *in vitro* with tsg and chd also contain greater numbers of engrafting HSCs, by transplantation in an immunodeficient mouse model.

## 27

### REGULATION OF NUCLEAR ARCHITECTURE

Katherine A. Harrison (Robin Wright)

Department of Genetics, Cell Biology, and Development, University of Minnesota

Increases in the level of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, induce assembly of membrane arrays derived from the endoplasmic reticulum. In the yeast, *Saccharomyces cerevisiae*, these membranes are called karmellae and consist of arrays of smooth membrane stacks that are closely associated with the nucleus. The presence of karmellae results in alterations in the molecular architecture of the nuclear envelope. For example, in yeast cells that lack karmellae, nuclear pore complexes are distributed uniformly over the surface of the nucleus. In contrast, as a result of karmellae assembly, nuclear pores become clustered on one side of the nucleus. Nuclear pore complexes allow molecules and molecular assemblies to pass across the nuclear envelope into the cytoplasm or vice versa. This process is essential for cell viability. In this study I wanted to examine the processes by which nuclear pore distribution changes to accommodate karmellae assembly. As a first step in this analysis, I have used a green-fluorescent fusion to Nup49p to follow the four-dimensional changes in nuclear pore distribution that accompany karmellae assembly. Nup49p is a nuclear pore complex protein that is essential for proper function of the nuclear pore, particularly the movement of newly assembled ribosome subunits from the site of assembly in the nucleolus into the cytoplasm. Data from this analysis will be presented.

## 28

### THE ROLE OF BUD1 IN *CANDIDA ALBICANS*

Cassandra Kistler-Anderson (Cheryl Gale)

Department of Pediatrics, University of Minnesota

*Candida albicans* is an important cause of fungal disease in individuals that are immunocompromised, such as premature infants, HIV patients, patients receiving immunosuppressive drugs, and surgical and burn patients with impaired skin and mucosal integrity. *C. albicans* can exist in three morphologic forms (yeast, pseudohyphae, and hyphae) and the ability to switch among these forms has been linked to the ability to cause disease. In budding yeast, small Ras-type GTPases have been shown to be important for early steps in yeast morphogenesis, daughter cell site selection and polarity establishment. I want to test the hypothesis that Bud1p (a Ras-type GTPase) is important for hyphal morphogenesis and daughter cell site-selection in *Candida albicans*. For this study, my approach was to analyze the morphogenesis phenotypes of a *bud1* knockout strain. I used the strategy of PCR-mediated gene deletion to create *C. albicans bud1*-null strains and verified the strain constructions by PCR. Isogenic strains containing wild-type *BUD1* were also constructed, to serve as controls in the phenotypic analysis. Initial null and control strain constructions and verifications have been completed. Phenotypic analyses are currently underway. Bud1p is predicted to cycle between GTP and GDP-bound forms through the action of its guanine-nucleotide exchange factor Bud5p and its GTPase-activating protein, Bud2p. I predict that mutations that affect any of the proteins in this module will result in similar defects in polarized growth and morphogenesis. The hyphal growth characteristics of the newly constructed *bud1* null strains, as compared to isogenic parent, *bud5* null, and *bud2* null strains (previously constructed by the Gale lab) will be discussed

## 29

### IN SEARCH OF THE CYTOCHROME *c* TRANSLOCATION CHANNEL IN THE MITOCHONDRIAL OUTER MEMBRANE

Harmony L. Tyner (Ronald Jemmerson)  
Department of Microbiology, University of Minnesota

A key step in programmed cell death or apoptosis is translocation of cytochrome *c* (Cyt *c*) from the space between the inner and outer membranes of mitochondria into the cytoplasm. Cyt *c* then binds Apaf-1 (apoptotic protease activating factor-1) serving as a co-factor for activation of the apoptotic enzyme, caspase 9. The release of Cyt *c* from mitochondria is dependent on the protein BID. Following cleavage by caspase 8, BID causes the oligomerization in the mitochondrial outer membrane of two apoptogenic polypeptides: BAK and BAX. Published data support the idea that BAK and BAX form channels allowing for the release of Cyt *c*. However, it has not been demonstrated that these polypeptides alone or in conjunction with some other polypeptide(s) actually form the channel for Cyt *c* translocation from mitochondria. To identify the channel-forming polypeptides we are cross-linking proteins in live or apoptotic HL-60 cells (a human pro-myelocytic leukemia line) using the membrane permeable cross-linker disuccinimidyl suberate (DSS), extracting the proteins from the cells in 0.5% Triton X-100 detergent, and immunoprecipitating Cyt *c* and any associated polypeptides using anti-Cyt *c* monoclonal antibodies. The proteins isolated are then electrophoresed in polyacrylamide gels under denaturing conditions (samples boiled in the detergent sodium dodecyl sulfate) and western blots are performed using antibodies reactive with Cyt *c*, BAK, BAX, and other potential channel-forming polypeptides e.g., porin, which forms the voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane. Preliminary results indicate that BAX and Cyt *c* are cross-linked in apoptotic cells consistent with the idea that Cyt *c* passes through a BAX channel during its translocation from mitochondria. Similar analyses of BAK and VDAC are underway.

## 30

### CANDIDA ALBICANS INT1P LOCALIZATION DEPENDS ON A REGION BETWEEN AMINO ACIDS 1229 AND 1382

Nicholas H Winning (Cheryl Gale)  
Department of Pediatrics, Department of Genetics, Cell Biology, and Development, University of Minnesota

*Candida albicans* is an opportunistic fungal pathogen whose virulence is dependent on its ability to form elongated cells called hyphae. The Int1 protein (Int1p, 1664 amino acids) is involved in hypha formation under some growth conditions, and null mutants have reduced virulence in mice. Int1p localizes at the mother-bud neck of budding yeast cells and at the septa of hyphal cells. These cellular sites are important for the regulation of morphogenesis. Analysis of the Int1p sequence reveals a Pleckstrin homology (PH) domain between amino acids 1528 and 1638. PH domains have been shown to be required for membrane localization of many proteins that contain them. In this study, we tested the hypothesis that the PH domain is required for localization of Int1p. Our approach was to construct strains expressing portions of Int1p fused to green fluorescent protein (GFP), either lacking or containing the PH domain, by PCR-mediated gene modification, followed by analysis of strains expressing the fusion proteins by fluorescence microscopy. Expression of fusion proteins was confirmed by Western blotting. Our analysis revealed that Int1p-GFP constructs containing amino acids 1229-1664, but not 1382-1664 or 1528-1664, have localization identical to the native full-length protein. In a previous study, we determined that Int1p constructs containing amino acids 1-1229 do not localize to the bud necks of yeast cells or to the septa of hyphal cells. Thus, taken together with our new findings, we conclude that a critical sequence for Int1p localization is between amino acids 1229 and 1382 and the PH domain is not required for localization. Future studies will address if the domain identified, and thus the localization of Int1p, has roles in *C. albicans* morphogenesis and virulence.

## 31

### IS NICOTINE ACTIVELY TRANSPORTED AT IMPORTANT BIOLOGICAL BARRIERS? ROLE OF P-GLYCOPROTEIN AND BCRP TRANSPORT PROTEINS

Tate N. Winter (William F. Elmquist)

Department of Pharmaceutics, University of Minnesota

Nicotine distribution in the body influences its pharmacological effect at important sites such as the brain and the placenta. Drug efflux proteins, present in the blood-brain barrier and the placental syncytiotrophoblast, can modulate xenobiotic distribution to these target sites. The purpose of this study was to determine if nicotine is a substrate of two efflux proteins, i.e., p-glycoprotein and the breast cancer resistance protein (BCRP), that are present in the blood-brain barrier and the placental barrier. MDCKII cells over-expressing either p-glycoprotein or BCRP were grown on semi-permeable polyester membranes in six-well Transwell® inserts. [<sup>3</sup>H]-mannitol flux across the monolayers was examined to verify the integrity of the apical tight junctions. In the MDR1-transfected cells, the directional flux of a prototypical substrate, digoxin, was examined to validate the functional expression of p-glycoprotein. Using this model, the directional flux of [<sup>3</sup>H]-nicotine was examined from 0 to 3 hours. The accumulation of nicotine in MDCKII cells transfected with the *bcrp1* gene was examined following a 3-hour incubation, and compared with wild-type cells. Mannitol flux was limited (<5%) and showed no directionality, suggesting limited transport by paracellular passive diffusion. Digoxin transport was significantly greater in the basolateral-to-apical direction, indicating the functional expression of the apically-directed p-glycoprotein transporter. There was no significant directional flux of nicotine in the MDR1-transfected cells. In preliminary experiments, nicotine accumulation was 25% less in BCRP-transfected cells versus wild-type cells. These results indicate that nicotine is not a substrate for the important drug efflux transport system, p-glycoprotein, and may be a weak substrate for BCRP. Therefore, the variability in the regulation or expression of p-glycoprotein is not an important determinant in nicotine disposition, and the subsequent pharmacological and toxicological effects of nicotine within the body. Further study on interaction of nicotine with BCRP and other candidate transport systems is underway.

## 32

### CELL CYCLE DEPENDENT EXPRESSION OF F-BOX PROTEINS

Remy E. Wong (Deanna M. Koepp)

Department of Genetics, Cell Biology, and Development, University of Minnesota

Ubiquitin mediated proteolysis has emerged as a fundamental mechanism to regulate proteins in control of the cell cycle. Ubiquitin ligase is the enzymatic complex responsible for conjugating ubiquitin to the substrate to be degraded. Within the ubiquitin ligase complex, the F-box protein is the protein responsible for substrate specificity. Fbw7, for which there are currently three known isoforms  $\alpha, \beta, \gamma$ , is a human F-box protein that has found to be involved with the development of several types of cancer. The goal of this experiment was to determine if the Fbw7 proteins were expressed in a cell cycle dependent manner. To test this, northern blots were run using RNA from 293T cells that had been a) synchronized by double thymidine block, and b) stopped at cycle phases using various drugs. Our hypothesis is that  $\gamma$  is cell cycle dependent, while  $\alpha$  and  $\beta$  are constitutively expressed. This is based on preliminary findings that implicate  $\gamma$  has an inhibitory role in the function of  $\alpha$  and  $\beta$  isoforms.

### 33

#### **THE ROLE OF S PHASE CHECKPOINT PROTEINS IN REGULATING ORIGIN ACTIVATION**

Mohamed Abdihalim, Miruthubashini Raveendranathan (Anja-Katrin Bielinsky)

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Checkpoint proteins are part of the molecular surveillance machinery that allows the cell to monitor the integrity of the genome. Checkpoint defects cause substantial problems in multi-cellular organisms because they give rise to damaged chromosomes and genetic instability, which can result in cancer. This is particularly important in S phase of the cell cycle, when genomic DNA is most susceptible to breakage. In the presence of the ribonucleotide reductase inhibitor, hydroxyurea (HU), the progression of replication forks from early-firing origins are blocked and late-firing origins are maintained in a prereplicative state. In wild-type cells, the stalled replication forks caused by HU activate the checkpoint kinase Mec1. Mec1 in turn activates Rad53, which then blocks the activity of other proteins that regulate origin activation. It has been shown that HU does not inhibit late origin firing in cells with mutant *RAD53* and *MEC1* checkpoint genes. *mec1* and *rad53* mutants have lost the S phase checkpoint system and thus, activate origins at inappropriate times. In order to determine if this deregulation occurs genome-wide, we monitored origin activation in these mutants, using yeast microarrays that contain all putative replication origins in yeast. As expected, in wild-type cells early origins fired in the presence of HU but late origins were inhibited. In the mutants, the activation pattern differed from wild-type cells because both early- and late-firing origins were activated. These data suggest that Mec1 and Rad53 are important regulators of origin timing in *S. cerevisiae*.

### 34

#### **YEAST ON DRUGS: ANALYZING THE EFFECTS OF ALTERED STEROL PRODUCTION ON DRUG SENSITIVITY**

Rebecca Long (Robin Wright)

Department of Genetics, Cell Biology and Development, University of Minnesota

Many cells are able to respond to changes in cellular physiology by altering specific aspects of their structure. For example, in human cells and in yeast, increased levels of the enzyme HMG-CoA reductase induce alterations of a cellular organelle known as the endoplasmic reticulum. In yeast, these altered membranes form karmellae, stacked smooth membrane arrays around the nucleus. Because HMG-CoA reductase is the rate-limiting enzyme in cholesterol synthesis, understanding the relationship between karmellae and HMG-CoA reductase regulation has important health implications. Three different yeast mutant strains were examined to see if the presence of increased levels of HMG-CoA reductase activity and karmellae made them more sensitive to three different drugs. These mutants were selected because they have abnormal lipid concentrations and are cold-sensitive for growth when they express elevated levels of HMG-CoA reductase. The three drugs used are Sulconazole, Miconazole, and Calcofluor. The first two drugs both target the activity of Erg11p, which catalyzes a late step in sterol production. Calcofluor interferes with cell wall assembly and was originally chosen as a negative control. These drug sensitivity experiments tested the hypothesis that the sensitivity of mutant strains to high levels of HMG-CoA reductase activity resulted from their inability to properly regulate sterol synthesis at steps following HMG-CoA reductase. If so, these mutants should be more sensitive than wild-type to drugs that target sterol synthesis, but have equal sensitivity to drugs that target other processes. Our results disproved this hypothesis, as the mutant strains and wild-type controls were all equally sensitive to the drugs. However, the experiment also revealed unexpected results that may be useful in future studies.

## 35

### **EVALUATION OF PERFORIN IN SIV SPECIFIC CD8+ T CELLS**

Jill M. Grandt, Ryan M. Sunderman, Cara B. White (Pamela J. Skinner)

Department of Veterinary and Biomedical Sciences, University of Minnesota

Every year, the severity of the HIV/AIDS pandemic increases. In 2003, HIV claimed the lives of an estimated 3 million people. There is an urgent need to gain insights into HIV pathogenesis and develop an effective vaccine. The overall goals of our research are to gain insights into the adaptive CD8+ T cell response to HIV and help evaluate vaccine efficacy. This study was initiated using simian immunodeficiency virus (SIV) infected macaques, a model system for HIV infections. During an SIV/HIV infection, the cytotoxic T lymphocyte (CTL) response is activated as an antiviral defense mechanism by the host. As part of the CTL response a pore-forming protein, perforin, is produced to kill virus-infected cells. The specific aims of our project are 1) to develop an objective method to quantify and characterize perforin levels, including subcellular localization in SIV specific CD8+ T cells and, 2) to gain insights into the effector status of the CTL response during SIV infection. Fresh tissue samples were cut and stained using MHC class I tetramers to stain SIV gag specific CD8+ T cells, a technique known as in situ tetramer staining. Tissues were also stained with perforin antibodies. Images from these sections were acquired using a confocal microscope. Two individuals using specific criteria scored the images independently. The objectiveness of the approach will be evaluated by comparing data collected by the two individuals. Results of these analyses will provide important insights into the perforin status of SIV gag specific CD8+ T cells in vivo.

