

17th Annual Life Sciences Undergraduate Research Symposium



Photo: Elizabeth Vinson-Lonsdorf

Jason Beyer (poster #2)



Photo: Jacqueline Baker

Matthew Heinicke (poster #1)

**Wednesday, April 30, 2003
Earle Brown Center**

**University of Minnesota
St. Paul Campus**

Oral Presentations

1:30-2:00 Rm 155

Poster Session

2:00-4:30 Rm 135

Reception /

Awards Program

4:30-5:30 Lobby / Room 155

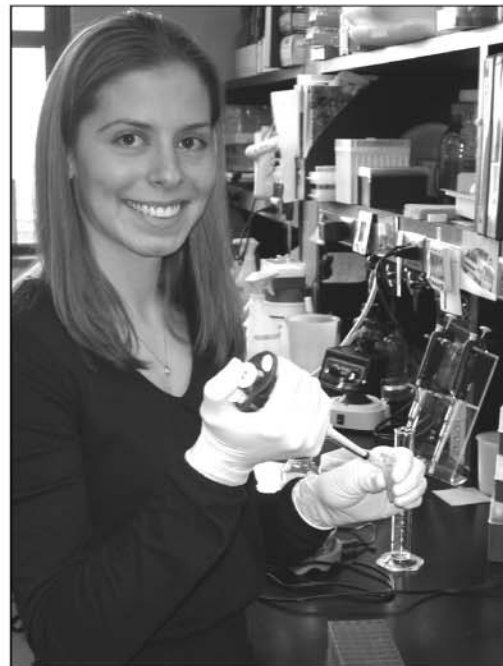


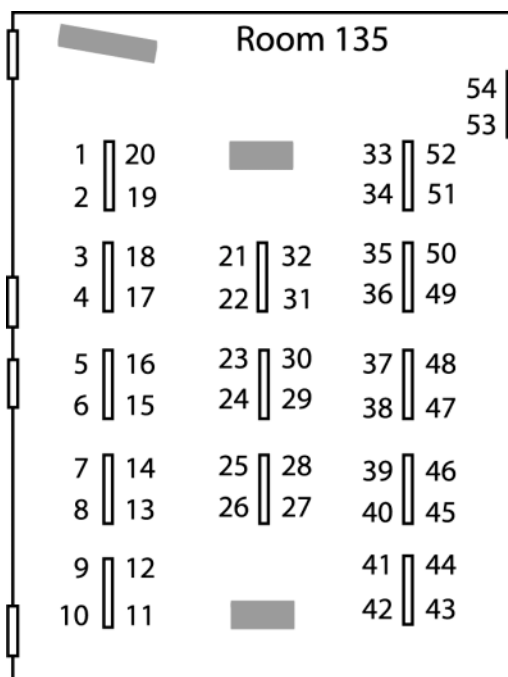
Photo: Rogene Schnell

Jennifer McNabb (poster #25)

COLLEGE OF **Biological
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UNIVERSITY OF MINNESOTA

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Sponsor: David Largaespada, GCD

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ABSTRACTS

01

MORPHOLOGICAL VARIATION IN THE OPTIC TECTA OF *Hyla chrysoscelis* AND *H. versicolor*, THE GRAY TREEFROGS

Matthew P. Heinicke¹ (Daniel J. Meinhardt²)

¹Department of Genetics, Cell Biology, and Development, ²Bell Museum of Natural History, University of Minnesota

A negative correlation between cell size and complexity of the optic tectum of the brain has been demonstrated previously in a sample of a number of species of frogs. Further, genome size has been shown to correlate positively with cell size in frogs. To this point, however, no one has examined if these relationships hold in diploid/polyploid species pairs of frogs. Several cell types are already known to be larger in the tetraploid *Hyla versicolor* (common gray treefrog) than in its externally identical diploid sister species, *H. chrysoscelis* (Cope's gray treefrog). We examined the morphological complexity of the optic tecta in these two species, as well as the size of cells in this region of the mesencephalon in order to determine if the above-mentioned relationships held in the case of a diploid/tetraploid species pair. Any differences between the two species would have implications in differentiating between them as well as demonstrating a case of non-selective morphological evolution. We sectioned, stained, and examined six brains each from *Hyla chrysoscelis* and *H. versicolor* in our study. Morphological complexity of the optic tectum was assessed following ranking systems from previously published studies. Cell size was approximated by measuring the nuclear diameter of 20 cells from each of three cellular layers per specimen. Preliminary results indicate that there is no difference between the two species of gray treefrog in cellular size or in morphological complexity of the optic tectum.

02

SOCIAL IMPACTS ON CHIMPANZEE HUNTING BEHAVIOR

Jason E. Beyer, Ian Gilby, and (Anne Pusey)

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

Chimpanzees (*Pan troglodytes*) hunt red colobus monkeys (*Colobus badius*) on a fairly regular basis. Despite considerable study controversy still exists over the factors that influence hunting behavior. Some studies suggest that male chimpanzees hunt to obtain meat to trade with allies for potential support and with females in exchange for mating. The purpose of this study is to test whether the presence of allies and/or sexually receptive females influences the decision to hunt. Encounters with red colobus are extracted from long hand notes taken from follows of chimpanzees at Gombe National Park. From these encounters I determine the group composition, whether or not the chimpanzees hunted, and if a kill was made. From this I am able to determine whether group composition effects the decision to hunt. At this point, the most influential factor appears to be the number of adult and adolescent males in the foraging party. This provides evidence for the meat in exchange for support from allies hypothesis but could also mean the decision to hunt is based on probability of success. I need to differentiate between these two ideas by testing the effect of ally presence. Another major factor appears to be the presence of sexually receptive females, which would support the meat for sex hypothesis. However, parties containing sexually receptive females tend to be larger because males tend to gather around these females and because these females are more likely to join large parties that have formed for other reasons. To clarify the answer to this question I will need to determine if males are in fact exchanging meat for mating. When my research is completed I will be able to draw definite conclusions regarding how hunting behavior is effected by social factors.

03

AN INVENTORY OF INSECT FAUNAL RESPONSE TO NATURAL BURNS

Tracy Dahl (Susan J. Weller)

Department of Entomology, University of Minnesota

After a forest fire, fire specialist insects recruit to burn areas and attack damaged and stressed trees. Some insects are involved in transmitting plant pathogens, a well-documented phenomenon in coniferous forests. However, only two studies have examined insect faunal response to fires in hardwood dominated tree stands. It is suspected, but not documented, whether oak bark beetles (Scolytidae) are a significant component of a fire response insect fauna in Minnesota. Oak bark beetles are involved in spreading oak wilt (a fungal pathogen), and the presence of the disease hastens the decline of oaks. The goal of this study was to investigate the timing of insect response to burns of known age, and determine whether oak beetles follow other colonizers or precede them.

Forest insects were collected by standard trapping methods summer 2002 in unburned and burned forests dominated by hardwoods. Ten traps were placed in each of two sites, one burned and one unburned. The burned site was 5 weeks post-burn at the time of sampling. Trap-catch insects were sorted to morphological species, identified to family and subfamily when possible, and prepared as scientific vouchers for the Insect Museum (University of Minnesota, St. Paul). Surprisingly, only one oak bark beetle individual was obtained. The numbers of individuals per species were compared between sites using standard statistics. There was a significant change in the species' composition caught in burned and unburned areas. There were many more tree-damaging beetles (Buprestidae, Scolytidae) in traps placed in burned areas. Long horn beetles (Cerambycidae) were nearly equally distributed among sites. The scolytids were pine specialists (*Ips*), not oak specialists, however. These results suggest that traps were set too late to sample fire specialist insects recruiting rapidly to a hardwood burn.

04

EFFECTS OF PH AND CALCIUM LEVELS ON RESPIRATION RATES OF ALASKAN SOILS

Gregory P. Snyder (Sarah E. Hobbie)

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

The effects of pH on soil respiration rates are not well understood. Although there has been much research suggesting that a circumneutral pH promotes higher rates of soil respiration than does acidic pH, this relationship is not always found. In a study done on soils from the Toolik Lake region of Alaska, soils taken from an acidic site (pH=3-4) showed a significantly higher rate of respiration than those taken from a nearby non-acidic site (pH=6-7). A proposed explanation for the unexpected difference in respiration rates involves the high levels of calcium found at the non-acidic site. It is believed that the divalent calcium cations may serve as a kind of cation bridge, holding negatively charged organic matter particles together and slowing their rate of decomposition. We tested that hypothesis by using potassium hydroxide or calcium hydroxide to vary the pH and/or calcium levels of samples taken from both the acidic and non-acidic sites at Toolik Lake. This allowed us to distinguish between the effects of increased pH per se, and the effects of increased concentrations of the divalent cation, Ca²⁺. The samples are being incubated at 4°C (a temperature comparable to soil temperatures found during an Alaskan summer) and respiration is being measured periodically. After 2 months of incubation we have seen that raising the pH of the acidic soils had a significant positive effect on respiration, as expected. However, samples treated with calcium hydroxide show a lower rate of respiration than those treated with potassium hydroxide, suggesting that higher calcium levels slow the rate at which organic matter decomposes compared to potassium hydroxide and partially offset the positive effects of higher pH on respiration. These results are consistent with our hypothesis.

05

PREFERENCE AND POTENTIAL FECUNDITY OF THE MILFOIL WEEVIL (*Euhrychiopsis lecontei*)

Kari A. Eichstaedt, (Florence K. Gleason), and Michelle Marko, Department of Plant Biology, University of Minnesota

Experiments were conducted involving the preference and fecundity of the aquatic weevil (*Euhrychiopsis lecontei*) on the invasive, exotic plant Eurasian watermilfoil (*Myriophyllum spicatum*) and native northern watermilfoil (*Myriophyllum sibiricum*). Female's first oviposition occurred approximately four days after emergence, regardless of which milfoil species they were raised on. Potential fecundity was assessed by measuring the number of eggs per day that Eurasian-reared and northern-reared weevils laid over 10 days. Six stems of Eurasian or northern or both were placed in 3-liter aquaria with one female. Eggs were counted and damaged stems were replaced daily. In no-choice experiments the weevil's rearing plant had a significant effect on the number of eggs females oviposited (ANOVA, $F=5.46$, $p=0.0012$). Eurasian-reared weevils oviposited more than northern-reared weevils, but the milfoil species the weevils oviposited on was not significant. In the choice experiment, Eurasian-reared weevils oviposited more than northern-reared females and both types preferred to oviposit on Eurasian watermilfoil. These results indicate that Eurasian-reared weevils show a preference for and have increased fecundity on Eurasian watermilfoil, and would have increased success as a biological control agent.

