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### α 1-ADRENERGIC ACTIVATION PATHWAY OF THE SODIUM/HYDROGEN ION EXCHANGER IN CCL-39 CELLS

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### ASSOCIATION AND ACTIVATION OF ERK1/2 WITH MICROTUBULE DURING SEA URCHIN EGG FERTILIZATION.

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Microtubules are long polymers consisting of α and β tubulin dimers. Microtubules provide several important cellular functions including structural support of the cell and chromosomal distribution during mitosis. Several different proteins are tightly associated with microtubules throughout the purification process. One of these proteins is the mitogen activated protein kinase (MAPK). MAPK has been found to be involved in the regulation of the assembly of microtubules in several cell lines. In the unfertilized egg, a large portion of tubulin is unassembled. Shortly after fertilization, tubulin is polymerized into microtubules. It is very likely that MAPK is involved in altering the dynamics of assembly in sea urchin eggs during the first stages of development. To determine if MAPK co-purifies with sea urchin egg microtubulin, microtubules were isolated by successive cycles of temperature-dependent assembly and disassembly. The product this procedure resulted in highly enriched preparation of microtubulin and microtubule-associated protein. The presence of MAPK was determined by immunoblot analysis. Phospho-specific MAPK antibodies were used to determine the activation level of the microtubule-associated MAPK. To investigate if fertilization of sea urchin eggs lead to the activation of MAPK, isolated eggs and sperm were incubated for various incubation periods. The level of MAPK activation was determined using a phospho-specific MAPK antibody. This data will help to determine the identity of two unknown proteins, which were implicated in an earlier study in microtubule assembly in sea urchin embryos.

### SYNTHESIS OF POLY(NAPHTHENYL KETONE) VIA POLY(NAPHTHENYL METHYLENE)

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Previous research showed how soluble poly(arylene methylene)s could be synthesized from 2-chloromethyl-napthalene via Friedel-Crafts self-condensation of the substrate in the presence of stannic chloride, $SnCl_4$. This polymer was selected as the starting material for this experiment. Selective oxidation of the methylene bridge in the poly(naphthenyl methylene), a benzylic position, will be attempted to form a ketone linkage. The product of this reaction should be a poly(naphthenyl ketone). This methodology is being attempted as previous direct methods have failed The extended conjugation of the polyketones should produce a polymer with greater electrical conductivity.

### CONTROL OF WILD-TYPE MRNA ACCUMULATION BY THE NONSENSE-MEDIATED MRNA DEGRADATION PATHWAY IN BAKER'S YEAST

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We are interested in the control of *CTF13* mRNA accumulation by nonsense-mediated mRNA degradation (NMD). NMD is responsible for accelerating the degradation of mutated mRNAs which contain a premature stop codon (nonsense mRNAs). These mutant mRNAs might otherwise accumulate and promote the production of their corresponding truncated proteins, which can lead to cancer and genetic disease. In baker's yeast, the NMD pathway also controls the abundance of some wild-type mRNAs, including that of *CTF13*. *CTF13* encodes a protein that functions in chromosome transmission during cell division and its mRNA has been shown to accumulate when NMD is inactivated. This mRNA accumulation suppresses the temperature-sensitive allele *ctf13-30*, which carries a missense mutation. We've been conducting experiments to address the hypothesis that NMD influences the synthesis of the *CTF13* mRNA by controlling the degradation of a mRNA encoding a protein regulator of *CTF13*. As a first approach, we've analyzed reporter mRNA expression from the *CTF13* promoter for NMD-dependent effects. The initial results of these experiments indicate a lack of NMD-dependent effects, and we are currently doing experiments to confirm these findings. As a second approach, we've isolated and characterized suppressors of *ctf13-30* in an effort to identify the putative *CTF13* regulator encoding a NMD substrate mRNA. Three genes have been identified. The effect of NMD on the suppressor gene expression and the suppressor genes effects on *CTF13* expression are presently being characterized Results from experiments now in progress will be shown.