## 36

### **INVESTIGATION OF THE BASIS FOR ACTIN-MITOCHONDRIA INTERACTIONS**

Senit R. Debesai, (Ronald Jemmerson)

Department of Microbiology, University of Minnesota

Actin plays a role in the movement of mitochondria in cells and its depolymerization sensitizes mitochondria to apoptogenic signals resulting in the release of cytochrome c and other proteins from the intermembrane space. The basis for the association of actin with mitochondria is not clear and is unknown in mammalian cells. To investigate this mechanism, monoclonal antibodies were obtained that are specific for actin, which was derived from the outer membrane of rat brain mitochondria, and the ability of the antibodies to precipitate mitochondrial polypeptides along with actin extracted from rat brain mitochondria was examined. The precipitated polypeptides were observed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate and their identities were determined by mass spectrometry. Preliminary results indicate that the inner membrane F0F1-ATP synthase complex and the adenine nucleotide transporter are among the polypeptides associated with actin. ATP has been shown to cause actin to disengage from mitochondria in yeast cells. This may allow mitochondria to locate within the cell depending on the ATP demand. Thus, it would not be unexpected for actin to be found in proximity to the ATP producing/ secreting machinery in mitochondria. The search for the putative outer membrane component that may link actin to inner membrane polypeptides continues

## 37

### PPXY(PY) MOTIF-MEDIATED HLATS2/SAVI INTERACTION

John D Andersen (Dr. Wufan Tao)

Stem Cell Institute, Division of Hematology, Oncology and Transplantation, Department of Medicine,  
University of Minnesota

In *Drosophila*, *lats* tumor suppressor has been demonstrated to play a role in negatively regulating cell proliferation and promotes apoptosis by interacting with *sav* and *hippo*, in a *lats-sav-hippo* tumor suppressor pathway. The studies of *Drosophila* suggested that *lats* interacts with the WW domain of *sav* through its PPXY(PY) motif. Two *lats* tumor suppressors, *Lats1* and *Lats2*, have been identified in mammals. *Lats2* negatively regulates G1/S transition by downregulating cyclin E/CDK2 activity. Preliminary studies in our lab show that human LATS2 protein interacts with MST1/2, a human homolog of *Drosophila hippo*, in a SAV1-dependent manner. Elucidating the mechanism in which the PPXP(PY) motif interacts with a WW domain of *Sav1* is the focus of this research. Using site-directed mutagenesis, we generated two *Lats2* mutants, *Lats2<sup>Y518A</sup>*, in which a point mutation altered the tyrosine residue in the PPXY(PY) motif, and *Lats2 $\Delta$ PPYP*, in which the entire PPXY(PY) motif is deleted. The mutant forms as well as wild-type of *Lats2* will be transfected into human embryonic kidney (293) stem cells. The interaction between LATS2 and SAV1 will be evaluated by Co-immunoprecipitation/Western Blot experiments.

## 38

### THE ROLE OF NEURAL CREST IN XENOPUS LEFT-RIGHT GENE EXPRESSION AND HEART DEVELOPMENT

Ann N. Neumann, Allison J. Frasier, Brad J. Martinsen, (Jamie L. Lohr)

Department of Pediatrics, University of Minnesota

Despite the recent advances in the treatment and diagnosis of congenital heart disease, we have a limited understanding of its causes and prevention. In order to gain a better understanding of congenital heart disease, developmental biologists use organisms, such as *Xenopus laevis*, to study the genetic and cellular processes involved in normal heart development. In this experiment, we investigated the role of neural crest in left-right gene expression and heart development. The importance of a subset of neural crest cells called cardiac neural crest in normal heart development has been demonstrated in chick, mouse, and zebrafish. We were interested in determining the relationship between early left-right signaling, which is known to be important in proper heart development, and neural crest in *Xenopus*. The neural folds of stage 15 embryos were ablated in the region of *Xslug* expression, a neural crest marker. The embryos were then analyzed using whole mount in-situ hybridization for two left-right markers, *Xnr-1* and *Xlefty*. We also examined the effects of neural crest ablation on heart looping. Preliminary results suggest that neural crest ablation results in abnormal bilateral expression of *Xnr-1*. Embryos also showed an increase in cardiac reversal rates and a high rate of loss of normal looping. Our data suggests that there is a contribution of neural crest to cardiac development in the *Xenopus* embryo and that early neural fold/neural crest ablation alters left-right signaling.

## 39

### UTILIZATION OF A YEAST TWO HYBRID SCREEN TO IDENTIFY PROTEINS THAT INTERACT WITH HS7, A CANDIDATE ONCOGENE

Meenal Kapoor (Kathleen Conklin)

Department of Genetics, Cell Biology and Development, Institute of Human Genetics, Cancer Center, University of Minnesota

Chromosomal rearrangements commonly found in tumor cells can lead to altered expression of oncogenes or tumor suppressor genes located in cis to the rearrangement. Thus, defining genes within or adjacent to sites of chromosomal rearrangements found in tumor cells can identify candidate genes that promote formation of the associated disease. Dr. Betsy Hirsh, Director of the Cytogenetics Laboratory at the University of Minnesota, identified a novel recurrent translocation that involves chromosomes 7 and 12 in pediatric patients with Acute Myeloid Leukemia (AML). Since its original description, this translocation has been identified in at least 30 additional cases of pediatric AML by Dr. Hirsch and by other investigators. We cloned a translocation breakpoint from one patient with a 7;12 rearrangement and have identified the common breakpoint region at 7q36 and identified a novel gene (HS7) within the common breakpoint region. We hypothesize that HS7 is up regulated in tumor cells due to this translocation. Over-expression of HS7 altered the growth properties of cells in culture, supporting its role in development of leukemia. We have found that HS7 localizes to the nucleolus (site of rRNA synthesis and ribosomal biogenesis) and that its over expression leads to an overall increase in translation. As one approach to investigate the function of HS7, we performed a yeast two hybrid screen to identify proteins that interact with HS7. Several interesting partners were detected, including Nopp140 and Microspherule Protein—two nucleolar proteins, and c-myc, which regulates synthesis of 5S rRNA and tRNA. The current hypothesis is that upregulation of HS7 increases ribosomal synthesis and processing which in turn leads to an overall increase in translational activity. The role of translation in transformation will be discussed.

## 40

### PURIFICATION OF THE VIRE2 NUCLEAR TRANSPORT PROTEIN AND ITS INVOLVEMENT IN GENE REPAIR

Carolyn Jean Presley (Clifford J. Steer)

Department of Medicine, University of Minnesota

Hemoglobin is the protein responsible for oxygen transport in the blood and is found in all red blood cells. Specific point mutations or deletions in the hemoglobin genes result in an altered hemoglobin protein product. Hemoglobinopathies are a genetically distinct group of blood diseases that result from abnormal or reduced amounts of hemoglobin proteins. Sickle-cell disease and some  $\beta$ -thalassemias are diseases caused by point mutations of the beta hemoglobin gene. Two gene therapy techniques have been developed to deliver either a wild-type copy of the hemoglobin gene (augmentation) or a small, short DNA oligonucleotide with a sequence designed to correct the mutation by stimulating the endogenous DNA repair machinery (targeted gene correction). Although targeted gene correction is a straightforward strategy, it is currently hampered by low correction efficiency. It is assumed that nuclear import is one of the limiting steps. The focus of this study was to enhance the nuclear import of the oligonucleotide using VirE2. This is a single stranded binding protein derived from a plant pathogenic bacterium, which has been demonstrated to increase localization of the DNA oligonucleotides. In fact, VirE2 has a unique affinity to bind single-stranded DNA transporting them into the plant nuclei. With the modification of the nuclear localization signals (NLS) of the VirE2, VirE2S20, has the capability of delivering single-stranded DNA into xenopus oocytes. In this study, the goal was to purify VirE2S20 that was overexpressed in *E.coli*. Transformation of the plasmid pET3b-VirE2S20 into the competent *E.coli* cells and induction with IPTG allowed large-scale production. Using a multi-step purification protocol, I successfully isolated and purified this protein. This will allow further study of VirE2S20-mediated targeted gene correction in the treatment of sickle cell disease.

## 41

### COMMUNICATION BREAKDOWN: DRUG EFFECTS ON CONNEXIN-43 TRAFFICKING

Andrea Zins (Ross Johnson)

Department of Genetics, Cell Biology, and Development, University of Minnesota

Intercellular communication is essential for life. Cells need communication for proliferation, development, and death. One important communication mechanism involves gap junctions, cell-to-cell channels that facilitate metabolic activities by allowing the direct exchange of ions and small molecules. Gap junctions are formed by an aggregate of docked hemichannels (HC). Each HC is composed of six connexin proteins. Defects in gap junction channels underlie several genetic diseases, including a neuropathy, skin disorders, congenital cataracts, non-syndromic deafness, and various juvenile heart defects. Connexin-43 (Cx43) is naturally expressed in mouse wild-type fibroblasts. An experimental model has been tested using three different drugs to block parts of the Cx43 trafficking pathway. Nocodazole was used to block any microtubule-dependent trafficking pathway, Brefeldin A to disrupt the Golgi Apparatus and therefore stop production and progression of the protein to the plasma membrane (PM), and Bafilomycin to block part of the endocytotic pathway. Bafilomycin is thought to dissipate the necessary proton gradient needed for endocytosed vesicles to fuse again with the PM. The drug effects were assayed in two ways. Experiments were performed using a dye uptake assay, which combines mechanical stimulation and low extracellular calcium levels to induce hemichannels to open and let dye into cells. As cells are treated with drugs, it is hypothesized that the amount of Cx43 on the PM diminishes and therefore dye uptake levels also diminish, as long as significant Cx43 turnover continues. Treated cells were also studied with immunofluorescence (IF) to evaluate the drug effects on the microtubule structure of the cells or the localization of Cx43 in the cell. It was determined that all three drugs inhibit dye uptake as a function of time, and IF preparations showed that the drug targets were indeed affected by drug treatment. Combination drug treatments, use of other drugs that inhibit endocytosis/recycling, use of antibodies to block hemichannels, and confocal microscopy will be used in the future to obtain more data for the present model of Cx43 trafficking.

## 42

### LOCATE AND ANNIHILATE: REGULATED PROTEIN DEGRADATION

Benjamin T. Miller (Deanna Koepp)

Department of Genetics, Cell Biology, and Development, University of Minnesota

Proteolysis has emerged as a mechanism whereby the cell is capable of degrading proteins that are abnormal or no longer necessary for cell functions or development. This mechanism is regulated by tagging the protein destined for degradation with ubiquitin. Ubiquitination of the desired protein is achieved through an enzyme cascade involving three separate enzyme complexes: E1, E2, and E3. One of the largest families of E3 complexes is known as the SCF family of ubiquitin ligases. One protein comprising the SCF complex is the F-box protein, and it is the F-box protein that is responsible for binding the substrate to the SCF complex for ubiquitination. One specific F-box protein, Fbw7, has been found mutated in human breast, endometrial, pancreatic, and ovarian tumors. There are at least three known isoforms of Fbw7:  $\alpha$ ,  $\beta$ , and  $\gamma$ , all identical in their C-termini but differing in their N-termini. There are three known substrates of Fbw7: cyclin E, Notch-1, and presenilin-1. There is preliminary evidence that isoform  $\gamma$  may play an inhibitory role in the turnover of cyclin E. The goals of this project were to (a) examine the role of isoform  $\gamma$  in cyclin E regulation, (b) the role of isoform  $\gamma$  during the cell cycle, and to (c) determine the expression of Fbw7 isoforms and their substrates in human tissues. To address (a) I transfected 293T cells with cyclin E DNA and with plasmids transcribing shRNA for each isoform. Protein expression of the transfected cells was then examined by immunoblotting. I expect to see decreased expression of cyclin E in cells transfected with  $\gamma$  shRNA. For aim (b) I transfected 293T cells with plasmids transcribing shRNA for each isoform and assayed the cells using flow cytometry. To determine (c) I employed Northern analysis. My results indicate expression of Fbw7 isoforms shows some tissue-specificity, but the substrates are present in all tissues examined

## 43

### A MODEL OF NRAS-DRIVEN MAST CELL DISEASE IN THE MOUSE

Jamie M. Jones, Stephen M. Wiesner, and (David A. Largaespada)

Department of Genetics, Cell Biology and Development, University of Minnesota Cancer Center,  
University of Minnesota

The *NRAS* gene is part of a gene family (*H-*, *N-*, and *KRAS*) that functions in signal transduction. The *NRAS* protein undergoes cycles of activation while bound to GTP and inactivation while bound to GDP. Point mutations in *NRAS* cause it to remain associated with GTP in the active conformation. Quite commonly, these activating mutations substitute glycine at position 12 of the protein for valine. *NRAS* is activated by a point mutation in as many as thirty percent of all cases of acute myeloid leukemia (AML).<sup>1,2,3</sup> However, the specific role of *RAS* in the development of myeloid leukemia has not been determined. In order to understand the contribution of an activated *RAS* oncogene to AML, transgenic mice were created in which an *NRAS(V12)* transgene is activated by an inducible system. The system has two parts. The tetracycline transactivator (*tTA*) transgene is expressed from a hematopoietic specific *Vav* promoter.<sup>4</sup> The *NRAS(V12)* transgene is expressed from a tetracycline transactivator-regulated promoter. The combination of the two transgenes will result in hematopoietic-specific expression of *NRAS(V12)* that can be silenced by treatment of mice with the tetracycline analog, doxycycline. As a result, the contribution of *NRAS(V12)* activation and signaling to hematopoietic malignancies can be determined. After testing the *Vav-tTA* and *TRE-NRAS* constructs *in vitro*, transgenic mice were created using pro-nuclear injection. *Vav-tTA* mice were then crossed with *TRE-NRAS* mice to create doubly transgenic animals. Recently we discovered that doubly transgenic animals have massive infiltrates of mast cells in the spleen and that all stages of mast cell development are represented in the peripheral blood of these animals. The disease is strikingly reminiscent of human mast cell leukemia or aggressive systemic mastocytosis. This work provides a mouse model for these diseases and may lead to a better understanding of *NRAS(V12)* regulated leukemia phenotypes *in vivo*.