06

MORTALITY AND SPATIAL DISTRIBUTION OF THE INVASIVE GREEN CRAB, *Carcinus maenas*: EFFECT OF THE BLUE CRAB

Cecilia A. Scheuerman¹ (Paul Jivoff²)

¹Department of Ecology, Evolution, and Behavior, University of Minnesota, ²Department of Biology, Rider University, Lawrenceville, New Jersey

In a variety of ecosystems, native species interact with non-indigenous species that affect these native ecosystems with their distribution, feeding, and habitat preferences. Understanding current marine invasions and how they are regulated is the most effective way to prevent further invasions in the future. By analyzing a marine species with multiple invasions in a variety of locations, a pattern of ecological constructs can be found to indicate the susceptibility of ecosystems to invasion. The green crab, *Carcinus maenas*, has a history of invasion and is rapidly developing a global distribution. On the east coast of North America, it is hypothesized that the native blue crab, *Callinectes sapidus*, regulates the local and regional distribution of the green crab. I tested this hypothesis, in the Great Bay-Mullica river estuary in southern New Jersey, using field data on the relative abundance of blue crabs and green crabs and a tethering experiment to determine the relative mortality of green crabs in areas of varying blue crab abundance. The field sampling, June-August 2002, show consistent trends of a negative correlation between the abundance of blue crabs and green crabs. Additional sampling (April-August) indicates a temporal shift in green crab habitat use driven by increasing blue crab abundance. In the tethering experiment, green crab mortality increases with escalating blue crab abundance suggesting blue crabs are a significant source of green crab mortality. These data support the hypothesis that blue crabs regulate the local distribution of green crabs and suggest that blue crabs may also regulate the regional distribution of green crabs on the east coast.

07

Managing the Evolution of Insect Resistance to Transgenic Plants

Zeina Dajani (Donald Alstad)

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

With damage and control costs exceeding \$1 billion per year, the European corn borer, *Ostrinia nubilalis*, is the most damaging insect pest of corn throughout the United States and Canada. Crop varieties genetically engineered to express the crystal-like proteins from *Bacillus thuringiensis* have several advantages over unprotected crops or those treated with pesticide varieties currently in use. The specificity and low-toxicity of *Bt* corn to non-target organisms make *Bt* ideal for the control of European corn borers. In order to ensure the continued effectiveness of *Bt* corn as a control agent, it is necessary to manage the evolution of insect resistance. A high-dose strategy coupled with a spatial arrangement of both *Bt* and refuge plots exploits corn borer preferences and movements, resulting in a delay in the evolution of pesticide resistance. Such a strategy rests upon the assumption of random mating between *Bt* and refuge fields. Using the presence of heterozygote deficiency as an indicator of non-random mating, we are currently conducting research in order to delineate the spatial scale of the random mating unit.

Alstad, D. N. and D. A. Andow. 1995. Managing the evolution of insect resistance to transgenic plants. *Science* 268:1894-6.

Alstad, D. N. and D. A. Andow. 1996. Implementing management of insect resistance to transgenic crops. *AgBiotech News and Information* 8:177-181

Ostlie, K.R., W.D. Hutchison and R.L. Hellmich. 1997. *Bt Corn and European Corn Borer: Long-Term Success Through Resistance Management*.

08

CONTRIBUTION OF FOUNDER PRONGHORN TO PRESENT PRONGHORN COLLECTION AT THE MINNESOTA ZOO

Nicole L. Untener (Kathryn Hanna)

College of Biological Sciences, University of Minnesota and Minnesota Zoological Gardens

It's important in captive breeding populations to avoid inbreeding. When the Minnesota Zoo breeds animals they pick out the animals that will represent the wild population best. This study was conducted to help the zoo determine how much of the founder population of *Antilocapra americana* (pronghorn) is still present in the current group. Reproductive histories of the eight founder *Antilocapra Americana* were examined. In addition two imported male *Antilocapra americana* pedigrees were examined and treated as founders because their genes were new to the group. Founder pedigrees were compared to the nine pedigree reports of the current *Antilocapra Americana* population. Two out of the eight founder's genes were lost. They were animals 1958 and 1959. A determination of the fraction of the founder *Antilocapra americana's* genetic material that was in each of the present pronghorns was made by looking at each of the present *Antilocapra americana's* family trees. Using this pedigree analysis the Minnesota Zoo can see which of the founders have been lost and breed the animals accordingly, while taking other factors like sex and age of the animals into account.

09

PRELIMINARY ESTIMATES OF GENETIC DIVERSITY OF THE GOLDENROD FLY (*EUROSTA SOLIDAGINUS*): ONE SPECIES OR TWO?

Sarah A. Endrizzi (Susan J. Weller)

Department of Entomology, University of Minnesota

The purpose of this project is to determine the genetic diversity of the goldenrod fly, *Eurosta solidaginus* (Tephritidae) for upper Midwest populations and compare it to a sister species, *E. floridensis*. Standard DNA extractions, PCR amplifications and automated sequencing were used to sample portions of the mitochondrial gene COI (666 bp) for 14 *E. solidaginus* individuals. Eleven individuals were sequenced for a 550 bp region of the nuclear gene EF-1 α . These flies were reared either on the goldenrod *Solidaginus altissima* or *S. gigantea*, and their hosts were located in either prairie or forest habitats. I found that EF-1 α is invariant within the goldenrod fly. The maximum COI sequence divergence was 1% among *E. solidaginus* individuals and it was 6% to the outgroup species *E. floridensis*. Sequences had typical insect AT bias, with an A:C:G:T ratio of 32%:14%:13%:40%. A phylogenetic analysis of the COI-COII region showed that mtDNA haplotypes track goldenrod host plant species not habitats. The flies obtained from *S. gigantea* hosts cluster together, however, the *altissima* flies do not. The data suggest that population structuring consistent with host race divergence has occurred, but are not definitive.

10

AFLP DIVERSITY STUDY OF WILD POTATO *SOLANUM*, SERIES *BULBOCASTANA*

Ryan L. Syverson, Marie J. Sanchez, and (James M. Bradeen)

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Approximately 225 tuber-bearing potato species are known to exist in the genus *Solanum*. The diploid series *Bulbocastana* (RYDB. Hawkes) includes 2 species: *S. bulbocastanum*, with 3 subspecies, and *S. clarum*. *S. bulbocastanum* evolved along side the late blight pathogen *Phytophthora infestans*. Consequently, *S. bulbocastanum* is a potential source of disease resistance genes for cultivated potato. In support of our ongoing efforts to characterize and isolate disease resistance genes for potato protection, we examined molecular diversity within *S. bulbocastanum* using Amplified Fragment Length Polymorphism (AFLP) markers. This study analyzed 17 populations, with 3 to 4 genotypes per population, of the wild diploid outcrossing species of the series *Bulbocastana*. Of these, 8 populations were *S. bulbocastanum* ssp. *bulbocastanum*, 6 were *S. bulbocastanum* ssp. *dolophilum*, and 2 were *S. bulbocastanum* ssp. *partitum*. One population was the species *S. clarum*. The data generated from the AFLP analysis were used to examine these populations in three ways: to determine the level of genetic similarity between the species within series *Bulbocastana*, to examine the taxonomic structure of *S. bulbocastanum* and its subspecies, and to analyze the effects of geographical location on inter-population and intra-population differences. Based on previously published results from a related wild diploid outcrossing species using Random Amplified Polymorphic DNA (RAPD) markers, we expect to find greater intra-population variation than inter-populations variation and poor correlation between geographical origin and genetic diversity. Research results will be presented.

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FUNCTIONAL ANNOTATION OF THE PORCINE SECRETOME

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Secreted proteins mediate both short-range and long-range intercellular signaling during the development and growth of multi-cellular organisms. Therefore, the livestock "secretome" is important for a diverse set of biological processes critical to production animal performance, and is a high-priority target for functional-annotation and gene-expression analysis. We propose to identify and annotate the porcine secretome using bioinformatics, biochemical, and embryological techniques. A "Secretome Workbench" will be developed and porcine EST consensus sequences will be subject to bioinformatic analysis to predict those encoding secreted proteins. cDNA clones corresponding to putative secreted proteins are being isolated from public porcine libraries for full-length transposon-based sequencing. In addition, because secreted proteins are co-translationally inserted into the endoplasmic reticulum, they are protected from proteolytic degradation when translated in the presence of microsomes. This provides a biochemical method for verifying our clones encode *bona fide* secreted proteins. Knowing what secreted proteins are expressed during pig production and reproduction will have important implications for the sustainability of the U.S. pork production by contributing to the improvement of animal performance by genetics or modified production methods.

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GAP JUNCTIONS: WHAT IS THE FUNCTION OF CONNEXIN 43 HEMICHANNELS IN DYE UPTAKE?

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Connexins are transmembrane proteins and the principle constructs of cylindrical channels on the cell surface called "connexons". A Connexon may function as an independent hemichannel, or dock with a second connexon and assemble into a gap junction. "Gap junctions" are collections of cell-to-cell channels that facilitate metabolic activities by direct exchange of ions and small molecules between the cytosolic domains of adjacent cells. This exchange via assembled connexons plays a role in patterning development, regulating growth, and transmitting impulses. The functions of connexons as independent hemichannels are unknown. My research involves developing methods to study independent hemichannel function. Investigations I perform involve hemichannels from wild type, mouse fibroblasts constructed from connexin 43 (Cx43). Cx43 is a transmembrane protein of 43,000 Daltons shown in prior experiments to be responsible for dye uptake into the cell upon mechanical stimulation in a low calcium environment. Study of functional inhibition of this dye uptake involves antibodies targeted to specific polypeptide chains in the extracellular domain of Cx43. My protocol involves applying 5(6)-carboxyfluorescein dye from a height of 25 mm (mechanical stimulation) to a culture plate with antibody treated, wild type fibroblasts expressing Cx43. Antibody treatments are added to cell cultures in various ratios to monitor concentration dependence, and fluorescent cells are scored under a microscope to determine the percentage of cells displaying dye uptake when compared to non-antibody treated control cell cultures. My results with the antibodies show a strong dependence on antibody concentration, with varying results based on the use of different kinds of antibodies. My goal is to demonstrate complete antibody inhibition of uptake for potential applications in several areas including: a.) hemichannel transport; b.) hemichannel function; and c.) the process by which gap junctions are formed.