1. Lee YY, et al. 1995. *Stem Cells*. 13:556-63.
2. Casey G, et al. 1993. *Pathology*. 25:57-62.
3. Radich JP, et al. 1990. *Blood*. 76:801-7.
4. Ogilvy, S, et al. 1998. *Blood*. 91:419-30.

## 44

### REGULATION OF A $\beta 1$ INTEGRIN PI3K/AKT VIABILITY PATHWAY BY PTEN

Nima Estharabadi (Craig Henke)

Department of Medicine, University of Minnesota

Integrins are cell surface receptors which mediate adhesion to the ECM. As such they regulate a variety of cellular functions including cell viability. We have found that a  $\beta 1$  integrin PI3K/Akt viability pathway regulates fibroblasts survival in collagen matrices. However, key molecules which regulate this viability pathway are unclear. We hypothesize that PTEN, a lipid phosphatase that is capable of inhibiting PI3K, is an important regulator of the  $\beta 1$  integrin PI3K /Akt viability pathway. To begin to approach this issue, we assessed PTEN levels as a function of time after plating fibroblasts onto type I collagen-coated dishes. We have previously found that Akt phosphorylation increases as a function of time after plating cells on type I collagen. Since, PTEN can inhibit Akt phosphorylation via its ability to inhibit PI3K, we sought to determine whether PTEN levels were altered in response to plating fibroblasts on type I collagen. No change in PTEN levels were seen when fibroblasts were plated on type I collagen as a function of time. Although we did not find that PTEN levels were altered when fibroblasts adhered to collagen, PTEN activity could be modulated by fibroblast adhesion to collagen. In order to determine whether PTEN regulates Akt activity in response to fibroblast attachment to collagen, additional studies to assess PTEN activity are required.

## 45

### **THE USE OF B-MODE ULTRASOUND TO IDENTIFY RECOVERY PATTERNS OF COLIC IN HORSES FOLLOWING SURGERY OR MEDICAL MANAGEMENT**

Molly M. Welle (Erin D. Malone)

Department of Clinical and Population Sciences, University of Minnesota

Ultrasonographic examinations are a valuable diagnostic tool in veterinary medicine. Sonographic examinations of the equine abdomen have become an important aspect of the diagnosis of equine abdominal disease and have recently been used to establish gastrointestinal activity patterns in normal horses. This project was designed to identify ultrasonographic intestinal activity patterns as observed following surgical or medical management of colics, in order to identify the typical progression as the cases recover. Cases included in the study were divided among four groups: impaction colics treated medically, large colon displacement and torsions (surgical), small colon enterolith and impactions treated surgically, and small intestinal lesions treated surgically. Those cases requiring surgery were examined ultrasonographically within 24 hours post surgery. Cases medically managed were examined ultrasonographically within 12 hours of presentation. Subsequent ultrasounds were carried out every 48 hours, with a final exam taking place upon clinical resolution. Only horses surviving to discharge were included in the study. Several aspects of the stomach, small intestine, large intestine, and caecum were investigated through the use of B-mode ultrasound. The stomach was significantly more visible upon clinical resolution than in the first exam in those cases requiring surgery. The small intestine was more visible in the first exam in those cases requiring surgery. There were significantly more contractions per minute in the last exam in the large intestine for the surgery cases. The caecum contained significantly more fluid in the first exam in the surgery cases. There were no significant differences in the motility or visibility of the organs of the GI tract of medically managed impactions. These findings should be useful in the monitoring of postoperative colics.

## 46

### **THE SLEEPING BEAUTY TRANSPOSON FOR CANCER THERAPY: REGULATED TRANSPOSASE AND ANTI-CANCER TRANSPOSON GENES**

Shruthi Ravimohan, Lara Collier, John Ohlfest, Won-Il Kim (David A. Largaespada)

Department of Genetics, Cell Biology, and Development. University of Minnesota

The Sleeping Beauty (SB) transposon vector system is a non-viral gene-transfer technology that may be useful for human gene therapy and insertional mutagenesis in model vertebrate organisms. The SB transposase can catalyze integration of transposon DNA and long-term transgene expression *in vitro* in 5–6% of adult mouse liver cells and 2–3% of mouse lung cells<sup>1,2</sup>. Although the SB vector system has advantages over viral vectors, an obstacle that this non-viral vector presents, is the inability to regulate transposase activity<sup>3</sup>. In order to be able to control the activity of SB transposase, we constructed fusions with the ligand binding domain of a tamoxifen-sensitive estrogen receptor (SB-ER<sup>TM</sup>)<sup>4</sup>. We tested the functionality and the tamoxifen inductibility of the SB-ER<sup>TM</sup> transposase in HeLa and U373 cell lines. Preliminary results indicate that the fusion protein is functional in both cell lines. However, the fusion protein does not seem to be tamoxifen regulatable and may be cleaved in HeLa cells. We are currently mutating the nuclear localization signal in SB to determine if this will result in tamoxifen regulation. In addition to constructing inducible forms of SB, we are investigating the use of SB in gene therapy of breast cancer. Here, we are targeting the epidermal growth factor receptors (EGFR), which have been documented to be overexpressed in breast cancer cells<sup>5</sup>, through the use of a fusion protein; epidermal growth factor-Herpes Simplex Virus thymidine kinase (EGF-TK). Our intention is to achieve tumor-directed integration of this suicide gene by SB transposase. Cells expressing EGF-TK will secrete it, followed by binding of EGF-TK to EGFRs and internalization. Upon administration of gancyclovir, a nucleotide analog, the cell will undergo apoptosis<sup>6</sup>.

1. Nat Genet. 25(1):35-41 (2000).; 2. Mol Ther.8 (3):501-7 (2003); 3. Mol Ther. 9(2):147-56 (2004);

4. *Current Biology*. 8:1323-1326 (1998); 5. *Oncogene*. 19; 23(7):1428-38 (2004); 6. *Cancer Gene Ther*.10 (1):64-74 (2003).

## 47

### **HOW ALZHEIMER'S DISEASE IS LINKED TO FREE RADICAL DAMAGE**

Ashley E. Lawson (Tinna Ross, William H. Frey II)

Alzheimer's Research Center, Region's Hospital, Department of Pharmaceutics, University of Minnesota

Free radicals resulting from oxidative stress can attack and permanently damage cell structures including receptors like the muscarinic acetylcholine receptor (mAChR), a slow, G-protein-coupled receptor involved in the formation of memory. Damage to the mAChR may result in decreased cholinergic function that contributes to the pathology of Alzheimer's disease and other neurological disorders such as Parkinson's Disease and stroke. Antioxidants are a class of compounds that have the ability to sequester free radicals from damaging the body. There is evidence that natural antioxidants, such as vitamin E, may decrease free radical damage to the mAChR and decrease incidence of Alzheimer's disease. The purpose of this project is to determine if various naturally occurring antioxidants will protect the mAChR from free radical damage. Binding assays are used to determine the activity of the mAChR, this involves the addition of tritium labeled QNB (3[H] QNB), a mAChR antagonist, to human cell membranes. Free radical damage is induced in the binding assay by the addition of heme, along with peroxide, which produce a source of free radicals. Varying concentrations of a specific natural antioxidant is added to the binding assay reaction to examine if the antioxidant will prevent heme-induced free radical damage of mAChR. Finally, a control group that contains no antioxidant is used to measure the binding of 3[H] QNB to the mAChR in order to determine the integrity of the receptor. My results show that resveratrol and taxifolin effectively protect the mAChR but alpha lipoic acid has no protective effect. These results suggest that natural antioxidants may provide a protective mechanism against free radical damage associated with Alzheimer's disease

## 48

### **IN VIVO DIFFERENTIAL GENE EXPRESSION IN PANCREATIC ISLET CELLS**

Forum D. Kamdar (Bernhard J. Hering, Brett K. Levay-Young)

Diabetes Institute for Immunology and Transplantation, Department of Surgery, University of Minnesota

Diabetes mellitus is a serious and chronic metabolic disorder where the body is unable to regulate blood glucose levels. In Type 1 diabetes mellitus (T1DM) the insulin-secreting  $\beta$  cells within the pancreas are destroyed due to an autoimmune attack on these cells, which leads to impairments in insulin production. Despite medical advances, individuals with (T1DM) must rely on daily insulin injections to regulate blood glucose. Whole pancreas transplants are often performed for people who are unable to control blood glucose with insulin injections. While whole pancreas transplants do allow individuals to eventually become normoglycemic and insulin-independent, the procedure comes with many risks associated with transplant surgery. Islet transplantation offers a solution for type 1 diabetics by transfusing donor islets cells into the hepatic portal vein of the recipient. Islets implant within the liver and begin producing insulin. One issue that arises is that some islets, from the same pancreas sample, become infiltrated by the immune system's T cells, while other islets are unaffected. My research aims to understand if there is differential gene expression in xenotransplanted human pancreatic islets that reverses diabetes in nude mice and those that do not reverse diabetes. Islets only comprise 2-3% of a pancreas; therefore to understand gene expression in islets the islets must be isolated from the surrounding exocrine tissue. This is made possible by laser capture microdissection (LCM). Laser capture is used to isolate immunofluorescent stained islets from both normal and infiltrated islets. RNA is isolated from the islets and then linearly amplified using T7 RNA amplification. The amplified RNA will be used to produce cDNA, which will be used in DNA microarray to study *in vivo* gene expression in islets.

## 49

### **THE SEARCH FOR CANINE MAPC AND THEIR USE IN GENE THERAPY FOR DEVELOPING A CURE TO HEMOPHILIA**

Jennifer A. Gravelle, Christina E. Clarkson, (Catherine M. Verfaillie)  
Stem Cell Institute, Department of Medicine, University of Minnesota

The Stem cell Institute at the University of Minnesota has recently discovered Multipotent Adult Progenitor Cells, MAPC, in the human, rat and mouse. These bone marrow derived cells have been shown to differentiate into mesodermal, neuroectodermal and endodermal cell types, undergo at least 60 cell doublings and express telomerase. The ability of MAPC to differentiate in to many different cell types lays a foundation for the treatment of many diseases using cell replacement therapy, while overcoming the need to use controversial embryonic cells. Hemophilia has many characteristics that make it a good target for cell replacement therapy. It is caused by a single gene defect resulting in a defective protein vital to the clotting cascade. In Hemophilia A, the non-functional protein is factor VIII, a plasma protein produced mainly by hepatic epithelial cells. The current therapy is transfusion of factor VIII, which requires pooling plasma samples from several individuals to obtain adequate amounts. This is expensive and increases the risk of infection with blood-borne pathogens. Cell replacement therapy would circumvent any issues of compatibility between donor and recipient and would also be much safer. The canine is an excellent large animal model for Hemophilia and an ongoing search for canine MAPC is underway. Bone marrow derived canine cells have been shown to differentiate phenotypically into endothelial cells that express vWF. These cells were transduced with a virus containing factor VIII and are also shown to express the factor VIII protein by an aPTT assay. More specific tests using PCR and western blot are being developed. Recently received bone marrow derived cells harvested from a factor VIII negative dog will be transduced by a lentivirus containing the factor VIII gene. Eventually cells differentiated in to epithelium, transduced with the virus and shown to express the factor VIII protein *in vitro* will be reintroduced in to the donor dog. This is a promising therapy that can one day be applied to humans.

## 50

### **THE ROLE OF REF-1 IN VASCULAR SMOOTH MUSCLE CELL APOPTOSIS**

Esther E. Kao (Jennifer Hall)  
Department of Medicine, Division of Cardiology, University of Minnesota

Vascular disease accounts for over 80% of the deaths in the diabetic population. The goal of this research is to understand the role of Redox Factor 1 (Ref-1) in vascular remodeling in response to injury. Recent studies attempting to determine the mechanism of vascular disease identified redox-signals as important modulators of inflammation in the blood vessel. Ref-1 is a 37 kD redox-sensitive bi-functional protein with a redox-sensitive domain as well as a DNA binding domain. It is involved in regulating cell growth, differentiation, survival, and death. It also has DNA repair activity. We used a wire-injury model to simulate vascular remodeling in response to balloon angioplasty. Vascular remodeling involves both proliferation and apoptosis of cells. Work *in vitro* has shown that loss of Ref-1 increases the susceptibility of cells to apoptosis. The goal of this research is to determine if down-regulation of Ref-1 *in vivo* (in the form of a hemizygous transgenic mouse) results in increased apoptosis. I am using TUNEL, caspase-3, and Hoechst stains to determine the levels of apoptosis. Another individual will be doing proliferation studies as well. In summary, we are assessing the role of Ref-1 in cell survival and pairing this data with parallel studies determining cell proliferation and downstream biochemical signaling pathways through which Ref-1 may be altering cell fate.

## 51

### **LONG-TERM GENE TRANSFER AND EXPRESSION IN HUMAN GLIOBLASTOMA USING THE *SLEEPING BEAUTY* TRANSPOSON SYSTEM**

Scott G Perkinson, John R Ohlfest (David A Largaespada)

Department of Genetics, Cell Biology, and Development, University of Minnesota

Gene therapy has the potential to become an effective component of cancer treatment by transferring genes that cause immunomodulation, tumor cell death, or inhibit angiogenesis<sup>1</sup>. Viral vectors have been the primary gene transfer vesicles used for intratumoral gene transfer to date. Plasmid-based vectors may be safer and more scalable than viral vectors. However, attempts at plasmid-based intratumoral gene transfer have been met with transient expression and poor gene transfer efficiency<sup>1</sup>. Here, we report integration and long-term expression of reporter genes into human glial tumors, growing in nude mice, using the *Sleeping Beauty* (SB) transposon system. A two-plasmid system was used, in which linear polyethylenimine (LPEI) was complexed with a GFP, NEO, or luciferase transposon plasmid and a SB transposase-expressing plasmid. SB-mediated transposition led to chromosomal integration of the NEO transgene in roughly 8% of tumor cells. Luciferase studies showed that SB facilitated long-term expression of the transgene in glial tumors. Glioblastoma and many other cancers over express the IL13 receptor<sup>2</sup>. Therefore, we constructed a plasmid in which we fused the HSV-TK suicide gene to the IL13 domain. We demonstrated that SB can be used with the secreted IL13-HSV-TK fusion to selectively kill human glial cells grown in mice. SB-mediated intratumoral gene transfer is a novel, non-viral technique that could be used to augment conventional therapy for glioblastoma or other cancers.