13

QUANTITATION COMPARISON OF TOTAL RNA LEVELS AT DIFFERENT AGES OF ADULT *Drosophila melanogaster*

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Previous studies on the correlation between aging and total RNA levels in *Drosophila melanogaster* indicated that total RNA levels (rRNA, tRNA, and mRNA) decline substantially with age (N.M.A. Tahoe and J.W. Curtsinger, unpublished). Total RNA was extracted from 30 long and normal-lived flies at ages 5, 13, 20, 33, 45, 55, 60 and 63 days post-emergence, with replication. It was found that in the latter ages, the RNA decline was more than 50% of initial levels. A sudden decline in RNA was detected in the earlier ages, 3 to 13 days, and a gradual decrease was found in the latter ages, 13 to 63 days post-emergence. Data also suggested that there was no correlation between longevity and total RNA levels; both long and normal-lived *Drosophila* have similar pattern of age-related RNA decreases.

The aim of this directed research project is to employ new RNA quantitative measurements, RiboGreen and Agilent 2100 Bioanalyses, to investigate the accuracy of previously obtained UV RNA concentration measurements. The reproducibility of each technique, as well as DNA contamination was also investigated. We have found that RiboGreen measurements were more reproducible and agreed well with the UV data. Concentrations from Agilent were found to be less reproducible and consistently low. This technique however, had the advantage of evaluating the integrity of RNA samples. Presence of two ribosomal peaks was evident in each RNA sample. These data confirm the usefulness of both techniques in the analysis of RNA samples and may help in understanding of the aging process.

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HEAD-DIRECTION ENSEMBLES RECORDED FROM AWAKE-BEHAVING RATS IN AN OPEN FIELD UNDER CUE-CONFLICT SITUATIONS

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Cells whose firing rate depend on the orientation of the rat in space can be recorded from the postsubiculum of awake, behaving rats. These cells are termed "head-direction cells" (Taube et al., 1990, *JNeuroscience* 10:420). Three rats were trained to forage for 20 minutes in a 1-meter diameter, black-walled, circular arena. Food pellets were dropped randomly at a Poisson interval with a 10-second mean. A white cue-card subtended 100° on the wall of the field. During training, the cue-card remained in the same position on the wall which provided a stable reference. After a two-week training period, the rats were chronically implanted with 12 recording tetrodes. The tetrodes were advanced into the postsubiculum over a period of one week. Up to 14 head-direction cells with different preferred directions were recorded simultaneously each day. These data constitute the first recorded ensembles of head-direction cells.

To investigate the stability of the behavioral correlates of these cells, two confusion paradigms were introduced. In the first, rats were faced with a cue-conflict between orientation of the cue-card and the arena itself. After foraging for 10 minutes, the rat was removed from the arena, while the cue-card was rotated 90°. The rat then foraged for another 10 minutes. After which, the rat was again removed and the cue-card rotated back to the original position. The rat foraged again for 10 minutes. In the second paradigm, the arena was spun without removing the rat from the arena. The rat foraged for 5 minutes, after which, the arena was spun for 720° at a rate of 90°/beat at 50 beats/minute. Spinning was timed with a metronome. The rat alternated between 5-minute foraging periods and 720° spins three times (forage, spin, forage, spin, forage).

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EFFECTS OF VITAMIN E ON MEMORY IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative condition characterized by memory loss, amyloid plaque deposition, and increased levels of oxidative damage in the brain. Vitamin E, a potent antioxidant, is currently used in AD therapy to slow the rate of cognitive decline. Whether vitamin E is capable of preventing memory loss in AD is unknown. To investigate the role oxidative stress plays in memory loss in AD, we used Tg2576, a transgenic mouse model of AD. These mice express a mutant form of the human amyloid precursor protein (APP) responsible for certain forms of familial AD. Tg2576 have been used as a model of memory loss and amyloid plaque deposition in AD. In the present study, Tg2576 mice were placed on vitamin E fortified chow (2000 IU/kg) at two months of age and were fed for approximately 10 months, at which time spatial reference memory and working memory were assessed using the Morris water maze. Compared to Tg2576 mice receiving a control chow, mice fed vitamin E showed significantly better spatial reference memory. Our findings suggest that vitamin E exerts a beneficial effect on memory in this transgenic mouse model of AD.

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ANTIOXIDANT OIL AUGMENTS CYTOTOXIC IMMUNE RESPONSE TO ATTENUATED *SALMONELLA TYPHIMURIUM*

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Currently, there is no effective treatment for unresectable hepatic metastases from colorectal carcinoma. Cancer immunotherapy utilizing the cytokine interleukin-2 has been investigated experimentally and clinically for more than two decades. This naturally occurring peptide induces the cytolytic activity of CD8⁺ cytotoxic T lymphocytes (CTL) and natural killer cells (NK). Unfortunately, the toxicity associated with systemic administration of interleukin-2 has limited its use clinically. We previously described the transformation of an attenuated strain of *Salmonella typhimurium* to deliver the human interleukin-2 gene (*Salmonella*-pIL2) directly to the liver. When administered orally in a murine model, *Salmonella*-pIL2 was shown to reduce hepatic colorectal metastases, mediated by CTL and NK. Recent studies have shown that plant oils rich in antioxidants also increase cellular immune response. Since the oral administration of *Salmonella*-pIL2 significantly increases CTL and NK populations, we postulated that the addition of antioxidant oil to a murine diet will further elevate NK and CTL populations. To test this hypothesis, animals were randomly placed into four groups: control, antioxidant oil only, *Salmonella*-pIL2 only, and *Salmonella*-pIL2 with antioxidant oil. Each study was initiated by gavage feeding animals 200 microliters of either saline or *Salmonella*-pIL2. For the duration of each study, the animals were fed either standard rodent chow or standard rodent chow supplemented with ten percent black raspberry seed oil by weight. Splenic lymphocyte populations and systemic cytokine levels were analyzed at the conclusion of 3, 7, and 14 day studies. We found significantly elevated CTL populations in the *Salmonella*-pIL2 with antioxidant oil group at all time points compared to the other groups. Furthermore, we observed that the NK population peaked on day 7 and was highest in the *Salmonella*-pIL2 with antioxidant oil group at all time points. These results suggest that high-antioxidant oil in conjunction with oral administration of *Salmonella*-pIL2 augments cytotoxic immune response and may prove to be a more effective treatment for unresectable hepatic colorectal metastases than *Salmonella*-pIL2 alone.

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

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Glucocorticoid-induced TNF receptor (GITR) has been recently identified as a potentially important molecule involved in the suppressive mechanisms of regulatory CD25+CD4 T cells. It is highly expressed on CD25+CD4 T cells, and stimulation of this receptor with the monoclonal antibody DTA-1 abrogates the suppressive affects of these cells *in vitro*. Furthermore, treatment of young BALB/c mice with DTA-1 results in the emergence of autoimmune gastritis, a disease known to be heavily controlled by regulatory CD25+CD4 T cells. We used the DO11.10 adoptive transfer system to measure antigen-specific CD4 T cell responses in the presence or absence of DTA-1. We found DTA-1 to work as an effective adjuvant in normal BALB/c and CD28 ^{-/-} hosts. We also learned that responder T cells transiently express DTA-1, maximally at 24 hours following antigen encounter. In fact, DTA-1 continues to function as an adjuvant even when it is given 24 hours after antigen. Finally, we discovered DTA-1 to work independently of CD28-mediated co-stimulation. These results show DTA-1 to be an effective adjuvant that works differently from other more commonly used adjuvants, such as LPS, as its effects are independent of CD28-mediated co-stimulation. Thus, targeting GITR may prove to be synergistic with other adjuvants, and therefore, helpful in future vaccine development.

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MECHANISMS OF CYTOPROTECTION IN PIG ENDOTHELIAL CELLS

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Many people die each year awaiting an organ transplant. Xenotransplantation, the transplantation of an organ between species, such as pig to human, may be a solution. One requirement for successful xenotransplantation is to protect the organ from rejection by the patient's immune system. Immediately after organ reperfusion hyperacute rejection takes place, due to pre-existing antibodies and complement activation. Avoiding hyperacute rejection may lead to accommodation, or the survival of the transplanted organ in the presence of antibodies and complement. Achieving accommodation is a potential strategy for xenograft survival. Galactose $\alpha(1-3)$ Galactose (α Gal), a carbohydrate expressed on pig cells, is the main antigen that activates complement. Primates do not express α Gal but have anti- α Gal antibodies. Previous studies in our laboratory have shown that ligand binding to terminal α Gal on pig endothelial cells induces protection of these cells from cytotoxicity by human complement. Therefore, I am studying mechanisms that may be involved in cytoprotection.

It is known that up-regulation of heme oxygenase-1 (HO-1) can induce cytoprotection. HO-1 cleaves an α -methene bridge in the heme ring, forming biliverdin, CO, and Fe^{+2} , followed by up-regulation of the iron storage protein ferritin. HO-1, CO, and ferritin may contribute to protection from complement and oxidative damage. I conducted experiments where up-regulation of HO-1 was induced with two separate agents: 1) ligand binding of the α Gal, and 2) stimulation with hemin. I measured the degree of cytoprotection from complement- and oxidative-mediated cytotoxicity induced in the endothelial cells and their expression of HO-1 using Western blotting. Results showed that both ligand binding of the α Gal and stimulation with hemin up-regulated HO-1 and caused protection from complement and oxidative injury. These findings suggest that HO-1 up-regulation might be a mechanism that may participate in accommodation. I will perform additional studies to understand these mechanisms of cytoprotection.