## 52

### **THE EFFECT OF AMES TRAPEZOID WINDOW ILLUSION ON AIMING ACTION**

Cheuk-Man Wong (Jürgen Konczak)

Department of Kinesiology, University of Minnesota

Visual illusions affect our perceptions of object properties and orientations. In contrast, previous studies suggested that visual illusions have no effect on our action. There is a debate on whether action is immune to visual illusion, implying that the visual information relevant for action and perception is processed separately in the brain (dorsal vs. ventral visual stream). In this study, we examine the effect the trapezoid window illusion on action. The flat trapezoid window creates an illusion of a window slanted in space about the vertical axis. The illusion is especially powerful during monocular viewing. We used three windows, a non-illusionary rectangular window and two trapezoid windows that had a longer edge on the left or right respectively. We recruited fifteen participants (age range: 19-22 years) and asked them to point with their index fingers to the edges of the display during binocular or monocular viewing. Our results indicate that participants responded to the perceived slant of the window during pointing indicating that the illusion affected action. The window illusions had less of an effect on action during binocular vision. This shows that stereoscopic vision is helpful in ignoring the perspective cues of the illusion. However, stereoscopic vision is insufficient to recover the true orientation of the illusionary display although the veridical information about the true orientation of the display was available in this case.

## 53

### **CELL SPRAYING TECHNIQUES FOR 3D TISSUE ENGINEERING**

Abhinav Arneja, Yaakov K Nahmias, (David J Odde)

Department of Biomedical Engineering, University of Minnesota

The short distance that oxygen and nutrients can diffuse before being consumed poses a limitation on the size of a three-dimensional tissue that can be constructed. This limitation can be overcome by creating a vascular network in the engineered tissue, which will greatly increase the rate of oxygen and nutrient transport. Human Umbilical Vein Endothelial Cells (HUVECs) form tube-like structures when cultured on Matrigel. We investigated the effect of the Extracellular Matrix mechanical properties on the induction of tube-like structures in HUVECs. HUVECs cultured on soft collagen gels (0.75 mg/ml) formed tube-like structures, while HUVECs cultured on rigid collagen gels (3.0 mg/ml) acquired a more flat morphology. Mesoscale patterning of endothelial cells on ECMs conducive to vascular network formation may provide control over their differentiation and allow their incorporation into an engineered tissue. Cell spraying is a novel technique for mesoscale patterning of cells on arbitrary substrates. Three different cell types, HUVECs, NIH 3T3s, and Hepatocytes, have been sprayed on both ECM coated surfaces and gels (Collagen, Matrigel) with little loss of viability. Mesoscale patterns (~100 $\mu$ m) of all three cell types were created by spraying suspensions of cells in media through a 100  $\mu$ m air slit on collagen gels. Cell spraying has also been successful in creating three-dimensional patterns of HUVECs on a two layered collagen gel. Current and future work will be focused on creating viable three-dimensional patterns of NIH 3T3 cells on collagen gels, and co-culture of HUVEC and NIH 3T3 patterns with parenchymal cell types such as Hepatocytes to demonstrate three-dimensional liver tissue engineering. Cell spraying and its applications in three-dimensional tissue engineering is a promising strategy for the mesoscale patterning of cells and makes the development of functional tissue *in vitro* a feasible goal in the near future.

## 54

### **RAFFINOSE INCREASES THE STABILITY OF SUCROSE COTTON CANDY**

Kellie M. Leinen, (Theodore P. Labuza)

Department of Food Science and Nutrition, University of Minnesota

Cotton candy is a treat loved by many young and old. Efforts have been made to make this product more than just a carnival delicacy; however, maintaining the amorphous sucrose system over time has proven to be problematic. Consumers have complained of receiving a collapsed, sticky, grainy product (Labuza & Labuza, 2004). Raffinose, a trisaccharide found in beans and beet sugar, has been discovered by other researchers to inhibit the crystallization of sucrose in solution (Smythe, 1967). This study explores the hypothesis that raffinose would increase the stability of an amorphous sucrose system. Two mixtures of cotton candy, one 100% sucrose and the other 95% sucrose and 5% amorphous raffinose, were made and stored at room temperature in three different relative humidity chambers, 11%, 33%, and 43%RH. The moisture content, DSC data to show glass transition, and x-ray diffraction patterns were obtained as a function of storage time to determine the stability of the different mixtures as a function of %RH. The data collected shows that raffinose slows sucrose crystallization in a dry amorphous state above the glass transition temperature and therefore improves the stability of amorphous sucrose systems.

## 55

### **SCHWANN CELL-MEDIATED CONTACT GUIDANCE OF AXONS IN ALIGNED FIBRIN GELS**

Ta-Chun Hang, Audrey Y. Gandadjaja, (Robert T. Tranquillo)  
Department of Biomedical Engineering, University of Minnesota

Extensive research has been done to find different means to augment neuronal axon regeneration. This is a highly researched subject because of various applications, such as the restoration of peripheral nervous system pathways following injury. Among these approaches, contact guidance has been shown to strongly promote nerve regeneration. To achieve contact guidance, Schwann cells (SCs) can be aligned along fibers of a fibrin gel formed in a tubular fashion as a nerve guide conduit for PC12 cells (immortalized neuronal cells that extend axons in response to NGF), similar to naturally exhibited processes in nerve regeneration. Our hypothesis is that spread Schwann cells will induce PC12 cells in co-culture to extend neurites within an aligned fibrin gel (that are more aligned than they would be in the absence of SCs) due to beneficial interactions. First, the cell co-culture was optimized in terms of media and NGF concentrations for maintaining viability of both cell types. Once completed, SCs were tested to demonstrate whether they spread and maintained an elongated morphology in the presence and absence of PC12s. Then a fibrin gel aligned by drainage of interstitial liquid during gel formation (Elsdale and Bard) will be seeded with SCs +/- PC12s and the degree of alignment of neurites relative to the fibrin alignment will be quantified. So far, it has been shown that PC12s and SCs will have sufficient viability for ~7 days when cultured in DMEM at 25-50 ng/mL NGF concentrations. At a high enough cell density, SCs in isotropic fibrin hemispheres (with identical medium conditions) exhibit remarkable spreading. These findings provide a basis for further experimentation using aligned gels, with eventual testing for nerve regeneration studies.

## 56

### **DETERMINATION OF PROTEIN KINASE C EXPRESSION IN CANCER CELL LINES ISOLATED FROM VARIOUS STAGES OF MELANOMA PROGRESSION**

Teodora Platikanova (James B. McCarthy, Cheryl L. Neudauer)  
Department of Laboratory Medicine and Pathology, University of Minnesota

Melanoma, a potentially deadly cancer with increasing incidence, progresses from radial growth phase (RGP) through vertical growth phase (VGP) to malignant melanoma. We have previously determined that insulin-like growth factor (IGF-1) stimulates VGP cell migration through the phosphoinositide-3 kinase (PI3K) pathway. Therefore, proteins activated after, or downstream of, PI3K may be important for melanoma cell migration. Here, we examined the expression of protein kinase C (PKC) isoforms in cell lines isolated from stages of melanoma progression since these proteins can be activated downstream of PI3K. We probed western blots with antibodies specific for each PKC isoform. No significant differences in PKC $\beta$ , PKC $\gamma$  and PKC $\iota$  expression were detected in contrast to PKC $\alpha$  and PKC $\zeta$ , which were increased in the RGP and malignant cell lines and PKC $\delta$ , which was increased in RGP cells. These results indicate that PKC $\alpha$ , PKC $\zeta$  and PKC $\delta$  may be important in signaling changes in melanoma progression. Future studies are planned to determine which of these three PKC isoforms are involved in IGF-1-stimulated migration. Understanding whether PKC is part of melanoma signaling and migration may lead to a new target for therapy of this disease.

## 57

### GENE THERAPY FOR PULMONARY FIBROSIS

Hong-Yiou (David) Lin, Karen A. Smith, (Peter B. Bitterman)

Department of Medicine, University of Minnesota

Eukaryotic translation initiation factor 4E binds to the 7-methyl guanosine cap of mRNA and together with eIF4A and eIF4G, forms the cap-dependent translation initiation complex eIF4F. A family of repressor proteins, designated 4E-BP (4E-BP1 is most abundant in lung and most tissues), antagonizes the action of eIF4E. Over expression of eIF4E, which is present in rate limiting amounts, stimulates cell proliferation and increases resistance to apoptosis. Chronic lung allograft rejection is characterized by accumulations of apoptosis-resistant fibroblasts in small airways leading to impaired gas exchange. When studied *in vitro*, these airway fibroblasts require aberrantly high levels of cap-dependent translation to remain viable, and undergo apoptosis when cap-dependent translation is normalized. As an *in vivo* proof of principle experiment in a murine model of chronic lung allograft rejection, we devised a gene transfer strategy to normalize airway fibroblast translation and trigger apoptosis. To date, our therapeutic vector, an adenovirus containing 4E-BP1, has been used to infect fibroblasts with aberrant activation of eIF4E *in vitro* and validated to normalize translation. We are now in a position to test the ability of our adenoviral vector to transfer 4E-BP1 into airway fibroblasts in the murine model and quantify its effect on airway fibroblast translation and apoptosis.

## 58

### DETERMINATION OF RNA SECONDARY STRUCTURE IN THE 5' NON-CODING REGION OF COXSACKIEVIRUS B1

Wade L. Schulz (Patricia Tam)

Department of Medicine, University of Minnesota

Coxsackievirus B1 (CVB1) is a plus-strand RNA virus and a member of the Picornaviridae family. Studies have shown that, unlike most cellular mRNA, translation initiation of picornavirus RNA is not cap-dependent. Instead, ribosome binding is mediated by an internal ribosome entry site (IRES) found in the 5' non-coding region (NCR). This IRES region and the surrounding NCR are composed of RNA secondary structures which form stems and loops. In a mouse model of CVB1-induced chronic muscle disease, myopathic virus causes acute symptoms as well as development of chronic disease. Previous experimentation has shown that a single mutation at nucleotide 706, which is downstream of the IRES and near the translation start site at nucleotide 743, changes the pathogenic phenotype of the virus to one that causes an acute infection but not chronic disease in our mouse model. We have performed computer modeling of the NCR secondary structure which has predicted a consistent structural change between the wild-type virus and the mutated form. The stem-loop containing nucleotide 706 changes from a length of 38 nucleotides in the wild-type virus to 59 nucleotides in the mutant. Through the use of ribonuclease digestion and reverse transcription with a fluorescently-labeled primer, we plan to determine the actual secondary structure of the two constructs. Determining how the mutation at nucleotide 706 alters RNA secondary structure is an important step in discovering how CVB1 causes chronic inflammatory myopathy.

Tam, PE, Messner, RP. *Coxsackievirus-induced chronic inflammatory myopathy: Virus variants distinguish between acute cytopathic effects and pathogenesis of chronic disease*. Virology 1997;233:199-209.

## 59

### **SARS-RELATED VIRAL ASSEMBLY: AN EXPERIMENTAL STUDY IN YEAST**

Lindsey R. Thompson (Robin Wright)

Department of Genetics, Cell Biology, and Development, University of Minnesota

SARS (severe acute respiratory syndrome), hepatitis, bronchitis, and other animal diseases are caused by coronaviruses. The coronavirus is enclosed within a membrane envelope with protruding glycoproteins that create a corona (crown) around the viral core. This envelope is derived from host cell membranes, which are also the site of viral RNA synthesis. Susan Baker, a professor at Loyola University, discovered that over-expression of the major viral membrane protein (MP-1) of mouse hepatitis virus resulted in accumulation of unusual cytoplasmic membranes. To test whether MP-1 is necessary and sufficient to induce these membranes, we collaborated with Dr. Baker to examine the effects of MP-1 expression in the yeast, *Saccharomyces cerevisiae*. Yeast expressing MP-1 did not assemble membranes that were similar to those observed in mouse cells. However, these yeast had altered vacuole morphology consistent with increased levels of autophagy. If so, yeast strains with defects in genes required for autophagy might die or grow slowly when MP-1 expression was activated. The growth rates of ten different autophagy mutants transformed with a control plasmid or an MP-1 containing plasmid were examined over 24 hours. In addition, we examined the growth of these strains on solid media at three different temperatures. In all cases, the mutant strains expressing MP-1 had the same growth rates as the control strains. Thus, it is unlikely that MP-1 affects vacuole morphology by activating the autophagy pathway. Instead, the alterations of vacuole membranes induced by MP-1 must originate from its effects on other cellular processes. Taken together, our studies suggest that MP-1 is not sufficient to induce membrane formations, but instead species-specific host factors must also be important for MP-1 protein function in viral assembly. Experimental strategies to understand the mechanism of coronavirus assembly may be inhibited by this species-specificity.

## 60

### **A NOVEL MODEL FOR ADDRESSING B CELL SOMATIC HYPERMUTATION**

Katie N. Lee (Timothy W. Behrens and Keli L. Hippen)

Department of Medicine, University of Minnesota

B cell production of high affinity antibodies is critical for the clearance of many pathogens. While many important experiments have been performed in conventional transgenic Ig receptor models and much has been learned from them about B cell immunity and autoimmunity, there are significant limitations to these models. For example, these models could not address two critical processes used to fine tune B cell responses, class switch recombination (CSR) and somatic hypermutation (SHM). In response to antigen and lymphokines, B cells switch from using the C<sub>μ</sub> constant region of the heavy chain to new isotypes, while maintaining their original antigen specificity. Affinity maturation describes an antigen driven process whereby mutations are introduced into the immunoglobulin loci (somatic hypermutation), and due to extreme competition for antigen, mutant immunoglobulin molecules with highest affinity for antigen dominate the response. Optimal class switching and affinity maturation requires B cell internalization of antigen and presentation to antigen specific T cells, which in turn provide 'help' in the form of cytokines and CD40 ligation. The importance of these fine tuning mechanisms is underscored by several human diseases resulting from an inability to generate class switched and affinity matured immunoglobulin molecules. This project makes use of an important new model system for understanding B cell immunity and autoimmunity, where the heavy and light chains that encode a Hen Egg Lysozyme (HEL)-specific B cell receptor have been knocked-into their respective loci. A chimeric antigen containing a B cell epitope (HEL), a fluorescent moiety, and a T cell epitope (I-Ea) allows tracking of antigen and peptide/MHC complexes *in vivo*. Initial experiments with this model system found class switching well in advance of germinal center formation. We will present evidence characterizing somatic hypermutation in this model system, and will address whether SHM can also occur outside of the germinal center microenvironment.

## 61

### **ANALYSIS OF CELL CYCLE GENES IN PRIMARY AND IMMORTAL CHICKEN CELLS**

Meghan A. Richardson (Douglas Foster)

Department of Animal Science, University of Minnesota

The cyclin genes of the cell are responsible for coding proteins that drive the cell cycle and are therefore closely tied to cell proliferation and division. The tumor-suppressing genes Rb and p53 are also instrumental in controlling the cell cycle. The Rb gene, when hypophosphorylated, suppresses passage into the S phase of the cell cycle. The p53 gene binds DNA to stimulate cell growth, is important in sensing DNA damage and can be inhibited by MDM-2. Because these genes are so closely tied to the proliferation of cells, their differential regulated expression would indicate cell growth and division. We studied the levels of expression of these genes in different types of avian cells treated with varying amounts of chicken embryo extract (CEE). Through the use of RT-PCR analysis we obtained levels of gene expression for both primary and immortalized cells. These numbers were normalized against the expression of GAPDH in each cell and then converted to set relative ratios of gene expression. The results provided evidence that primary cells treated with CEE in some instances showed an elevated expression of the relevant genes, Rb, p53, and several of the cyclins. Immortalized cells treated with CEE showed elevated expression of the MDM-2, and Cyclin D2 genes. These results indicate that proliferation and division was taking place. This information will be helpful in establishing the most beneficial conditions for cell proliferation.

## 62

### **DIRECT PHYSICAL CHARACTERIZATION OF PRRSV VIRION PROTEINS**

Brenda J. Saxton (Michael P. Murtaugh)

Department of Veterinary and Biomedical Sciences, University of Minnesota

Precise and specific knowledge of the physical characteristics of a viral particle provides the basic information about surface antigen candidates for neutralizing antibodies, receptor-binding structural candidates for inhibitors, and essential proteins that initiate the infection process. Surprisingly little, indeed almost nothing, is known about the North American PRRSV virion. Our picture is formed largely by analogy and inference to work on the mouse cousin, LDV, and the European PRRSV, Lelystad virus, from indirect methods that are subject to multiple interpretations, or from scant data that has not been confirmed. By contrast, direct physical methods based on mass spectrometry can directly identify proteins in the virion without recourse to antibodies, labeling devices or other limitations. The methods are unbiased, facilitating the identification of proteins that are unexpected, unknown, or not predicted previously. Unbiased methods of analysis are critical to understanding novel viruses like PRRSV that by definition behave in unknown ways. We are using mass spectrometry and a PRRSV protein sequence database to directly identify peptide fragments of PRRSV proteins in virions and to determine the relative amounts of these proteins. This information will help to identify likely and unlikely targets for immune protection, to better understand the infectious process, and to guide the development of strategies for reducing or preventing disease in infected pigs. At a minimum, it improves the present situation in which research on the control and prevention of PRRS is based on uncertain hypotheses and assumptions, where certain facts can be obtained.

## 63

### **SURFACE INTERFACE EFFECTS IN INDUCED PROTEIN DIMERIZATION**

Jessie L. Kerns, (Carston R. Wagner, Jonathan C. T. Carlson)  
Department of Medicinal Chemistry, University of Minnesota

Protein dimerization is an important mechanism of biological regulation. Chemically induced dimerization, moreover, has the potential to modulate cell membrane receptor signaling, selectively antagonize cellular processes and control gene expression. *E. coli* DHFR and bis-MTX are an important model system for chemically induced dimerization. A Chemical Inducer of Protein Dimerization (CID) for DHFR is a dodecanediamine linker ( $C_{12}$ ) joining two molecules of Methotrexate (MTX), inducing a dimerized state in DHFR. At the interface of the proteins in this system, position 19 (wild-type alanine) of one protein is in close proximity to position 23 (wild-type asparagine) of the other. Mutations at these sites have the potential to create steric and charge-charge interactions and effect the thermodynamic stability of the dimeric species. The thermodynamic stability of the dimer is assessed using equilibria associated with the dimerization system. The first such equilibrium constant ( $K_{eq}$ ) describes the state of the ligand (bis-MTX). The second equilibrium constant ( $K_c$ ) isolates the interaction of the interface. The ratio of these constants ( $K_{eq}/K_c$ ) is used to study the relative stability of various dimeric species. Thus far, several mutated proteins have been produced and a number of them have been analyzed. Mutations have been selected to reduce or increase steric interaction from wild-type, or to create a charge-charge interaction. The results are as follows: N23A (wild-type asparagine at position 23 mutated to alanine) yields a dimeric species 3 times more stable than wild-type; preliminary results on A19N indicates it is 3 times less stable; N23H at neutral pH (uncharged histidine residue) has no effect on dimeric stability; A19K has a destabilizing effect roughly 2.5 times that of wild-type; and A19K coupled to N23H at pH 6 (charged histidine residue) is 4 times less stable than wild-type. Still in process is the analysis of these proteins as well as a pH profile of mutants containing amino acids affected by pH.

## 64

### **INVESTIGATION OF THE EGG-BINDING PROPERTY OF RAT CRISP-1 SPERM PROTEIN**

Miranda L. Bernhardt (Kenneth P. Roberts)  
Department of Genetics, Cell Biology, and Development, University of Minnesota

The rat *Crisp-1* gene encodes the glycoprotein couplet of Proteins D and E, also known as Crisp-1 or acidic epididymal glycoprotein (AEG). Crisp-1, a member of the CRISP family of cysteine-rich secretory proteins, is secreted by the epididymal epithelium and binds to sperm as they travel through the epididymis. Crisp-1 has been implicated in the process of sperm/egg fusion in rat and mouse, but the functional domains in Protein D and/or E responsible for this process have not yet been determined. The objective of this project is to determine what region of Crisp-1 is responsible for its egg-binding property. In order to investigate this, a fusion protein between Crisp-1 and Green Fluorescent Protein (GFP) was synthesized and purified. Rat Crisp-1 cDNA was linked to GFP cDNA and subcloned into the bacterial expression vector pBAD/gIII. The vector was transfected into *E. coli* cells, and the cells were induced to produce recombinant protein. The recombinant fusion protein was partially purified by affinity chromatography using a histidine tag also encoded by the vector. Once sufficient purified protein has been collected, experiments will be performed to test whether this fusion protein will bind to rat eggs, if native Crisp-1 will compete for the same binding sites, and if shorter peptide sequences contained in Crisp-1 will also compete for these binding sites. Fusions between GFP and truncated forms of Crisp-1 may also be created to localize the functional region of Crisp-1.

## 65

### **CATALOG OF PROTEIN CHANGES PERTAINING TO AGE-RELATED MACULAR DEGENERATION IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS**

Kristin M. Berg, Xiao Feng, M.D., Rebecca J. Kappahn, Tim W. Olsen, M.D (Deborah A. Ferrington)  
Department of Ophthalmology, University of Minnesota

Age-related macular degeneration (AMD) is the leading cause of blindness in aging populations. It is characterized by a gradual decrease of central vision, and the accumulation of drusen between the retinal pigment epithelial (RPE) cell layer and choroid layer. Although the cause is unknown, antioxidants seem to help slow down the progression of the disease. Therefore, it is hypothesized that the oxidation of polyunsaturated fatty acids in cell membranes, producing 4-hydroxy-2-nonenal (HNE), which can then modify amino acid residues, plays a role in the progression of AMD. A second hypothesis is that specific proteins will exhibit altered expression levels with AMD. Human donor eyes were collected from the Minnesota Lions Eye Bank, evaluated for their level of AMD, and dissected to retrieve the retinal and retinal pigment epithelial cell layers. In order to determine modifications and expression level changes, proteins from the RPE layer were subjected to two-dimensional gel electrophoresis. Parallel gels were run per sample, for Western blotting to detect HNE modifications, and silver staining to detect expression level changes. Select proteins were then extracted from the gels and subjected to in-gel trypsin digest. Mass spectrometry analysis was performed to confirm their identities. Preliminary results demonstrate decreased expression levels of select proteins, and increased HNE modifications with advanced levels of AMD. The proteins affected could identify defective cellular pathways that either precede or are a consequence of the progression of AMD, thus creating a target on which to base possible pharmacologic therapies.

## 66

### **EFFECTS OF DESICCATION ON RNA AND PROTEIN EXPRESSION IN *E. COLI***

Jason A. Motl (Janet Schottel)

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota

My lab is interested in producing biocatalysts and biosensors linked to green fluorescent protein (gfp) for environmental testing using the bacteria, *E. coli*. Since the bacteria will be in a desiccated state on these biosensors until water immersion, we are researching the effects of desiccation on the bacteria on survival rate, protein expression and RNA expression. By understanding the changes in protein and RNA expression we may be able to engineer hardier bacteria to increase biosensors sensitivity. We are analyzing protein and RNA expression changes in *E. coli* prior to dehydration and over a period of time of rehydration after dehydration. These changes are being monitored via sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) and agarose gels, respectively. So far we have observed protein expression changes but have not been able to positively identify the proteins.

## 67

### **DETERMINING THE SPECIFICITY OF CCF10 PEPTIDE PHEROMONE FOR *ENTEROCOCCUS FAECALIS* PHEROMONE BINDING PROTEINS, PRGZ AND PRX**

Kathryn R. Goeden (Gary M. Dunny)

Department of Microbiology, University of Minnesota

*Enterococcus faecalis* cells containing the antibiotic resistance plasmid pCF10 respond to the chromosomally encoded heptapeptide pheromone cCF10 (LVTLVFV), which induces the conjugative transfer of pCF10 from a donor cell to a recipient cell. Pheromone response involves binding by the secreted lipoprotein PrgZ, and its subsequent import into the cell and interaction with the negative regulator PrgX. This mating system is very specific for cCF10, suggesting that the amino acid sequence of the pheromone is a critical determinant of its activity. The purpose of this study was to ascertain the specific amino acid residues of cCF10 that determine specificity for interaction with PrgZ and PrgX. In order to do this, oligonucleotide-directed random mutagenesis of cCF10 coding sequence was used to create variants of cCF10. Cell supernatants from bacteria carrying these mutations were screened for pheromone activity using a clumping assay. Pheromone induction leads to the visible formation of cell “clumps” of responding donor cells. Mutants leading to reduced activity were sequenced, and resulting amino acid changes were deduced. Analysis of the peptides resulting from mutated sequences supports previous findings that suggested the amino terminal end of this peptide is important for the cCF10’s activity, and essential internal residues were also identified. Intermediate induction activity was observed with mutations at the carboxy terminal end, and a possible ‘super pheromone’ was found when an isoleucine was substituted at this position. This would suggest that the carboxy terminal end does play a role in the pheromone’s induction activity. A *lacZ* fusion was used to test the level of the super pheromone’s activity via  $\beta$ -galactosidase activity. The super pheromone exhibited higher activity than cCF10 at physiological levels. Analysis of the biological activity of synthetic cCF10 variants containing various amino acid substitutions suggested that interaction with PrgX is a more important determinant of pheromone activity than interaction with PrgZ.

## 68

### **EXPRESSION AND FUNCTION OF NITRIC OXIDE SYNTHASE (NOS) IN *SALMONELLA ENTERICA* SEROVAR *TYPHIMURIUM***

Amanda J. Helvig (Lucy Vulchanova and David R. Brown)

Department of Veterinary & Biomedical Sciences, University of Minnesota

Nitric oxide (NO) is an important mediator of intercellular communication and antimicrobial defense. This gaseous substance is generated by NOS-catalyzed oxidation of L-arginine. In mammals, inducible NOS (iNOS) expressed in macrophages generates NO to kill phagocytosed pathogens, such as *Salmonella typhimurium* (Michel & Feron, 1997). NO is also generated by the intestinal epithelium, where it may decrease mucosal barrier function and facilitate enteropathogen invasion (Kubes, 2000). I have discovered that *S. typhimurium* itself expresses specific immunoreactivity for iNOS. Thus, this microorganism may express the same enzyme that is used by host cells to kill it. This finding is supported by a report of the isolation of iNOS protein from *S. typhimurium* (Choi *et al.*, 2000). I have hypothesized that NO generated by NOS in *S. typhimurium* may contribute to its growth characteristics, mucosal invasion or intracellular survival. To address this hypothesis, I am examining (1) the effect of the NOS inhibitors on *S. typhimurium* growth, and 2) the role of NO in *S. typhimurium* invasion of porcine intestinal mucosa explants at extended time periods post infection. At present, I have found that NOS inhibitors do not seem to alter *S. typhimurium* growth. I am in the process of examining the effects of NOS inhibitors on intracellular internalization of *S. typhimurium*. These studies will provide important new information on the role of NO in host-pathogen interactions.

Choi, D. W., H. Y. Oh, S. Y. Hong, J. W. Han, and H. W. Lee. 2000. Arch. Pharm. Res. 23: 407-412.

Kubes, P. 2000. Gut. 47: 6-9.

Michel, T. and O. Feron. 1997. J. Clin. Invest. 100: 2146-2152

## 69

### THE INVADER: A NOVEL mRNA ASSAY

Rania A. Habib, Daniel B. Leslie\*, Paul S. Vietzen\*, (David L. Dunn\*, Karen R. Wasiluk\*)  
Department of Microbiology and \*Department of Surgery, University of Minnesota

Gram-negative bacterial sepsis causes more than 200,000 deaths in the United States annually. The outer membrane of gram-negative bacteria contains lipopolysaccharide (LPS) that is released into the bloodstream during infection. LPS binds and activates host macrophages, resulting in the secretion of various cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), an early marker of sepsis, which can ultimately lead to death in surgical patients. Two standard methods used to measure TNF- $\alpha$  include: **1)** ELISA, which is very expensive and measures both active and inactive TNF- $\alpha$ , and **2)** the WEHI bioassay, which takes two days to perform and is subject to variability. We hypothesized that an invader assay specific for TNF- $\alpha$  mRNA would provide data comparable to that obtained with the bioassay. We incubated cells from a murine macrophage cell line (RAW 264.7) with media alone, media with 10 ng *P. aeruginosa* LPS  $\pm$  either polymyxin B, a known LPS antagonist, or a test peptide (PV00) for 3 hours. Inhibition of TNF- $\alpha$  is correlated to LPS-neutralization. We removed the supernatant for measurement of TNF- $\alpha$  using the WEHI bioassay. We then lysed the cells and used the invader assay to quantitate TNF- $\alpha$  mRNA. Differences in the mean values were analyzed using a two-tailed t-test with significance at  $p < 0.05$ . Experiments were performed in triplicate. Both the invader assay and WEHI bioassay demonstrated an increase in the quantity of detectable TNF- $\alpha$  mRNA and bioactivity, respectively, in RAW 264.7 cells stimulated with LPS compared to unstimulated cells ( $p < 0.05$ ). LPS was neutralized by both polymyxin B and PV00 (both  $p < 0.05$ ), although the degree of LPS-neutralization was less for PV00 than for polymyxin B. The TNF- $\alpha$  mRNA invader assay is a novel assay, developed by our laboratory, that can be used to detect LPS-neutralization by LPS antagonists.