19

USING HEAT SHOCK PROTEIN 70 WITH BREAST CANCER PEPTIDES TO ACTIVATE CYTOTOXIC T CELLS

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A subset of heat shock proteins are potent immune adjuvants. They function by stimulating dendritic cells and delivering peptides for subsequent presentation to T cells. Once activated by the dendritic cells, T cells can then kill tumors that express these peptides. Our long-term goal is to activate breast cancer-specific killer T cells using heat shock proteins and tumor-associated peptides. The specific goal of this project is to make recombinant human heat shock protein 70 (rhHSP70) in bacteria and yeast. We attempted to make the recombinant protein from both because the bacterial derived protein is often contaminated with lipopolysaccharide (LPS), which is an endotoxin that binds to the same receptor as rhHSP70 on dendritic cells. Persistent problems occurred with the yeast expression system therefore we produced the rhHSP70 in bacteria and removed any contaminating LPS. This protein will be tested for peptide binding using competitive inhibition, HPLC, and gel electrophoresis. We will then determine how well rhHSP70 activates human dendritic cells, and induces peptide specific human cytotoxic T cell responses.

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INVESTIGATING POSSIBLE MOVEMENTS IN A MEMBRANE TRANSPORT PROTEIN

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The lactose permease is a member of the major facilitator superfamily (MFS) that includes symporters, uniporters, and antiporters. The members of this superfamily transport a wide variety of substrates, such as sugars, amino acids, Krebs cycle intermediates and antibiotics. Particular regions among these proteins are conserved and have been implicated in having a general role in their structure and function. In previous studies of the lactose permease, evidence showed that mutations within this conserved region greatly decreased its transport function¹. These results suggest that disturbances in these areas cause an inability of the mutant permease to make the necessary transmembrane movements that allow lactose to access the cell.

One goal for this project was to further investigate this proposal by first attempting to define the water/lipid boundary of the amino acid residues associated with transmembrane segment 9 (TM-9) and loop 9/10. Using PCR mediated site-directed mutagenesis we were able to change amino acid residues F308, A309, T310, S311, A312, and L313 located at the hypothesized boarder of TM -9 to cysteine residues. Oregon Green 488 maleimide (OGM), a fluorescent sulfhydryl reagent (which reacts specifically with cysteine side-chains) that is impermeant to the lipid bilayer, was then used to determine if a site-directed mutant was accessible to this reagent. After the reaction, the protein was purified via Ni-NTA chromatography and then subjected to SDS-PAGE. The level OGM labeling was determined by a Molecular Dynamics STORM fluorescence imaging system (blue fluorescent-chemifluorescence mode). The results obtained thus far suggest that certain residues are buried within the membrane while others are accessible to the aqueous solvent. Future studies will be investigating the movement of these boundaries by examining how the conformational structure of the transmembrane protein changes while transporting lactose.

¹Jessen-Marshall, A.E., Parker, N.J., and Brooker, R.J. (1997) *J. Bacteriology* 179, 2616-2622

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EFFECT OF METFORMIN ON FRUCTOSE-2,6-BISPHOSPHATE LEVELS IN LIVER CELLS

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Type II Diabetes is characterized by a resistance to insulin resulting in increased plasma glucose levels (hyperglycemia). Approximately ninety percent of people diagnosed with diabetes have Type II diabetes. Metformin, a dimethylbiguanide, has been used since 1995 in the US for treatment of type II Diabetes. This orally administered drug has the effect of lowering plasma glucose levels as well as decreasing hepatic glucose output. While the clinical effects of this drug are known, its cellular mechanisms are not. Fructose-2,6-bisphosphate (F26P₂) regulates the glycolytic/gluconeogenic pathways by allosteric effects on the key enzymes of these pathways, 6-phosphofructo-1-kinase (PFK 1) and fructose-1,6-bisphosphatase (FBPase 1). F26P₂ is both synthesized and degraded by the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. Increased levels of F26P₂ have been shown to increase the rate of glycolysis in liver cells as well as decreasing the rate of gluconeogenesis. This study examines the hypothesis that Metformin acts by increasing the levels of F26P₂ in liver cells, thereby creating the physiological effect described. FAO cells, a well differentiated hepatoma cell line, were treated with differing concentrations of Metformin for different lengths of time and the levels of F26P₂ were assayed. Preliminary data shows that after normalizing F26P₂ levels with protein concentration, the stimulatory effects of Metformin on F26P₂ could not be seen.

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EXPRESSION LEVELS OF α -CRYSTALLIN PROTEINS IN YOUNG AND OLD RAT RETINAL PIGMENT EPITHELIUM

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The degeneration of the retinal pigment epithelium (RPE), which supports the function of the photoreceptors in the sensory retinal layer, is associated with the onset of age-related macular degeneration (AMD). Currently, AMD affects approximately 30% of people over the age of 75. Early AMD detection is characterized by the loss of RPE pigmentation associated with aging. This hypertrophy of the RPE subsequently leads to photoreceptor death and the loss of vision. The α -crystallin proteins, members of the small heat shock protein family, play a vital role in RPE function by acting as molecular chaperones, which prevent aggregation of misfolded and damaged proteins. The over-expression of heat shock proteins occurs when a cell encounters a stressful environment, such as high temperature, exposure to oxidizing agents, pH extremes, and nutrient limitation. Previous studies in the Ferrington lab demonstrated an increase in oxidative stress in the sensory retina of aged rats. It has also been reported that α B-crystallin is over-expressed in response to heat shock treatment and oxidative stress and protects the RPE from apoptosis. The expression levels of α A and α B-crystallin, which together form the multimeric α -crystallin protein, were studied in young and old rat RPE using Western immunoblotting technology. Analysis of the immunoblot revealed that more α -crystallin was expressed in young rat RPE. This may indicate that the old rats have inadequate protection from protein unfolding, making the cell more susceptible to oxidative damage.

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CHARACTERIZATION OF HAMSTER N-ACETYLTRANSFERASE USING SITE DIRECTED MUTAGENESIS

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N-acetyltransferase (NAT) enzymes are important in metabolic transformation of arylamines and have been linked to several types of human cancers. Recent X-ray crystallography of NAT from *Salmonella typhimurium* shows that active site residues include a catalytic triad (cysteine, histidine, aspartic acid) similar to that of the cysteine proteases. In cysteine protease enzymes such as papain, mutation of the aspartic acid residue of the catalytic triad to asparagine resulted in over 66% recovery of wild-type activity. To compare the similarity of NAT catalysis to that of the cysteine protease family, mutagenesis of the corresponding aspartic acid to asparagine and alanine was performed in the recombinant hamster NAT2 gene. Also, a tyrosine residue neighboring the aspartic acid was mutated to phenylalanine in order to investigate its role in stabilizing the catalytic triad. Hamster NAT2, with over eighty percent homology to the human enzyme, is an important model for studying catalysis of NAT enzymes, as it can be stably overexpressed and purified to homogeneity in large quantities. Expression levels of the three mutant proteins were compared to wild-type hamster NAT2 protein using SDS-PAGE and Western Blot. Activities of the proteins were measured using transacetylation assays. The expression and activity levels of the mutant proteins indicate a striking difference between the catalysis of this NAT model and the cysteine protease family of enzymes.

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REGULATION OF THE GENE EXPRESSION OF FRUCTOSE-1,6-BISPHOSPHATASE BY HIGH LEVELS OF FRUCTOSE-2,6-BISPHOSPHATE

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Currently, there are 17 million Americans, 6.2% of the population that have diabetes mellitus (DM). While less than 1% of individuals under 20 years old have type 1 DM, the prevalence of type 1 or type 2 DM have reached over 20% in individuals 65 years and older. Thus, an understanding of the cause of DM has become a critically important public health problem. One of the ways in which we can gain insight on DM is in the study of glucose metabolism. Hepatic 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase is an important regulatory enzyme of glucose metabolism. 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase regulates hepatic glucose output by controlling the level of fructose-2,6-bisphosphate, an allosteric activator of the glycolytic enzyme 6-phosphofructo-1-kinase and an allosteric inhibitor of the gluconeogenic enzyme fructose-1,6-bisphosphatase. Previous experiments overexpressing a bisphosphatase-deficient 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase in normal or diabetic mice showed significant increases in the levels of fructose-2,6-bisphosphate for both groups. The normal mice showed a slight lowering of blood glucose levels while there was a marked reduction of blood glucose levels in the diabetic mice. These results confirmed the critical role played by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase in glucose metabolism. We then wanted to determine whether high fructose-2,6-bisphosphate decreases gluconeogenic flux by the down-regulation of fructose-1,6-bisphosphatase gene expression, beyond its negative allosteric effects on this enzyme. Western blotting was performed on liver samples from both normal and diabetic mice, which were treated with adenovirus to elevate their levels of fructose-2,6-bisphosphate. The results showed no differences in the amount of fructose-1,6-bisphosphatase in the liver cells of all mice in the experiment. These findings suggest that the effect of fructose-2,6-bisphosphate on fructose-1,6-bisphosphatase is purely allosteric.

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GLOBAL ANALYSIS OF mRNA DECAY IN NORMAL VERSUS MALIGNANT HUMAN T LYMPHOCYTES

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Precise regulation of messenger RNA decay is important for the normal regulation of gene expression. We hypothesized that abnormal mRNA decay may contribute to the deregulated gene expression that occurs in cancer. To test this hypothesis, microarray analysis was used to compare, on a genome-wide basis, mRNA decay rates between normal primary human T lymphocytes and the malignant human T cell lines H9 and Jurkat. Actinomycin D was added to normal or malignant human T lymphocytes to stop transcription by RNA polymerase II, and total cellular RNA was harvested at 0, 45, 90, and 120 minutes. This RNA was used to probe Affymetrix microarrays, and mRNA decay curves were generated for each expressed gene. Data from three independent experiments were analyzed using a first order decay model, and RNA half-lives (with 95% confidence intervals) were determined for each of approximately 7000 expressed transcripts. Numerous regulatory transcripts encoding oncogenes, kinases and phosphatases involved in signaling, and growth-factor-responsive proteins were found to be up-regulated in H9 and Jurkat cells compared to T cells. In contrast to the up-regulated transcripts in H9 cells, up-regulated transcripts in Jurkat cells were found to be more stable. In both H9 and Jurkat cell lines, numerous transcripts encoding cell cycle control proteins, signaling intermediates and transcription factors were repressed and destabilized compared to T cells. Furthermore, we found significant differences in the profiles of transcript decay between Jurkat and H9 cells. The use of Affymetrix microarrays in this study allowed the identification of numerous transcripts that are significantly up- or down-regulated at the level of mRNA stability in malignant human T lymphocytes.