## 70

### BACTERIOPHAGE TRANSDUCTION OF BIOLUMINESCENT GROUP A STREPTOCOCCUS

Matthew M. Schaefer (Patrick Cleary, Haesun Park)  
Department of Microbiology, University of Minnesota

The use of genetically engineered luminescent M49 group A streptococcus (Xen 20) allows for tracking bacteria during an infection in mice. The *lux* cassette, which provides the genes needed for luminescence, is integrated into the open reading frame immediately downstream of the *aroE2* gene of this strain. Our laboratory has constructed a collection of mutants in a serotype M1 strain, 90-226, which is better adapted for virulence studies in mice. The goal of this project was to genetically transfer the *lux* cassette from Xen 20 into the 90-226 background to produce a bioluminescent strain for further study of virulence. A bacteriophage lysate of strain Xen 20 that carries the *lux* and *kan*<sup>R</sup> cassette was used to transduce M1 (90-226) cells. Kanamycin was used to select for recombinants, which were then screened for luminescence. All of the *kan*<sup>R</sup> colonies were luminescent. Among them ten of the brightest colonies (90-226 *lux*<sup>+</sup>) were further characterized. The growth rates of transductants were similar to that of the parental strain 90-226 when measured by change in optical density over time. The luminescence per cell of the 90-226 *lux*<sup>+</sup> was higher than Xen 20. One strain (90-226 *lux*<sup>7</sup>) was chosen for further investigation and experimentation. RFLP will be used to confirm that 90-226 *lux*<sup>7</sup> is M1 as opposed to M49. PCR was used to confirm that *lux* was inserted in the same location in 90-226 *lux*<sup>+</sup> as it was in Xen 20 by amplifying the DNA region known to be the integration site in Xen 20. Finally strain 90-226 *lux*<sup>7</sup> was used to infect mice, and as predicted it produced detectable levels of light and colonized nasal-pharynx tissue more effectively than the M49 strain.

## 71

### **USE OF CARBONATE TO REDUCE *ESHERICHIA COLI* O157:H7 IN WATER**

Julie A. Kuruc (Francisco Diez-Gonzalez)

Department of Food Science and Nutrition, University of Minnesota

*Escherichia coli* O157:H7 is a food-borne pathogen that infects more than 70,000 Americans every year, costing the U.S. economy over \$700 million dollars. Cattle are a natural reservoir of *E. coli* O157:H7 and these bacteria are commonly shed in their feces. Post-harvest strategies such as carcass rinsing and irradiation can lower the incidence of infections caused by ground beef. However, in order to control future outbreaks, pre-harvest strategies at the farm level should be implemented. On farms, water can be a vehicle of transmission of *E. coli* O157:H7 among cattle populations. The goal of this project was to develop an effective water treatment to kill *E. coli* O157:H7 based on the antimicrobial properties of carbonate. Two types of carbonate, sodium carbonate and sodium sesquicarbonate, were used to obtain the desired pH of the solution at various concentrations. The carbonate solution was inoculated with *E. coli* O157:H7 strain ATCC 43895. The experiment was carried out at room temperature and samples were taken at 0, 3 and 6 hours. At six hours, a 4 g/L carbonate solution (pH 11) had the ability to cause a six log reduction in the viable count of *E. coli* O157:H7. When the pH of the solution was decreased, the cell inactivation declined. Increasing the concentration to 6 g/L carbonate at pH values less than 11 did not enhance the killing of strain ATCC 43895. These results indicate that carbonate at pH 11 would be effective at controlling *E. coli* O157:H7 in water. Future research would involve determining if the carbonate solution has the same effect on other *E. coli* O157:H7 strains and different bacterial species. In addition, determining the acceptability of carbonate solutions as a water source for cattle would have to be established.

## 72

### **GREEN FLUORESCENCE PROTEIN TRANSFECTION IN PRIMARY PIG FETAL FIBROBLAST AND IMMORTAL PIG ENDOMETRIAL GLANDULAR EPITHELIAL CELLS**

Aili V. Salo (Scott Fahrenkrug, Byung-Whi Kong)

Department of Animal Science, University of Minnesota

The cells of many mammals have the ability to take up and express DNA which is applied externally under normal environmental conditions, however this occurs under a relatively low efficiency rate of transfection. To help get around this problem several methods have developed in an effort to improve transfection efficiencies. These transfection methods include direct microinjection, electroporation, and using chemical means such as the calcium phosphate method and the liposome method. While there is much known about the individual methods, for example that direct microinjection has a high efficiency for transfection but that it also carries the downfall that the conditions for this method kills all of the cells present, not much is known about what the best chemical method of transfection of DNA is. Through this project our goal was to do just this, to test transfection efficiencies of primary pig fetal fibroblasts (PFF) using the chemical methods of liposome (using lipofectin) and calcium phosphate. In addition we also wanted to test and compare in the same manner with immortal pig endometrial glandular epithelial (PEGE) cells. From our experiment we have found that using by the liposome method and the calcium phosphate method that the green fluorescence protein (GFP) was expressed in both cases, but that there was relatively no difference between these two methods in the efficiency of transfection of the primary PFF.

## 73

### **EXPRESSION OF GROWTH AND DIFFERENTIATION FACTORS IN TYPE II DIABETES**

Joshua T. Wilson-Grady (David A. Bernlohr)

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Adipose fatty acid binding protein (A-FABP) is expressed in murine white and brown fat and facilitates efficient lipolysis through solubilization of intracellularly-derived lipids. Obese FABP null exhibit reduced lipolysis and attenuated characteristic of type II diabetes including lower fasting glucose and improved glucose and insulin tolerance tests. Conversely, obese FABP transgenic mice (over expression in fat) have increased lipolysis and exhibit potentiated characteristics of type II diabetes including elevated fasting glucose, impaired insulin and glucose tolerance tests, and mild cardiac hypertrophy. Secretion of adipose hormones linked to energy metabolism (adiponectin, resistin and TNF $\alpha$ ) is altered in such animal models of the metabolic syndrome linking adipocyte fatty acid metabolism to adipokine secretion and systemic energy metabolism. The laboratory has recently discovered that the expression and secretion of GDF-3, a member of the bone morphogenetic protein family, is increased at the level of mRNA and protein in FABP null mice. To further establish a connection between type II diabetes, FABP function, and the GDF family of secreted proteins, three model system of adipose biology have been examined: lean vs. obese C57Bl/6J mice; differentiating 3T3-L1 preadipocytes; and FABP null or transgenic mice. In all studies RT-PCR was used to monitor expression of GDF-3 as well as other members of the GDF family (GDF-5 and GDF-15). The results indicate that GDF-3 mRNA is the only member of the family to be expressed in murine adipose tissue and is not significantly affected by diet-induced obesity. Insulin sensitive FABP null mice, however, exhibit increased adipose expression of GDF-3 mRNA, while insulin resistant FABP transgenic mice down regulate GDF-3 expression. Interestingly, all three GDF family members are expressed in cultured adipocytes. These results are consistent with a model for GDF-3 involvement in the development of obesity linked insulin resistance in the mouse and form the basis for future examination of the role of GDF-3 in type II diabetes.

## 74

### **PITUITARY SUPPRESSION BY DEXAMETHASONE ATTENUATES THE COMPENSATORY ADRENAL GROWTH RESPONSE**

Anu Elayaperumal (William C Engeland)

Department of Surgery, Department of Neuroscience, University of Minnesota

After unilateral adrenalectomy, the remaining adrenal has the ability to compensate for the function of the removed adrenal by cell hyperplasia in a phenomenon known as compensatory adrenal growth (CAG). In response to the surgical stress required for CAG, activation of the Hypothalamic-Pituitary-Adrenal (HPA axis) occurs. Pituitary hormones like ACTH have been proposed to play a role in CAG. In order to determine if ACTH is responsible for the proliferative response, the synthetic glucocorticoid dexamethasone, was used to block ACTH release. Adult male rats were injected with either saline or dexamethasone two hours before unilateral adrenalectomy (ULA), sham adrenalectomy (SHAM), or no surgery (NO SURG). After two days rats were sacrificed, and adrenal glands were collected, weighed, and sectioned. The amount and zonation of proliferating cells was determined using immunohistofluorescent labeling against antibodies for Ki67 (a proliferative marker), P45011 $\beta$  (a fasciculata cell marker), and P450aldo (a glomerulosa cell marker). Cells were counted using unbiased stereological methods in specific adrenal zones - zona glomerulosa and zona fasciculata (inner and outer). Adrenal weights for dexamethasone-treated rats were significantly lower than rats receiving saline. The number of proliferating cells in ULA, SHAM, and NO SURG groups following saline reflected normal CAG. However, in all surgical groups, dexamethasone resulted in significant decreases in cell proliferation of the outer fasciculata. The difference in adrenal weight suggests that CAG occurred normally in the saline group compared to the dexamethasone treated group. The decrease in proliferation after dexamethasone suggests that blockade of ACTH suppresses CAG. These results implicate ACTH or other pituitary derived hormones in the CAG response.

## 75

### ISOLATION OF PUTATIVE CHICKEN STEM CELLS

Eileen F. Kerkhoven, (Douglas Foster, Shelly Christman, Linda Foster)

Department of Animal Science, University of Minnesota

Chicken embryonic stem cells hold a plausible key to the advancement of avian reproduction and the potential generation of transgenic birds. The purpose of this research project is to determine if it possible to establish and definitely characterize an immortal chicken embryonic stem cell line. The approach taken was to isolate embryonic chicken cells from six-day-old embryos and culture them without a feeder layer for possible growth of chicken embryonic stem (ES) cells. To date our research indicates that these cells were phenotypically and analytically identified as having the potential characteristics of chicken embryonic stem cells. The cells remained in culture for five months to passage 28, which is considerably longer than normal embryonic cells. The putative ES cells were positive for telomerase at passage 26, but then the cells differentiated due to a presumed lack of needed growth factors. These findings are significant since the putative ES cells grew for a long duration of five months without differentiation. They were telomerase positive throughout culturing and they were unique in that they were grown without utilizing a feeder layer.

## 76

### ROLE OF CORTICOTROPIN-RELEASING FACTOR IN ACUTE OPIATE DEPENDENCE

David M. Atkinson (Jonathan C. Gewirtz)

Department of Psychology, Department of Neuroscience, University of Minnesota, Twin Cities

The induction of negative affect (withdrawal) following cessation of drug use may serve as a potent reinforcer of continued substance abuse. Corticotropin-releasing factor (CRF), a neuropeptide involved in stress and anxiety, has been implicated in the manifestation of anxiety-like symptoms of drug withdrawal from *chronic* exposure. The objective of this study was to determine whether intracerebroventricular (ICV) infusions of the CRF antagonist astressin affects the severity of the anxiety-like state during repeated withdrawals from *acute* doses of morphine, as measured through the acoustic startle reflex. Acoustic startle has been found to be elevated during anxiety-like states in both humans and animals, including the state of withdrawal from an acute dose of morphine (withdrawal-potentiated startle). In this study, rats were infused ICV with either astressin (5 µg in 2 µl DI water) or 2 µl vehicle (DI water) immediately prior to naloxone-precipitated withdrawal. Astressin significantly attenuated withdrawal-potentiated startle on Day 2, but not Day 1 of acute morphine exposure. A separate experiment found no significant effect of astressin on startle in non-withdrawn, drug free animals, demonstrating that the effects of the antagonist were due to its action on specifically the withdrawal state. These results suggest that there is a recruitment of the CRF system as dependence escalates from repeated exposures to an opiate, and that it takes only a single dose for this plasticity to occur. Understanding these initial changes will aid in developing more effective therapies for individuals in different phases of drug dependency.

**77**

**OREXIN A-INDUCED FEEDING AND ACTIVITY: ROLE OF THE LOCUS COERULEUS**

Amber L Martell (Cathy Kotz)

Department of Food Science and Nutrition, Minnesota Obesity Center, University of Minnesota

Obesity and associated health problems are today becoming the number one health problem. Some individuals appear to be more resistant to weight gain, even when consuming the same amount of food and engaging in similar physical activity levels as those who are overweight. These obesity-resistant individuals appear to expend energy that is not accounted for by their basal metabolic rate or by formal physical activity. The brain and associated neurotransmitters/neuropeptides regulate feeding behavior and energy expenditure. Orexin A (OXA) is produced in neurons of the lateral hypothalamus (LH), which project to many areas of the brain involved in feeding behavior and physical activity. When injected into the LH and the ventricular system, OXA causes short-term hyperphagia and enhanced physical activity, whereas OXA loss or loss of its receptor is associated with narcolepsy, a disorder of REM sleep. The phenotype of OXA knock-out models is obesity, which results from inadequate energy expenditure due to lack of physical activity in these animals. These findings indicate that OXA is important to maintaining physical activity; however, knowledge of brain mechanisms regulating physical activity is scarce. The locus coeruleus (LC) is an area in the hindbrain involved in activity with heavy projections from OXA-containing LH fibers. We hypothesized that the LC is vital to OXA-induced activity, but not to OXA-induced feeding. To test this, we lesioned, or 'deactivated' the LC, then injected these animals with OXA and measured feeding and activity. Our data show that deactivation of the LC produced no change in activity or feeding produced by OXA, suggesting that the LC is unrelated to OXA-induced feeding and activity.

**78**

**THE ROLE OF N-WASP IN CELL MOTILITY**

Kristyn E. VanderWaal (Lorene Lanier)

Department of Neuroscience, University of Minnesota

The neuronal growth cone is the key structure for finding and making synapses in developing neurons. Growth cones follow specific cues in order to move to the final location of the synapse. The mechanism of this movement is important for understanding neuronal pathfinding and eventually in artificially regrowing neurons. Motility is caused by the interaction of actin in the cytoskeleton with the actin binding protein Arp2/3. Neural Wiskott Aldrich Protein (N-WASP) is then necessary to activate Arp2/3; however, the exact method of activation is not well understood. My experiment attempts to discover whether N-WASP is the only protein necessary for activation of Arp2/3. Since N-WASP must be activated by phosphorylation, our plan is to mutate N-WASP's phosphorylation site so N-WASP cannot interact with Arp2/3. Our hypothesis is that when the site is mutated, Arp2/3 will not function normally. To study N-WASP, two types of motile cells were used: fibroblasts and neurons. N-WASP acts differently in each type of cell, although fibroblast motility is better understood. In order to observe N-WASP in living, moving cells, a cyan fluorescent protein (CFP) was fused to N-WASP. Then, this fusion protein was mutated to mimic phosphorylation. Finally, the mutant and wild type fusion proteins were studied in both fibroblasts and neurons using transfection and recombinant adenoviruses.