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PROTEOMIC APPROACHES TO THE IDENTIFICATION OF PROTEINS SECRETED BY BRAIN TUMORS

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An important tool that is rapidly advancing towards the detection, diagnosis, and treatment of cancer is proteomics. Proteomics attempts to characterize proteins and compare the variation in protein expression levels in normal and diseased states. Therefore, proteomics can play an important role in the discovery and development of drugs against cancer. Three main steps have been established in proteomics: the separation of proteins by 2D-PAGE, the identification of proteins by mass spectroscopy, and the analysis of proteins using bioinformatics. Currently, there is little research in brain tumor proteomics. My research involves the use of proteomics to identify secreted growth factors by brain tumors, which may be used as potential targets for developing vaccines against brain tumors. To identify these growth factors, human brain tumor cell line U87, T98, and U373 were grown in tissue culture flasks for five days. The supernatant was collected, precipitated, and run on 2D-PAGE. The gels were compared to the control, DMEM, which was also run on 2D-PAGE. The results revealed two unique growth factors about the size of 13kD and 45kD in the U87 cell line. These proteins are currently being identified using mass spectroscopy.

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DIFFERENTIATION OF CANINE MULTIPOTENT ADULT PROGENITOR CELLS INTO CARDIOMYOCYTE-LIKE CELLS

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An important aspect of treatment for congestive heart failure and numerous other cardiac diseases is understanding the pathophysiological and biochemical changes occurring. Combining knowledge of cardiogenesis and the potential of Multipotent Adult Progenitor Cells (MAPCs), bone marrow derived stem cells which form all three tissue lineages and possess high levels telomerase activity, cell therapies can be created in the future to reduce issues associated with cardiac diseases. Preliminary data (unpublished) have suggested that human MAPCs may differentiate into cells expressing cardiac muscle markers. Experiments similar to those performed on human MAPCs are being carried out on mouse, rat, and canine MAPCs. Preliminary data indicate the canine MAPCs differentiate into fat tissue and endothelium. In addition, early cardiac markers in canine MAPCs are being analyzed by quantitative PCR and immunohistochemistry

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ACUTE PANCREATITIS AND PERICARDIAL EFFUSION: AN EXPLORATION OF A RARE ASSOCIATION

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Acute pancreatitis is the sudden inflammation of the pancreas, a large endocrine gland which lies behind the duodenum. It secretes digestive enzymes, insulin and glucagon to control the metabolism of sugars, fats, proteins and carbohydrates. Inflammation occurs when, under abnormal conditions, the digestive enzymes attack the pancreas. Symptoms begin with severe pain in the upper abdomen and may be accompanied with a swollen and tender abdomen, nausea, vomiting, fever, and a rapid pulse. Approximately 80,000 cases occur yearly in the United States. About 80% of cases of acute pancreatitis are caused by excessive alcohol use and/or by gallstones that block the flow of digestive enzymes. Although most people recover fully from acute pancreatitis, complications like pseudocyst and pleural effusion may develop. In rare cases, a patient with acute pancreatitis may develop pericardial effusion.

The pericardium is a tissue sac that surrounds and protects the heart. Normally, there is a small amount of fluid between the pericardium and the heart that helps cushion the heart and reduce friction between the heart and other structures in the chest when the heart beats. In pericardial effusion, too much fluid collects within the pericardium, preventing normal filling of the heart, which can reduce its ability to pump blood. Three hypotheses on the mechanism of pericardial effusion in acute pancreatitis have been proposed: 1. the development of chemical pericarditis due to circulating enzymes, 2. fistulous connections between pericardial and abdominal cavities, and 3. a lymphatic transport of amylase, a pancreatic digestive enzyme. After a thorough reading of medical journals and patient cases, we support the second hypothesis. The author is assisting in a review of 411 medical records of patients with severe acute pancreatitis at a teaching hospital to obtain clinical evidence in the identification of the rare association of pericardial effusion and acute pancreatitis.

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UP-REGULATION OF SPROUTY1, A NEGATIVE INHIBITOR OF RAS/MEK/ERK CASCADE, FOLLOWING VENTRICULAR UNLOADING IN HUMAN HEART FAILURE

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Background: The guanine nucleotide-binding protein Ras plays a direct role in myocyte remodeling; however the molecular mechanisms mediating Ras regulations remain poorly defined. **Methods and Results:** we screened a compendium of gene profiles from 15 paired human left ventricular samples at the time of left ventricular assist device (LVAD) implant and at the time of transplantation for novel genes regulating Ras signaling. From this analysis we identified Sprouty1, an evolutionary conserved gene that acts as an intrinsic negative inhibitor of Ras signaling. Sprouty1 was significantly up-regulated following ventricular unloading with an LVAD. Interestingly, ventricular unloading induced a consistent up-regulation in Sprouty1 irrespective of the underlying etiology leading to heart failure. Furthermore, Sprouty1 expression was elevated in the ventricular as 28 days following ventricular support and remained elevated out to 521 days. In parallel, Sprouty1 protein expression was also up-regulated following the ventricular unloading. The up regulation of Sprouty1 protein expression was accompanied by a significant increase in post translational modification. **Conclusion:** to our Knowledge, these findings are the first to define Sprouty1 expression in the heart and suggest that Sprouty1 may serve as an intrinsic regulatory mechanism governing Ras signaling in the failing heart in response to alterations in workload.

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RED HOT CHILI PEPPERS AND ALOPECIA AREATA

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Capsaicin is the active ingredient that makes chili peppers and other peppers of the genus *Capsicum* hot. Capsaicin binds to a membrane receptor, Vanilloid Receptor Subtype 1 (VR1), localized on sensory neurons and within the central nervous system. It is also expressed in cultured human keratinocytes. Activation of VR1 by heat, protons, or vanilloid compounds leads to the release of neuropeptides, like Substance P (SP), involved in proinflammatory responses. Application of capsaicin or SP in C57BL/6 mice suggests that SP is involved in the murine hair growth cycle. Furthermore, SP and capsaicin were each shown to successfully induce hair regrowth in treated mice. Alopecia areata is a complex auto-immune disease involving hair loss that has been shown to have a neurological component in its pathogenesis. It can be limited to the scalp or involve all hair-bearing surfaces of the body. Reports demonstrate that some alopecia areata patients have an unusual organization of nerves and altered expression of SP around affected hair follicles. Preliminary clinical trials in Turkey indicate topical capsaicin treatment is effective in inducing hair regrowth in alopecia areata patients. In this experiment, two healthy patients with alopecia areata totalis, of greater than two years duration, applied 0.075% topical capsaicin cream to the scalp for 180 days. Immunostaining of 4mm punch biopsies from the scalp show increased SP expression around the bulge region of the hair follicle. Vellus hair regrowth was also observed. Duplicate samples from these patients, as well as scalp skin biopsy specimens from age and sex-matched controls, were immunostained and analyzed with antibodies to VR1. Given these initial findings, further analysis may prove capsaicin useful in the treatment of alopecia areata.

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THE EFFECT OF PROGESTERONE AND ESTRADIOL ON LUTENIZING HORMONE AND FREE ALPHA INHIBIN LEVELS IN A MARE WITH AN OVARIAN TUMOR

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In mares, the presence of an ovarian tumor has been associated with a high level of inhibin, notably the type of inhibin known as “free alpha inhibin.” Another hormone, known as lutenizing hormone (LH) also has been reported to be higher in concentration in mares with ovarian tumors in comparison to control mares. The reproductive hormones progesterone and estradiol are known to negatively affect the release of LH in the normal mare. In this study, injections of a solution containing progesterone and estradiol were given to a mare with an ovarian tumor. Injections of progesterone and estradiol may decrease the amount of LH released and thus, bring about a decrease in the plasma LH levels. This decrease in LH would, in turn, decrease free alpha inhibin levels and the size of the ovarian tumor.

An Arabian mare housed at the University of Minnesota Teaching facility was used in this case study. Blood samples were taken prior to, during, and after the thirty days in which the progesterone and estradiol treatment was given. Plasma samples were collected and tested for levels of LH and free alpha inhibin. The level of free alpha inhibin in the equine plasma was analyzed utilizing a method known as enzyme-linked immunosorbant assay (ELISA). Levels of LH were detected utilizing radioimmunoassay, or RIA. Upon analysis of the assay data, it was determined that both LH and free alpha inhibin decreased over time. This study suggests that injections of progesterone and estradiol may prove to be a short-term, potentially more cost-effective method, in comparison to surgery, to stunt the growth of an equine ovarian tumor.

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STEM CELL CHARACTERIZATION AND INDUCED RESTORATION OF DAMAGED MYOCARDIUM IN THE CANINE MODEL

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The increasing demand for human organs for transplantation has caused an interest in the development of novel tissue engineering techniques. The recent discovery of pluripotent stem cells within the bone marrow of adult mammals has suggested that adult stem cell therapy may be a viable option for future transplant patients [1,2]. Stem cell induced restoration of myocardium (SCIRM) has been conducted in the canine model. Preliminary data shows engraftment in the area of infarct after four months as well as increased global perfusion around the area of infarct in the experimental animals. Here we report the results of the characterization of the injected cells *in vitro* including the results of the differentiations of the injected cells into the three germ layers: mesoderm, endoderm, and neuroectoderm. The cell populations have been analyzed for cell markers CD44, CD45, and MHC class I and have shown portions of the population to be negative for all three of these hematopoietic markers. Functional assays are currently underway to further characterize these cells. This data, while preliminary, suggests the presence of multipotent adult progenitor cells (MAPC) amongst the heterogeneous injected cell population.