## 79

### **BRAIN GENE EXPRESSION WITH STATIN DRUG TREATMENTS**

John B. Capen, Leslie N Johnson, (W. Gibson Wood)  
Department of Pharmacology, University of Minnesota

Cholesterol is an essential molecule for life. It is the precursor to steroids, hormones and a vital component of eukaryotic cell membranes. Although this molecule is necessary, it is also the focal point for many health problems. High cholesterol (hypercholesterolemia), has been associated with coronary heart disease, heart attacks, and strokes. Statin drugs lower cholesterol levels by raising HDL concentrations while lowering triglyceride and LDL concentrations. These drugs inhibit HMG CoA-Reductase from converting HMG CoA into Mevalonic Acid (the rate limiting step in cholesterol biosynthesis). By inhibiting these enzymes, the statin drugs help to lower cholesterol in the body. In the last few years, there has been a noted link between cholesterol levels in the brain and Alzheimer's disease. Various studies have shown that mice with high cholesterol diets, high cholesterol biosynthesis in the hippocampus, or subjected to both of these controls have an increase in Alzheimer's disease like symptoms. Treatment of these mice with statin drugs has worked effectively to slow the progression of Alzheimer's disease as well as delay onset. In our experiment, normal female mice (C57Bl6) were given Simvastatin, Lovastatin, or Pravastatin for 21 days, sacrificed (via cervical dislocation), and the brains were analyzed via microarray for changes in gene expression levels. Upon collection of data, four genes: *igfbp3*, *mct2*, *npylr*, and  $\alpha$ -hemoglobin, were selected to check accuracy of the microarray. RT-PCR was performed from mice brains and resolution was done via gel electrophoresis. It was determined that these drugs induced a change in the expression level of many genes in the brain. The four genes analyzed were all up-regulated and known to be neuro-protective. Though further investigation is needed, these data lead to the possibility of the use of these drugs as treatments for Alzheimer's disease, or as a possible means of prevention.

## 80

### **THE 2A6 GENE AND NICOTINE METABOLISM**

Catherine Pham (Mary Dempsey, Sharon Murphy)  
Department of Biochemistry, Molecular Biology, and Biophysics, Cancer Research Center, University of Minnesota

Smoking and use of tobacco products are key risk factors for a number of cancers. Over 85% of lung cancer deaths, or 132,000 deaths in 2002 alone, are attributable to smoking in the U.S (American Cancer Society, 2002). Many smokers are interested in quitting but find that it is extremely difficult to quit despite repeated cessation attempts. Nicotine is well recognized as the major addictive agent in tobacco products. In smokers, nicotine is rapidly and extensively metabolized. Nicotine clearance occurs primarily through its conversion to cotinine. One of the key enzymes involved in the metabolism of nicotine is cytochrome P450 2A6 (gene name CYP2A6), which is a good catalyst of nicotine 5'-oxidation that leads to the formation of cotinine. Recently, a number of gene polymorphisms were detected in CYP2A6. Based on preliminary evidence, investigators hypothesize that well known differences in the percent conversion of nicotine to cotinine in individual smokers may be explained, in part, by gene polymorphisms in CYP2A6. In at least one case, where a CYP2A6 polymorphism leads to no P450 2A6 product, there is clear evidence nicotine metabolism is altered. Ultimately, further characterization of CYP2A6 polymorphisms in different populations will contribute to our understanding of nicotine metabolism and may provide insight about observed variation in both susceptibility to nicotine addiction and risk for smoking-related cancers.

**81****MONITORING URINARY GLYCOSAMINOGLYCAN EXCRETION IN EXPERIMENTAL MICE USING AN AUTOMATED METHOD**

Christopher M. Erickson (Chester B. Whitley)

Department of Pediatrics, Gene Therapy Center, and Institute of Human Genetics, University of Minnesota

Mucopolysaccharidosis (MPS) diseases are genetic lysosomal storage disorders caused by the body's inability to produce certain enzymes. This enzyme deficiency produces storage of excess cell deposits, leading to progressive cell damage. The main obstacle to successful treatment is early diagnosis. However, the development of an automated method measuring urinary glycosaminoglycan (GAG) excretion has raised the possibility for newborn screening (Whitley *et al.*, Clin. Chem. 35:2074-2081, 1989). A high urinary GAG level corresponds to a lack of enzymatic breakdown, and a signal for an MPS disease. We hypothesized that like past studies with human specimens, this method could be used to distinguish normal from MPS subjects in mice. We also explored urinary GAG levels in experimentally treated MPS mice. A direct 1,9-dimethylmethylene blue method for quantifying GAG, originally developed for MPS patients, was used. The results in the table below show large differences in urinary GAG (expressed as milligrams GAG per gram creatinine) for normal and affected human specimens. The results for mice also indicate significant differences in urinary GAG for normal, untreated, and treated MPS mice. Therefore, this automated method may be beneficial in following treatment in experimental MPS mice by quantifying urinary GAG. The effectiveness of this test further supports its use both for mice, and potentially for newborn screening in the future.

	#subjects	#samples	Avg. mg GAG/g Cr	Range
Human (Normal)	7	26	9	0-52
Human (MPS Affected)	20	47	355	22-2,018
Mice (Normal)	7	29	59	0-183
Mice (MPS Affected)	15	66	322	38-2,907
Mice (MPS Treated)	9	32	146	0-782

**82****TIME AND TIDE WAIT FOR NO CRAB: MANIPULATION OF CIRCATIDAL RHYTHMS IN AN INTERTIDAL FIDDLER CRAB**

Jayna DeVore, (Frank Barnwell)

Department of Ecology, Evolution, and Behavior, University of Minnesota

Biological rhythms are integral to the timing of many crucial functions. Circadian (~24-hour) rhythms keep organisms in synchrony with the day-night cycle and are commonly entrained (set) by light. Circatidal rhythms (~12.4 hours) are used by animals that live in the intertidal zone to program their activities to anticipate the changing stages of the tidal cycle. I studied circatidal rhythms in an intertidal fiddler crab, *Uca tetragonon*. Members of this species are rhythmically active at the time of low tide, but it is not known how their rhythms are entrained to the low tide phase of the tidal cycle. It was found that crabs that had been freshly collected from a beach with low amplitude (>.5 meter) tides exhibited a weak tidal activity pattern in the lab that aligned with the times of low tide on the home beach. Crabs that were maintained in non-tidal aquariums for two months continued to exhibit this same pattern when placed in activity recorders. However, crabs kept in a 12.4-hour artificial tidal aquarium developed strong circatidal rhythms, and those kept in 12.0-hour tidal aquarium showed 12.0 hour rhythms. Both of these patterns were much pronounced than the tidal activity patterns of freshly collected crabs whose circatidal activity had not been reinforced through the use of the artificial tides. Interestingly, the circatidal activity rhythms produced by treatment in the tidal aquariums were found to be entrained to the time of the artificial high tides. As this is the opposite of what occurs in nature, it raises the question of what factor of the artificial tide is entraining circatidal activity patterns. Further work to identify this factor may provide insight into the entraining factor for crabs in nature.

## 83

### **DTA-1: AN ANTIBODY AGAINST GLUCOCORTICOID-INDUCED TNF RECEPTOR INCREASES CLONAL EXPANSION INDEPENDENTLY OF CD28-MEDIATED CO-STIMULATION AND REGULATORY CD25<sup>+</sup>CD4 T CELLS**

Richard E. Osness, Jr. (Alexander Khoruts)

Department of Medicine, University of Minnesota

Glucocorticoid-induced TNF receptor (GITR) has been recently identified as a direct T cell costimulatory molecule that may also be involved in the suppression mediated by regulatory CD25<sup>+</sup>CD4 T cells. However, the effects of GITR stimulation on the immune response in vivo are currently largely unknown. We used the TCR transgenic adoptive transfer system to measure the responses of antigen-specific CD4 T cells in vivo in the presence or absence of DTA-1, an agonistic monoclonal antibody specific for GITR. Stimulation of GITR promoted cell cycle progression and recall antigen responsiveness of antigen-specific CD4 T cells. The antibody was an effective adjuvant in normal mice and mice lacking regulatory CD25<sup>+</sup>CD4 T cells. Thus its dominant effects were mediated by direct targeting of the responder CD4 T cells. Surprisingly, we found the expression of GITR on responder CD4 T cells and effects of GITR stimulation on their proliferative response to be independent of CD28 costimulation. However, survival signals delivered by GITR stimulation were at least in part CD28-dependent. The costimulatory properties of the GITR molecule may aid in future vaccine development.

## MENTOR KUDOS ...

### Agronomy and Plant Genetics

For Paul Porter,  
Thank you very much for all your help and support. I have enjoyed working with you and I value your feedback and input. Thank you.

~ Lillian Magidow

### Animal Science

For Douglas Foster,  
Thank you for all your help through this whole process. There's no way I could have finished this all on time without you.

~ Meghan Richardson

For Douglas Foster,  
Great thanks for being welcoming, quick witted, motivational, understanding of my bold independence on this project, and for being the greatest director behind the scenes of this complicated film. You truly are the silent father of the lab.

~ Eileen Kerkhoven

### Biomedical Engineering

For David Odde and Koby Nahmias,  
Thank you for allowing me an opportunity to do research in your lab, for acquainting me with the scientific process, and for your support throughout my research. Working in your lab has provided me with a newfound respect and appreciation for the pursuit of knowledge.

~ Abhinav Arneja

For Robert Tranquillo,  
Thank you for giving me the opportunity to work in a research laboratory. I have gained valuable knowledge about lab and its protocols that I would not have otherwise.

I hope that one day I will be able to use this knowledge to improve on the research I perform myself. Thanks for everything!

~ Ta-Chun Hang

### Biochemistry, Molecular Biology, and Biophysics

For Howard Towle,  
As a beginning scientist, you stumble, get discouraged and frustrated. I felt same but thanks to the unquavering support of Professor Towle, I have been able to endure and understand that science is easier said than done. You were there to discuss the problem with me, suggested different review articles and you taught me how to handle the unforeseen. You have been wonderful. You will even take us out for lunch and I have been fortunate to have you.

~ Fausta Ditah

For Claudia Schmidt-Dannert,  
Thank you for the opportunities you have provided and the introduction to the fascinating world of metabolic engineering. I have learned a great deal this year and am honored to have worked under your guidance. For Kevin Watts, You are a gifted scientist, a fabulous mentor, and an extraordinary teacher. It has been a privilege. Thanks.

~ Lisa Lenarz-Wyatt

For Howard Towle,  
Dr. Towle was the greatest mentor one could ask for...always willing to help and answer questions!

~ Mohamed Moussa

Biochemistry, Molecular Biology, and Biophysics, cont'd

For Dave Bernlohr,  
I thank you for taking this advisor-less transfer student under your wing. Though I am new to the lab, you guide me and have faith in me, as though I have worked here longer. I have already learned so much, and I look forward to the opportunities for learning that the future holds. Most of all, I thank you for instilling an interest of science and research in me. What I learn in your lab will be invaluable to my future. Thank you.  
~ Joshua Wilson-Grady

For Howard Towle,  
I would like to give my deepest appreciation to Dr. Towle for his guidance and tremendous support. Dr. Towle is truly the best mentor, academically and personally. What I have learned in his lab will become one of the most valuable assets in my life. I will never forget how he helped me go through the difficult times and I will treasure the joys we have shared with Lin, Brennon, Niko, Angela, and Fausta. Dr. Towle's encouragement and inspiration have helped me get closer to my goals. I wish this mentorship could last a life time.  
~ Xiaosong Liu

For Janet Schottel,  
Thanks for being there in lab throughout my misadventures with malformed gels, buying chemical reagents, and trying to get the digital camera to take a picture. I really appreciated this opportunity and without your help I am sure I would have had many more stories/problems.  
~ Jason Motl

To Sharon Murphy,  
Sharon has shown me how to persevere and continue when frustrations and other obstacles lie in the path toward future goals. Her guidance and encouraging words in the laboratory have affected my outside life as well. Thank you Sharon for being a great mentor.  
~ Catherine Pham

CBS

For Kathryn Hanna,  
I greatly appreciate Kathryn Hanna for helping me become more detail oriented. It has made my research more precise and emphasized the useful information by eliminating clutter.  
~ Ryan Laux

Chemistry

For Gianluigi Veglia,  
MIL GRAZIE PER TUTTI, IL MIO CAPITANO!  
~ Kim Ha

For Mark Distefano,  
I thank Mark for the invaluable opportunity to become involved with work in his lab for the past two years (biological chemistry, protein preparation, and cancer research). I have learned many valuable skills and the importance of research. You have been an excellent mentor, instructor, and advisor. I appreciate your understanding of and assistance with my undergraduate education and long-term goals in life. My utmost thanks also to Dr. JuHua Xu, Dr. Tamara Kale, Laila Albers and the rest of the Distefano group for their encouragement and support.  
~ Benji Mathews

### Chemistry, cont'd

For Mark Distefano and Lab,  
I am thankful to have had this research opportunity; my undergraduate education would have been incomplete without it. To Mark Distefano, thank you for your expertise, encouragement, and the support you have continually shown for me. To Amy Tann, I am very appreciative for your unrelenting patience and the countless hours you devoted to assist me with this project and further my understanding. Finally, to everyone else in lab, thank you for creating a positive environment; it has been a pleasure working beside such great people!

~ Matthew Reeves

### Clinical and Population Sciences

For Erin Malone and Eli Hendrickson, I would like to thank Dr. Malone and Dr. Hendrickson for their time and patience throughout the research project. This experience has been invaluable, and I am very grateful to both of you. I couldn't have done it without you!

~ Molly Welle

### EEB

For Craig Packer,  
I would like to thank my advisor and mentor Dr. Craig Packer. I have worked with Craig as an undergraduate research assistant since 1999. During this time Craig has given me guidance, support, and friendship. Thank you for giving me everything I needed, exactly when I needed it. I could not have asked for a better mentor.

~ Holly MacCormick

For Jennifer King,

Thank you for this incredible opportunity. I have learned more from you these past two semesters than I ever could have imagined. Thank you for always guiding me but never pushing. You always made me feel that I had total control and ownership over this project. That allowed me to feel passionate and excited about it. You are an incredible role model, scientist, and teacher who has impacted my life in so many ways. Thank you.

~ Elizabeth Brodeen

### Fisheries, Wildlife, and Conservation Biology

For Andrew Simons and Tony Gamble,  
Thanks for the wonderful opportunity. I have developed many skills that I will need in the future.

~ Krsna Rangarajan

For Andrew Simons, Jacob Egge, Pete Berendzen, Tony Gamble, and Brett Nagle,  
I could not have done any of this without them.

~ Michael Bush

For Jim Perry and Mark Williams,  
I want to thank you for your patience, guidance, expertise, and allowing me to pursue my dreams. Thank you for making the sky within my reach and making possibilities seem limitless. No matter where life takes me, I'll always think of you starting me in the right direction. I've learned a tremendous amount of biology and laboratory skills and cannot wait to continue this research with you!