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siRNA-MEDIATED SUPPRESSION OF APOPTOTIC PATHWAY PROTEINS

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In response to stress, cells can be induced to die through the activation of certain apoptotic pathways. We are interested in understanding how these pathways operate in B-lineage acute lymphoblastic leukemia (ALL). Caspases, a family of aspartate-specific cysteine proteases, play an important role in these pathways. An early step involves release of cytochrome c from the mitochondria as a result of cellular insult. The cytochrome c complexes with APAF-1, ATP and procaspase-9 to form the apoptosome. Caspase-9 becomes activated and goes on to activate downstream caspases. Interestingly, in several B-lineage ALL cell lines, a 4 amino acid peptide inhibitor of caspase-9 was shown to enhance cell death in response to apoptotic stimuli, a result not observed with other caspase inhibitors. In order to investigate this phenomenon, we have begun to employ RNA interference (RNAi) to specifically suppress proteins involved in the pathway. RNAi involves the introduction of 21-nt double-stranded RNA molecules known as small inhibitory RNA (siRNA) into cells. The siRNA targets the complimentary mRNA for destruction, resulting in transient reduction of protein levels. Using the JURKAT T lymphoblastic lymphoma cell line transfected with green fluorescent protein (GFP), we demonstrated by flow cytometry reduced GFP expression in cells co-transfected with GFP-specific siRNA. However, the low transfection efficiency made this method impractical for further studies. Using electroporation with FITC-conjugated siRNA, we were able to demonstrate in JURKAT and several B-lineage cell lines that 20-50% of cells had taken up siRNA. In BLIN-4L cells, we showed by Western blotting reduced levels of APAF-1 and caspase-2 in cells that had been electroporated with the respective FITC-conjugated siRNA. Current studies are evaluating the response of leukemic cells deficient in APAF-1 or caspase-2 to stress-induced cell death. This has the potential to reveal the role of specific apoptotic pathway components in drug/stress-induced death of human leukemia.

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IDENTIFICATION OF ADIPOCYTE LIPID BINDING PROTEIN (ALBP) INTERACTING PROTEINS USING YEAST TWO-HYBRID ANALYSIS

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Obesity and diabetes are pathologic conditions that affect a relatively large percentage of the U.S. population and are continually increasing in abundance. These conditions often stem from problems occurring within adipocytes. Adipocytes are dynamic cells in which fatty acids are stored and hydrolyzed from triglyceride droplets in response to the body's metabolic needs. Adipocytes are also generally accepted as a complex cell type involved in generating a number of signals, which include hormones, growth factors, and cytokines. Fatty acids are minimally soluble in the aqueous cytoplasm due to their long hydrocarbon tail. Adipocytes and other lipid-metabolizing cells have alleviated this problem by evolving an intracellular fatty acid-binding protein (FABP) that is able to traffic and promote the solubility of free fatty acids. Adipocytes express two fatty acid-binding proteins, the products of the FABP4 and FABP5 genes. Adipocyte lipid binding protein (ALBP), the protein product of FABP4, has been discovered to interact with and activate hormone sensitive lipase, an enzyme that hydrolyzes fatty acids from the triglyceride droplet within the adipocyte. We hypothesize that other proteins interact with ALBP in the adipocyte. The yeast two-hybrid method was used to screen a rat adipocyte cDNA library to identify any protein-protein interactions that occur with ALBP.

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A NEW MEMBER OF THE *RIM101* PATHWAY LINKS ALKALINE SENSING AND ENDOCYTOSIS

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Candida albicans is the most common pathogenic fungus in humans. It has the unique ability to infect a wide range of tissues, thus it must respond to diverse environments. One important environmental condition that affects *C. albicans* physiology is extracellular pH. The most striking effect being on its morphology. In acidic environments, *C. albicans* grows in the yeast form and in alkaline environments, it grows in the filamentous form. *RIM101* encodes a transcription factor that regulates pH responses and its activity depends on upstream members of the *RIM101* pathway. A genomic two-hybrid screen in *S. cerevisiae* identified proteins that may interact with members of the *RIM101* pathway. The most significant candidates were Snf7p and Vps4p. Thus, we hypothesized that *SNF7* and *VPS4* encode members of the *RIM101* pathway. To test this hypothesis, we created *snf7* and *vps4* deletion mutants in *C. albicans*. If these mutants have similar phenotypes to a *rim101Δ/Δ*, then this supports our hypothesis. These phenotypes include the inability to filament in an alkaline environment and growth defects on LiCl and alkaline media. Our studies have shown that the *snf7Δ/Δ* acts like a *rim101Δ/Δ*, while *vps4Δ/Δ* does not. Thus, we conclude that *VPS4* is not a member of the *RIM101* pathway. From the two-hybrid analysis, we predicted that *SNF7* encodes an upstream member of the *RIM101* pathway. To test this hypothesis, we integrated the constitutively active member *RIM101-405* allele, which bypasses the requirement of the upstream *RIM101* pathway members, into the *snf7Δ/Δ* mutant. *RIM101-405* rescued growth and filamentation defects of the *snf7Δ/Δ* mutant. Thus, we conclude that *SNF7* encodes an upstream member of the *RIM101* pathway. Snf7 protein is required to fuse the endocytic vesicles to the vacuole. This suggests a possible link between endocytosis and the *RIM101* pathway.

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FUNCTIONAL ANALYSIS OF GSK3 AND ITS ROLE IN REGULATION OF FLAGELLAR LENGTH IN CHLAMYDOMONAS

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Chlamydomonas reinhardtii is a unicellular green alga widely used as a model system for understanding the regulation of the assembly and disassembly of cilia/flagella. Chlamydomonas have two anterior flagella that are kept at an equal and discrete length. Mutations in genes that regulate flagellar length result in abnormally short or long flagella. Our lab has shown that glycogen synthase kinase 3 (GSK3) is present in flagella and may be involved in the regulation of flagellar length. For example, if cells are treated with lithium, a known inhibitor of GSK3, the flagella grow to 1.5 times the wild-type length. To learn more about the role of GSK3 in regulation of flagellar length, I used site-specific mutagenesis to change a lysine at amino acid 88 to an arginine (K88 Δ R). This change disrupts the ATP binding site of GSK3 and results in a kinase dead mutation. In other systems this mutation has been shown to produce a dominant negative effect when introduced into cells through transformation. I hope to see the same in this experiment. I inserted the mutated GSK3 into pSaD (a Chlamydomonas vector) and I am in the process of transforming the pSaD-GSK3 construct along with a selectable marker into Chlamydomonas wild-type cells. I will then characterize transformants to test for a possible flagellar phenotype.

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ENHANCER/SUPPRESSOR SCREEN FOR TARGET RAPAMYCIN (TOR) INTERACTORS

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Target of Rapamycin (TOR) is a serine/threonine protein kinase that plays an important role in cell growth and proliferation during development. While this response is initiated by nutrient conditions, the mechanisms and upstream regulators of TOR remain poorly understood. We have generated lines of flies which overexpress dTOR in the developing eye (eyeTOR), leading to reduced size and altered morphology of the adult eye. We have used the altered level of signaling resulting from dTOR overexpression as a background for a genetic screen to reveal potential dTOR interactors. Transposable elements were mobilized to create a library of flies containing random insertional mutations throughout the *Drosophila* genome. These mutants were crossed to eyeTOR flies. An insertional mutation that disrupts a gene regulating TOR signaling is expected to reduce the gene copy by 50%, thereby leading to an enhancement or suppression of the eyeTOR phenotype in the progeny. Candidate interactors with TOR, including one amidase, have been identified through multi-generation screens. To confirm the role of these candidate interactors in the dTOR signaling pathway, we are examining their genetic interactions with other TOR signaling components, with TOR loss of function phenotypes, and with TOR overexpression phenotypes in other tissues.

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LEUCINE AMINOPEPTIDASE IN *CAENORHABDITIS ELEGANS*

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Leucine aminopeptidases (LAPs) are part of a family of metalloenzymes which are expressed in many organisms. Catherine Kirkpatrick has found that *Drosophila* LAP CG7340 interacts with a component of the Wnt/Wingless pathway, and is expressed in the developing gut and excretory system. This research is designed to determine the function of LAPs in *C. elegans*, which has two LAP genes, ZK353.6 and W07G4.4. These are structurally similar to LAPs found in *Drosophila*. RNA interference (RNAi) techniques were used to determine whether LAPs are involved in Wnt signaling. In RNAi techniques, double-stranded RNA from the gene of interest is introduced to the organism, inducing a loss of function phenotype. RNAi of either LAP alone and of both simultaneously did not produce embryonic lethality, suggesting that LAPs are not involved in Wnt signaling. No phenotypes were observed in larvae or adults. The tissue expression patterns of LAPs were studied by injecting individuals with vectors encoding GFP fusion proteins. These proteins from both LAPs are expressed in the gut of late embryos, larvae and adults, suggesting an involvement in digestion, and in different amphid neurons, suggesting an involvement in neuropeptide generation or degradation. W07G4.4 is expressed in the vulva of L4 larvae and adults.

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DETERMINING THE SPECIFICITY OF cCF10 FOR THE BINDING PROTEINS PrgZ AND PrgX

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The antibiotic resistance plasmid pCF10 has become a model system for studying conjugation in *Enterococcus faecalis*. Enterococcal cells containing this plasmid responds 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. These mutants were then screened for pheromone activity using a clumping assay, which allows for quantitative analysis of induction by the visible formation of cell “clumps” of responding donor cells. Mutants that had reduced binding were sequenced, and their amino acid sequence was deduced. Analysis of some of these mutants supports previous findings that suggested that the amino terminal end of this peptide is important for the pheromones activity. Essential internal residues were also identified.

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ANALYSIS OF GENES CODING FOR GROUP A STREPTOCOCCAL M-PROTEIN AND PYROGENIC EXOTOXINS: A 45-YEAR PERSPECTIVE

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Historically, group A streptococci (GAS) frequently caused serious infections such as rheumatic fever and scarlet fever. Following the development of antibiotics in the late 1940s, the incidence of these life-threatening infections was greatly reduced. However, in the late 1980s there began an unexpected, and unexplained, resurgence of rheumatic fever and invasive infections such as necrotizing fasciitis and toxic shock syndrome. In an effort to understand the reason(s) for this resurgence, many studies have focused on possible changes in the streptococcus, especially in the M-protein, a virulence factor, and in the streptococcal pyrogenic exotoxins (SPEs) produced by these bacteria. Several studies associated M-types 1 and 3 with these invasive infections, and others found an association with a specific toxin, SPE A. However, these studies lacked sufficiently large numbers of viable streptococci isolated before the 1980s to demonstrate that changes in the streptococci had, in fact, occurred.