~ Kelsey Dahl

*Fisheries, Wildlife, and  
Conservation Biology, cont'd*

For Anne Kapuscinski,  
I have known Anne for over two years now. She has been a wonderful mentor, supervisor, and friend to me. She has helped me identify my goals and taught me to achieve them. Thank you Anne for believing in me.

~ Ozge Goktepe

*Food Science and Nutrition*

For Franciso Diez-Gonzalez,  
Thank you very much for your willingness to let me pursue this research project in your lab. This has been an enjoyable and valuable experience.

~ Julie Kuruc

For Ted Labuza,  
Thank you so much for the opportunity to work in your lab and on this particular project. I have gained so much knowledge that is helping me now and I know will help me later in life. Thanks again!

~ Kellie Leinen

For Cathy Kotz,  
Thanks for the great opportunity to be involved in research. I appreciate your time and advice and support for all my projects. Andy Thorpe, thanks for teaching me everything I know about research. I appreciate all your time, concern, and advice! Kevin Silverstein, thank you so much for all your time, patience, and advice on graduate schools. I have enjoyed learning some of the skills in bioinformatics and hope to utilize them in the future.

~ Amber Martell

*Genetics, Cell Biology, and  
Development*

For Mary Porter,  
Thank you for giving me the opportunity to do research in your lab. The experience has taught me more than I can ever learn in a classroom, and I will always be grateful for it. I have had a wonderful experience working with the lab these past two years.

Thanks again.

~ Irene Dorweiler

For Michael Simmons,  
I would like to thank Dr. Simmons for providing me with the opportunity to do research with him in his lab. You were kind, helpful, and very patient explaining the ins and outs of the experiments. I learned a lot by working with you. Thank you for the memorable and rewarding experience.

~ Abraham Gol

For Deanna Koepp,  
I'd like to thank you for your patience in helping me learn safe laboratory technique. I know there were times I had you worried, but I'm glad you have a sense of humor. Thank you for making my research experience memorable and enjoyable!

~ Ben Miller

For Pete Magee,  
My first year at the University of Minnesota, Dr. Magee's course, Heredity and Human Society, confirmed my decision to major in Genetics, Cell Biology, and Development. Now as a senior, I feel very privileged to complete research in his lab. I greatly appreciate Dr. Magee's guidance and support. This project has given me greater confidence in pursuing research in the future. Thank you!

~ Charissa Lewis

GCD, cont'd

For David Largaespada and Steve Wiesner,

I would like to thank both of you for allowing me to be a part of this research. The past year has been a great experience and I have learned a tremendous amount. You have both been great role models and I admire your knowledge and dedication to this research. Steve, I can not thank you enough for your patience and support. I appreciate the time you have spent teaching me everything I need to know in lab and for always being there to help when I have questions or concerns. Thanks again! Sincerely, ~ Jamie Jones

For Kathleen Conklin,

Working in your lab was a great learning experience. It opened the doors of the real world of science to me, and the amount I absorbed was beyond what I thought I could comprehend. Today, due to that experience, I feel well-trained and confident to step out into the world and utilize my knowledge and skills. I know that I have brought exciting results at times and failures at others. But in the end, I would just like to thank you for taking me in with no experience. The lab was truly a home away from home for me!  
~ Meenal Kapoor

For David Largaespada,

Since I joined the lab two years ago, I have learned one very important lesson: getting things to work 50% of the time is really good and 10% ain't bad either. I have felt privileged to have a mentor like David who has given me projects that play to my strengths and have the potential to turn into papers. He has given me the opportunity to use the science I have learned in class in a work environment. I would like to thank David for these things

and for always being the guy who chips in a little extra cash at Happy Hour.

~ Paul Lobitz

For Jeff Simon,

Thank you so much for allowing me to be a part of your lab for the past few years. It has been one of the best parts of my experience at the University of Minnesota. I have really enjoyed experiencing the highs and lows of research with you and the great people in your lab. Thanks for sharing your advice and knowledge with me. I have learned so much and am excited to keep learning for the rest of my life. Thanks! ~ Sarah Malmquist

For Ross Johnson,

Thank you for being my research advisor, mentor, and friend. My time in lab was incredibly fun and intellectually stimulating. I appreciate the time you spent guiding and teaching me. Thank you for everything!  
~ Andrea Zins

For David Largaespada,

I would like to thank Dr. Largaespada for the opportunity he has provided me by allowing me to work in his lab. It has been a great experience, one I will cherish for a long time to come. I would like to thank him, more importantly for helping me make one of the most crucial decisions, till date, concerning graduate school and future career goals. I would like to extend my gratitude to my supervisors Lara Collier and John Ohlfest for their support and patience. ~ Shruthi Ravimohan

For Deanna Koepp,

Thank you for giving me the opportunity to do high caliber research in a high caliber lab. I never knew that ubiquitin could be so interesting. You have truly made my experience at the University an enjoyable and memorable one. ~ Remy Wong

### Kinesiology

For Jurgen Konczak and Lab,  
Thanks for teaching me the valuable skill and knowledge related to research. I enjoy working with you all and the smell of coffee in the lab!  
~Mandy Cheuk-Man Wong

### Laboratory Medicine and Pathology

For Cheryl Neudauer,  
I would like to thank Cheryl, from the bottom of my heart, for being my mentor, my advisor, my teacher and my friend. I am incredibly thankful for everything you have done for me. You are the one person that has made the biggest impact in my undergraduate life, helping me grow both personally and educationally! Thank you!!!  
~ Teodora Platikanova

### Medicinal Chemistry

For Rick Wagner and Patrick Hanna,  
I would like to thank you both for your guidance, flexibility, and humor. Working in your lab this past year has been the most valuable and influential part of my undergraduate education. I would also like to thank the other members of the Wagner-Hanna lab for all of their assistance and patience. Thank you all!  
~ Caleb Bates

For Carston Wagner,  
Thank you for allowing me the opportunity to work in your lab, and for your patience.  
~ Stephen Hinkin

For Carston Wagner and Jonathan Carlson,  
Dr. Wagner, Thank you for the mentorship and guidance you've provided throughout this project. Your sincere effort to cater

a research experience to undergraduates has been successful and invaluable. I appreciate the experience and lessons I've gained under your supervision. Jonathan Carlson, I consider myself extremely lucky to have had a mentor such as yourself in this endeavor. Your patience and tutelage in conveying your impressive experience and knowledge is appreciated more than you know. I am grateful to have you as a friend and colleague and wish you the best of luck.  
~ Jessie Kerns

### Medicine

For Craig Henke,  
I would like to take this opportunity to thank my mentor for all of the help and encouragement that he has given me throughout my research. I have thoroughly enjoyed working in a lab as a result of the great atmosphere and tone your lab provided me! I appreciate your understanding of and assistance with my undergraduate education and long-term goals in life.  
~ Nima Estharabadi

For Keli Hippen,  
Thank you for the invaluable opportunities and experiences you have given me this past year. It has given me an entirely new way of thinking and I am so enthralled to be a part of your lab.  
~ Katie Lee

For Peter Bitterman,  
It has been a very wonderful experience working in Dr. Bitterman's lab, and I am looking forward to the challenges that lie ahead.  
~ Hong-Yiou (David) Lin

### Medicine, cont'd

For Clifford Steer,  
Thank you Cliff for all that you have done for me. You are one in a million! I couldn't be more grateful for finding a mentor like you. Thanks again!  
~ Carolyn Presley

For Patricia Tam,  
Thank you so much for the opportunity to do research in your lab. Thank you for all of your help with finding a project, developing a protocol, and learning all of the procedures necessary. I would also like to thank everyone in the lab for all of your help throughout the last year. Thanks!  
~ Wade Schulz

For Catherine Verfaillie,  
I would like to thank Dr. Verfaillie for giving me the opportunity to do research as a high school student. It's been absolutely amazing to work at the Stem Cell Institute. Dr. Verfaillie has been an inspiration for me, and it has been a tremendous honor to work for her.  
~ Nicole Ali

For Wufan Tao,  
I would like to thank my mentor, Dr. Wufan Tao, for his support, encouragement and excitement in directing my research. It has been a great pleasure to work with him and his colleagues. I would also like to thank Jing Pai and Hengning Ke for their support.  
~ John Andersen

For Jennifer Hall,  
Thank you for welcoming me into your lab and for taking so much time to explain the background and direction of our work! It has been a challenging experience and I thank you for granting me what Ami calls "a real research experience." I also want to

thank Qinglu, Ami, and Neeta for all their help in the lab. I enjoyed working with you all. Thank you all so much.

~ Esther Kao

### Microbiology

For Ron Jemmerson,  
Over the past year, you have been my employer, professor, and research mentor and I would like to thank you for each opportunity I've had to work with you. The learning experiences I've had in your lab are priceless. I truly appreciate your guidance, patience and flexibility. Thank you.

~ Senit Debesai

For Gary Dunny,  
Thank you for allowing me the freedom to think critically about my project and for your guidance, input, and support. Being in your lab has made me a better scientist.  
~ Kathryn Goeden

For Patrick Cleary and Haesun Park,  
Thank you for giving me the chance to learn so much. I have learned so much in the past few months! Thank you for taking the time to teach me.  
~ Matthew Schaefer

For Ron Jemmerson,  
It has been a privilege to work with you in your lab - your patience and insight are gifts that have enhanced the way I look at science and research. Thank you for sharing your wisdom and enthusiasm with me!  
~Harmony Tyner

### Neuroscience

For Lorene Lanier,  
You have been the best mentor anyone could ask for! Thank you so much for your individual attention, your advice, and the chance to do my own project in your lab. I have learned so much from you and I will remember this experience my entire life.  
~ Kristyn VanderWaal

### Ophthalmology

For Deb Ferrington,  
Thank you for all of your support and encouragement over the past few years. I've learned so much from working with you that classes alone cannot teach. I've also learned to never give up, and to always keep a positive outlook. Your example has made such a positive impact on my life, I cannot even begin to say thank you.  
~ Kristin Berg

### Pediatrics

For Chester Whitley,  
I cannot thank you enough for all the knowledge and experience I have gained with my time in your lab. I greatly appreciate all the guidance you have given me on the road to medical school. Thank you very much for all of your support and confidence in me! Sincerely,  
~ Christopher Erickson

For Jamie Lohr and Lab,  
I would just like to let you guys know how much fun I've had in the last year working in the lab. You have shown me what science is really about which is something I never would have learned in the classroom. I appreciate all of the time you have spent and continue to spend teaching me new and exiting aspects of life in, and outside, of

the lab. I have enjoyed getting to know each of you and look forward to spending more time with you this summer. Thanks for such a wonderful opportunity!  
~ Ann Neumann

For Cheryl Gale and Lab,  
I would like to thank Cheryl for helping me get research experience, and for teaching me scientific writing. Also, thanks to Maryam Gerami-Jejad for teaching me molecular biology and techniques and to Mark McClellan and Danielle Hausauer for helping me with Western blotting.  
~ Nicholas Winning

For Cheryl Gale,  
Thank you so much for your help over the past year. Both your help with this project as well as with my graduate school applications have been a tremendous assistance to me. Working with you has been a great addition to my undergraduate experience. Thank you so much for everything! The connection between my lab work and your work in the hospital has helped me connect to the bigger picture. Thank you and Best Wishes!  
~ Cassandra Kistler-Anderson

### Pharmaceutics

For William Frey and Tinna Ross,  
My work in the Alzheimer's research center is my first experience with research. Dr. Ross's, Dr. Frey's, and Elizabeth's patience and willingness to answer my questions have made the experience very memorable and worthwhile. I would additionally like to thank Dr. Ross for "starting from square one" with me and being so willing to teach me. ~ Ashley Lawson

### Pharmaceutics, cont'd

To William Elmquist and Lab,  
I would like to give a special 'thanks' to Dr. Elmquist and all those in his lab who have greatly assisted me in my research effort. Your help and encouragement has helped me realize where my future direction will lie. Thanks a million.  
~ Tate Winter

### Psychology

For Jonathan Gewirtz,  
Thank you for the opportunity to gain a truly exceptional research experience. I have grown tremendously as a result of being a part of your laboratory, both as an independent thinker and as a member of a team.  
~ David Atkinson

### Surgery

For William Engeland,  
Thank you for giving me the wonderful opportunity to be a part of the laboratory! Through your guidance I have learned and gained much experience working in the lab. Thank you for challenging and advising me on various methods of scientific research. This has truly been a great undergraduate experience!  
~ Anu Elayaperumal

For Dr. Dunn, Dr. Wasiluk, Dr. Leslie, Dr. Vietzen, Mike and Karen,  
Thank you all so much for giving me the opportunity to work in your lab. It was through this project that I learned the pain, frustration and excitement of research. I will never forget the guidance and life lessons each of you have contributed. You all made the last year wonderful, despite the tedious

troubleshooting I faced in this project. Thank you for giving me a head start into the real world.  
~ Rania Habib

For Bernhard Hering and Brett Levay-Young,  
Thank you for the opportunity to conduct challenging and cutting-edge research. I am very appreciative of all of your continued support and advice throughout my project. I look forward to working with you in the future. Thanks again!  
~ Forum Kamdar

### Urologic Surgery

For Ken Roberts & Lab,  
I would like to thank you for allowing me the opportunity to get involved with research. This experience has shown me what it really means to be involved in science, and how to get through all its ups and downs. I have gained knowledge and skills I need to become a scientist, as well as an appreciation for what I learned from stories about "the old days." Thank you for the incredible amount of support, patience, and knowledge you have given me. You and everyone in the lab have made my undergraduate research experience wonderful.  
~ Miranda Bernhardt

### Vet Pathobiology

For Michelle Wagner and Jim Mickelson,  
I would like to thank you both for being so helpful and encouraging during the research and writing of my thesis. I greatly enjoyed working with you and learning from you and cannot imagine a better lab in which to have done my research.  
~ Erin Bequette

*Vet Pathobiology, cont'd*

For David Brown and Lucy Vulchanova-Hart,

Dave and Lucy have been wonderful advisors for the past year. I have learned so much from both of them. Thanks for everything you guys!

~ Amanda Helvig

*Veterinary and Biomedical Sciences*

For Pam Skinner,

Thank you for having us do research in your lab. We have had a great experience and learned a great deal of information.

Thanks for all the fun and facts! *In vivo*,

~ Jill Grandt & Ryan Sunderman

For Kent Reed,

Thanks Kent for all the opportunities you've given me. Most undergrads are not presented with publications and conferences. I've learned 10-fold working in your lab.

~ Todd Knutson