The objective of this study was to examine lyophilized samples of streptococci, many of them non-viable, dating back to the late 1950s, and to determine if changes had occurred in either the M-protein or the presence or molecular structure of SPE toxins. A polymerase chain reaction was developed to examine these mainly non-viable streptococci, originally isolated in the late 1950s to mid 1960s, for M-protein and SPE toxin genes (*speA*, B, and C). DNA sequencing was used to identify possible genetic variation in these genes. Preliminary findings suggest that the genes coding for M-types 1 and 3 (*emm-1* and *emm-3*) in these strains occur in multiple allelic forms. Several M3 strains were found to carry the gene for SPE A (*speA*). Of these, at least two allelic variants were observed. One of these forms (*speA3*) has been associated in the literature with increased streptococcal virulence. Studies are ongoing to determine whether this variation is associated with changing strain virulence.

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UTILIZATION OF YEAST TWO HYBRID TECHNIQUE TO DETECT PROTEIN-PROTEIN INTERACTIONS WITH THE PRODUCT OF THE CANDIDATE LEUKEMIA GENE, *RasGRP2*Matthew P. Abdel[†], KJ Morgan[‡], and (DA Largaespada[‡])[†]Department of Biochemistry, Molecular Biology, and Biophysics, and [‡]Department of Genetics, Cell Biology, and Development, University of Minnesota

Several techniques may be utilized to detect protein-protein interactions, including yeast two hybrid and mammalian two hybrid assays. In this particular project, the yeast two hybrid technique was used to determine what, if any, protein-protein interactions exist between the protein product of the putative oncogene, *RasGRP2* (also called *CalDAG-GEF D*), and other genes. *RasGRP2* is a guanine nucleotide exchange factor for Rap 1, a small GTPase downstream of the Ras signaling pathway.^{1,3} *RasGRP2* is a member of a family of at least 4 genes encoding exchange factors that are activated by calcium and diacylglycerol.² More importantly, it is activated by proviral insertion in multiple BXH-2 acute myeloid leukemias.^{1,4,5} There are two isoforms of the gene: the full-length *RasGRP2* gene and the C-terminally truncated *RasGRP2b* gene.² In these experiments, the full-length *RasGRP2* gene product was thought to be essential in BXH-2 myeloid leukemogenesis. As so, it was hypothesized that *RasGRP2* may interact with proteins involved in several signaling pathways, in addition to the Rap1 pathway. Initial results from the yeast two hybrid experiments indicate that *RasGRP2* may indeed interact with up to as many as 9 other proteins. Final sequencing of each gene must be completed to verify their identities. In addition, repeat experiments of the yeast two hybrid must be performed and confirmation will be obtained with pairwise comparisons using the mammalian two hybrid technique.

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INTERGENERIC GENE TRANSFER BETWEEN *ESCHERICHIA COLI* AND FILAMENTOUS STRAINS OF CYANOBACTERIA

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Natural products, or secondary metabolites, are created by many species of microbes and are represented in over 25% of human therapeutics. Historically, the search for new biologically active compounds from nature has focused predominantly on the soil-dwelling gram-positive actinomycetes. A more contemporary approach of finding novel natural products has involved the examination of a variety of microorganisms including the photosynthetic gram-negative cyanobacteria. Recent research on cyanobacteria has uncovered numerous new secondary metabolites that are structurally and functionally diverse, and demonstrate great potential for medicinal application. One compound, cryptophycin, is generated by cyanobacterial strains, *Nostoc sp.* ATCC 53789 and *Nostoc sp.* GSV 224. Cryptophycin has generated significant interest for its ability to induce apoptosis in cancer cells by through binding and depolymerization of microtubules. A serious limitation in the development of cryptophycin as a clinical anti-cancer therapeutic is that production through fermentation, using naturally producing strains, has proven difficult. In order to devise a viable route to large-scale production of cryptophycin and generate novel analogs inconceivable through total chemical synthesis, the cloning of the genes responsible for the compounds production and shuttling these genes to genetically engineered heterologous hosts has been a major initiative. An important component of this research initiative is the identification of cryptophycin biosynthetic pathway gene cluster within the genome of the natural producer, *Nostoc sp.* ATCC 53789. Progress toward this has been made in the form of cloning genes from *Nostoc sp.* ATCC 53789 into an *E. coli* cloning host containing portions or full copies of genes suspected to be required for the biosynthesis of cryptophycin. For unequivocal evidence that the suspected genes are involved in the production of cryptophycin, a gene transfer system between *E. coli* and the *Nostoc sp.* was developed. The design, results and implications of these experiments toward the overall research aims of producing anti-cancer agents through microbial biotechnology is presented.

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LOCATING MOLECULAR MARKERS LINKED TO RESISTANCE TO NET BLOTCH IN BARLEY (*Hordeum vulgare*)

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Net blotch, caused by *Pyrenophora teres* f. *teres*, is a disease that greatly affects barley (*Hordeum vulgare*) throughout the world. Since resistance is believed to be the best approach to controlling this disease, breeders have worked to produce varieties that confer net blotch resistance to barley. The objectives of this study were to identify molecular markers linked to net blotch resistance derived from a wild barley accession referred to as Wadi Qilt 23-38. Published molecular maps were studied to determine locations on the barley chromosomes that may contain quantitative trait loci (QTL) responsible for net blotch resistance. A region on chromosome 6 was identified from previously reported research, and four markers within this region were tested to determine whether they are associated with net blotch resistance. A recombinant inbred line population derived from a cross between Wadi Qilt 23-38 and Harrington was developed. Wadi Qilt 23-38 exhibits resistance to net blotch and Harrington is susceptible. The population was grown in the greenhouse and inoculated with the net blotch pathogen. Upon infection, the plants were scored for severity of disease on a 1-10 scale. Leaves from each line were harvested and DNA was extracted. Four simple sequence repeat markers mapping to chromosome 6 were scored on the population. The genotypic results were compared with the net blotch severity results. These analyses showed that these four markers explained between 27% and 31% of the variation for net blotch resistance. These data will allow barley breeders to employ marker-assisted selection to develop net blotch resistant varieties.

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BIODEGRADABLE POLYMERS: PALLADIUM CATALYSTS FOR LACTIDE POLYMERIZATION

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The polymer industry has played a large and important role in our everyday lives. Almost every object that we use in everyday life contains some type of synthetic polymer. While these plastics are versatile, the materials tend to be non-biodegradable and accumulate in our landfills. A material known as polylactide has been gaining more attention due to its biodegradable properties. It is also derived from a renewable resource, lactic acid, which can easily be obtained from corn. This material is being investigated for medical applications such as bone screws and timed-release drug implants. The material, once implanted, would be broken down by the body's own processes to leave only lactic acid.

The molecule lactide is formed from the dimerization of lactic acid molecules. Ring strain in lactide is the driving force for ring opening polymerization to polylactide if a catalyst is present. New catalysts for the synthesis of polylactide from the cyclic ester lactide are of interest in both industry and academic settings. The goal of my project was to produce a catalyst with the transition metal palladium that would be highly active, provide stereocontrol and molecular weight control, and polymerize lactide at elevated temperatures in solventless conditions, which are desired for industry. Palladium metal has been used to catalyze other reactions including the polymerization of olefins. If successful, it would be the first example of palladium in lactide polymerization. The syntheses of several potential palladium catalysts and results of their lactide polymerization behavior will be presented.

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STUDYING POWDERY MILDEW-MEDICAGO INTERACTIONS USING EXPRESSION PROFILING

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The fungus *Erysiphe pisi*, also known as Powdery mildew, is a pathogen of legume plants such as peas and alfalfa. A genetically-related species, *Medicago truncatula*, is also infected by powdery mildew. Since *M. truncatula* ecotypes have small diploid genomes and fast generation times, they can serve as excellent genetic models for related legume species. Reactions of *M. truncatula* to the pathogen varies from complete resistance, as in the Jemalong 6 ecotype, to partial resistance, as in the A20 ecotype, to complete susceptibility, as in DZA315.16. One goal of our lab is to study the differential expression pattern between infected and non-infected *M. truncatula* ecotypes, using microarray analysis. My specific objective is to analyze the expression pattern in Powdery mildew inoculated and non-inoculated Jemalong 6 ecotype. This process involves extracting total RNA from infected and healthy plants, preparing cDNA from the total RNA using reverse transcriptase, and then using the cDNA strands as hybridization probes against a 6K clone micarray prepared from *M. truncatula* cDNA libraries. By comparing the hybridization of genes from infected plants to those from healthy plants, we can determine which additional genes are expressed in the infected plants, and more specifically, which genes may lead to resistance in the plants. We are currently in the process of collecting and analyzing data from the microarrays, and will reach our conclusion later in the term.

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M63: A DEFECTIVE CYTOKINESIS MUTANT IN *CHLAMYDOMONAS REINHARDTII*

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Chlamydomonas reinhardtii is a biflagellate, unicellular green alga, surrounded by a cell wall, typically used as a model system to study flagellar motility and assembly, photosynthesis, and other biological processes. Cytokinesis is a universal cell process whereby two daughter cells separate, each with a nucleus and cytoplasmic organelles. In *Chlamydomonas*, this process has been previously described using immunofluorescence and transmission electron microscopy. An array of microtubules termed the phycoplast forms at the anterior end of the cell and extends around the cell. The cleavage furrow follows the phycoplast microtubules and divides the cell into two daughter cells. Little is known about the molecular components of cytokinesis in this organism. Insertional mutagenesis is a method used to generate mutations that results in a “tagged” gene that can be isolated. The M63 strain, generated by insertional mutagenesis, has a phenotype of defective cytokinesis. Some cells fail to complete cytokinesis, leading to large, multi nucleate cells with many flagella. An isolated 17kb genomic fragment has rescued the mutant genotype when transformed into mutant cells. Smaller plasmid subclones were tested to find the location of the M63 gene. The sequence of the 17kb region was analyzed to identify predicted open reading frames, including one that encodes a protein kinase. Identification of the M63 gene can provide information on molecules involved in cytokinesis and elucidate the mechanism of cytokinesis.

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A σ 3-DEPENDENT MECHANISM OF IMMUNE RESPONSE EVASION IN REOVIRUS

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Viruses are utterly dependent upon the transcriptional and translational machinery of the cells they infect to continue their life cycle. Infection with certain strains of reovirus, an archetypical member of the *Reoviridae* family, results in host shutoff, an event that selectively inhibits cellular translation but allows translation of viral proteins to continue. The goal of our research is to determine how reovirus translation is able to continue effectively in the presence of cellular translation inhibition. One mechanism of host shutoff is mediated through the induction of the cytokine type I Interferon (IFN). In eukaryotic cells, IFN is induced in response to viral infection. Through a signal cascade, IFN then stimulates the production of the double-stranded RNA activated protein kinase (PKR), an enzyme that inhibits protein synthesis in the cell through the phosphorylation of translation initiation factor eIF2 α . RNase L, a nuclease that destroys both cellular and viral single-stranded RNA, is also induced by IFN signaling. The PKR proenzyme is directly activated by dsRNA and RNase L is indirectly activated by dsRNA through the modification of 2'-5'-oligo(A)synthetase. Thus, PKR and RNase L effectively terminate the replication and dissemination of IFN-sensitive viruses. Our hypothesis is that σ 3, a 41 kilodalton reovirus protein, binds dsRNA, thus preventing the activation of PKR and the RNase L activation factor 2'-5'-oligo(A) synthetase. In cells infected with viral strains that induce host shutoff, we suspect that punctuate subcellular localization of σ 3 leads to areas of refuge where PKR and RNase L activation is locally blocked because σ 3 has bound the dsRNA needed to activate these antiviral molecules. As a result, viral translation continues while cellular translation is inhibited in the rest of the cell where σ 3 concentrations are much lower. To test our hypothesis, we have employed two approaches. First, we have used metabolic labeling to examine both cellular and viral total protein synthesis in order to determine the extent of host shutoff. Second, we are using immunofluorescence and confocal microscopy to determine the subcellular co-localization of σ 3, total and active phosphorylated PKR and 2'-5'-oligo(A) synthetase. To date we have discovered that strain Dearing does not induce host shutoff and has a diffuse cellular concentration of σ 3, while strain Jones induces host shutoff and has a perinuclear σ 3 localization.

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Propagation of Avian Pneumovirus in Turkey Turbinate and monkey Vero cell lines

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Currently, Avian Pneumovirus (APV) propagation techniques are most commonly carried out in monkey Vero cell lines, however, since APV specifically infects avian species, ideally, propagation of the virus should be done in a homologous avian cell line. A life span extended line of turkey turbate cells (TT1 cells) and immortal monkey Vero cells were tested and compared for their potential to propagate APV. Light and fluorescent microscopy were used to enumerate APV plaques by staining and immunofluorescent antibody techniques, respectively. Viral titers were determined in quadruplicate in 96 well plates containing either 1×10^4 TT1 or Vero cells. The sixth passage TT1 cell line titer was positive (plaque forming units/milliliter, PFU/ml) at 10^4 PFU/ml, while the Vero cell APV titer was 10^6 PFU/ml. These results proved comparable to the current techniques used for propagating APV. The turkey turbate cell line works well enough to accomplish the purpose of APV propagation, but is not a significant improvement in terms of efficiency to initiate a change from the current virus propagation methods. As the TT1 cell passages increased, there was a noticeable decrease in the APV titer. To avoid this problem, an immortalized turkey turbate cell line would be ideal. The next step for determining the efficacy of APV propagation in TT1 cells lines will be to test the virus with an in vivo infection model. Development of a highly efficient APV vaccine will be valuable for large flocks, since APV annually causes significant economic losses to Minnesota's turkey industries.

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GROWTH AND ISOLATION OF REOVIRUS REASSORTANTS FROM STRAINS DEARING AND JONES

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Reoviruses of the family *Reoviridae* have a genome that consists of 10 discrete double stranded RNA segments: three large (L), three medium (M), and four small (S), surrounded by a capsid made up of eight structural proteins. Like influenza viruses, reoviruses can evolve by creating unique progeny virions through a process called reassortment in which there is an exchange of individual segments between the genomes of different strains. This reassortment occurs when cells are infected with more than one strain of reovirus. It was previously shown in our lab that reovirus strain Jones grew in a particular cell line, but Dearing was unable to replicate. To determine what viral gene is responsible for this difference, reassortants with different combinations of gene segments from the two parental strains were generated for use in growth experiments. In this study, we isolated viruses from cells that were coinfecting with strains Dearing and Jones. Double stranded RNA was isolated from these viruses so that the genome segments could be examined by SDS-polyacrylamide gel electrophoresis to determine the parental origin of each gene segment. Thus far, seven reassortants have been isolated and identified. They appear to be monoreassortants in which all gene segments, except for one, are Dearing in origin. Further experiments will use these Dearing and Jones reassortants in an attempt to determine what is responsible for the variation in growth phenotypes.

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GENE THERAPY FOR LUNG FIBROSIS

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Pulmonary fibrosis occurs when airway fibroblasts do not undergo apoptosis after fulfilling their function resulting in gas exchange abnormalities. This apoptotic resistance derives in part from an aberrantly activated translation initiation apparatus. Our objective is to chemosensitize fibroblasts to proapoptotic drugs by decreasing cap-dependent translation using members of the 4E-BP translational repressor family. To accomplish this, we propose to develop an adenoviral vector harboring a mutant of wild type 4E-BP1, (4E-BP1 S65A which has a serine replaced with alanine at the sixty-fifth amino acid rendering it resistant to physiological inactivation), and use it to infect target fibroblasts. 4E-BP1 S65A decreases cap dependent translation by sequestering a key component of the translational machinery, eIF4E. pShuttle CMV, a plasmid containing 4E-BP1 S65A, was linearized with PME1 restriction enzyme prior to electroporation into *E. coli* BJ5183 Ad-1 for recombination with the pAdEasy plasmid already in the cell. Recombinants were selected by taking advantage of the Kanamycin (pShuttle CMV) resistance and Ampicillin sensitive (pAdEasy 1) of the recombinant gene. AdenoBP1IRESeGFP recombinants were harvested and transfected into *E. coli* DH5-alpha, which are recombination deficient, to produce more plasmid. AdenoBP1IRESeGFP was transfected into 293 cells for adenoviral production. After titering the adenoviral vector containing 4E-BP1 S65A for infectious particles/ml, it was used to infect naïve 293 cells for viral production. High titer virus was used to infect human and murine fibroblasts. Successful introduction of the therapeutic gene into target lung fibroblast cells was indicated by the green fluorescent tag of 4E-BP1 S65A and by western blots showing the presence of exogenous 4E-BP1 S65A. Therefore, we successfully produced a potentially therapeutic adenoviral vector which is ready for preclinical *in vivo* testing.

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SNP DEVELOPMENT FOR INTEGRATIVE MAPPING IN THE TURKEY (*MELEAGRIS GALLOPAVO*)

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The primary turkey linkage map is based on expressed sequences and was developed using restriction fragment length polymorphisms (RFLPs) detected with cDNA clones. The mapping families used to create this map were based on a F-2 backcross design. A second-generation linkage map is currently being developed based on polymorphic microsatellites (simple sequence repeats) utilizing a new robust mapping population. While the primary linkage map provides greater information on the functional genome (e.g. expressed genes), Southern blot detection is laborious and requires great quantities of genetic material. Therefore, an alternative approach is needed to integrate the linkage data of the primary map with that of the emerging microsatellite-based map. This project is designed to develop SNP (single nucleotide polymorphism) markers to integrate the two maps. By amplifying 5' and 3' untranslated regions and introns from genomic DNA, SNPs were discovered in the genes of the original cDNA clones. These are being used to genotype the F2 progeny of the new resource family.

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MECHANICAL CUTANEOUS HYPERALGESIA IN THE RAT MODEL

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Recent evidence suggests that cannabinoids may have analgesic effects that occur through peripheral mechanisms. Since skin blisters are painful, we investigated whether cannabinoids applied directly to a blister might decrease the pain. The aim of my research is two fold: 1) to develop a model to study hyperalgesia following a skin blister in the rat, and 2) to test the effects of various cannabinoid drugs in alleviating the hyperalgesia. Once animals are anesthetized with halothane, a suction blister is made on the plantar surface of the hindpaw using a vacuum (45-60 mm Hg), applied through a 3 mm diameter capsule. Shortly after formation of the blister, all animals exhibited mechanical hyperalgesia as measured by an increase in the percentage of paw withdrawals to controlled mechanical stimuli applied to the blister lesion. The average percent of trials in which a withdrawal response occurred was 0-20% before the blister, and this response increased to 70-100% following the blister. A cannabinoid drug, WIN 55,212-2 at a concentration of 20ug/uL was applied directly to the blister, and did not alter the mechanical hyperalgesia. It is concluded that a suction blister in rats produces profound mechanical hyperalgesia, but that cannabinoids are not effective in alleviating this form of hyperalgesia.

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TESTING THE ROLE OF BETA-CATENIN IN THYMIC POSITIVE SELECTION

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Thymic selection is responsible for developing the repertoire of T-cells present in the body. Positive selection promotes the development of the thymocytes that eventually make up the body's T-cell repertoire. Interactions of the T-cell receptor (TCR) with specific self-peptide/MHC complexes are essential for the positive selection process. Relatively few self-peptides, one of these being Beta-Catenin, appear to participate in the interactions. We have studied the eight peptide sequence of Beta-Catenin that is involved in the positive selection process. To better understand the dynamics of the interactions between this sequence and MHC/TCR, we studied the effects of mutations to the eight peptide sequence using specific assays that measure ability to bind the TCR and MHC. Beta-Catenin proteins with mutations that disrupt binding will be prevented from participating in the positive selection process. We plan to express the mutants in a mouse using a knock-in construct and examine the extent to which they effect the positive selection process. Our aim is to develop a mouse model that may provide important insights into selection in general as well as the specific role that self peptides play.