### UNCOVERING THE *IN VITRO* PROPERTIES OF C4 PYRUVATE, ORTHOPHOSPHATE DIKINASE REGULATORY PROTEIN, RP, A MOST UNUSUAL DUAL REGULATORY PROTEIN KINASE/PHOSPHATASE

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Pyruvate,orthophosphate dikinase, PPDK, is a central enzyme of the $C_4$ photosynthetic pathway found in maize. Because it is a photosynthetic enzyme, it is inactivated at night and activated during the day. A specific protein kinase called PPDK regulatory protein (RP) is responsible for this light/dark regulation via reversible phosphorylation of PPDK. Little is known about the functional properties of RP because of its extreme instability once removed from the leaf for study. Recently, we have developed antibodies specific for the inactivated (phospho-Thr -456) form of $C_4$ PPDK. These polyclonal antibodies were generated against a synthetic phosphopeptide composed of 20 residues flanking the Thr-456 region of the maize $C_4$ polypeptide. We have employed these antibodies as a new tool for assessing RP kinase activity in an *in vitro assay*. This assay utilizes rapidly extracted RP from maize leaves for the subsequent *in vitro* phosphorylation of recombinant maize PPDK. Phosphorylation of the target enzyme is then quantitated by immunoblot analysis of phospho-PPDK A comprehensive analysis of the *in vitro* properties of RP with respect to dark/light regulation of PPDK will be presented.

### THE EFFICACY OF MUSSEL RELOCATION AS A RESOURCE MANAGEMENT TOOL: AN EXPERIMENT IN THE ST. CROIX RIVER

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Increasing threats to the native mussel community in the St. Croix River (e.g. bridge construction, zebra mussel outbreaks) make it necessary to study the efficacy of relocating mussels to less-threatened parts of the river. To determine the effects of relocation on mussel growth and survival a three-year *in situ* experiment was conducted at Wild River State Park, Minnesota. In 1997 a 25 $m^2$ study grid containing 25 cells was placed near the confluence of the St. Croix and Sunrise rivers (reference site), and another was placed at the eastern boat launch at Wild River State Park (relocation site) Each cell was randomly assigned one of the following treatments (1) double resident mussel density, (2) addition of 10 pimplebacks *(Quadrula pustulosa)*, (3) addition of 10 spikes *(Elliptio dilatata)*, (4) addition of 10 pocketbooks *(Lampsilis cardium)*, and (5) control (no manipulation occurred during the first year). In 1997 mussels were collected

from the reference site, placed into study grids, and individuals from the first four treatments were measured, weighed, and marked. In 1998 and 1999 mussels were measured and weighed. Those found without a number were recorded as "new" and marked; those missing from the 1998 or 1999 census were recorded as "missing", and the rest were recorded as "recovered", "control", or "dead" as applicable to their status. Preliminary examination of data indicates no difference in growth or mortality between treatments. Mortality was low (5%) compared to similar studies (Cope and Waller 1995). Results suggest that relocating mussels to similar habitats may be an effective strategy for conserving mussel populations living in potentially harmful parts of the St. Croix River.

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Suggested Reading: Cope, W. G. and D. L. Waller. 1995. An evaluation of freshwater mussel relocation as *a* conservation and management strategy. *Regulated Rivers: Research and Management* 11:147-155.

### Competitive PCR Products as Positive Controls in PCR-based Assays for Tick-Borne Pathogens

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The deer tick, *Ixodes scapularis,* carries several diseases, including Lyme disease and human granulocytic ehrlichiosis. These are serious diseases and they are difficult to diagnose by conventional methods. Polymerase chain reaction (PCR) based tests have the potential to detect fewer organisms sooner after infection, but have the problem of false positives caused by DNA contamination. If modified pathogen DNA molecules are used as positive controls rather than genomic pathogen DNA, the likelihood of contamination and false positives will be reduced. My work involved developing such modified positive controls, using a technique of creating deletions by PCR. Because these products are of different sizes than genomic pathogen DNA, contamination of reactions with the positive control can be readily identified.

### Factors Contributing to the Spatial Distribution of Herbaceous Plants in a Minnesota Oak Woodland

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Ecologists struggle to explain the spatial distribution and patchiness of plants. The goals of this study, which took place at the Cedar Creek Natural History Area in East Bethel, MN, were (1) to determine if plant species and functional group distributions are associated with specific environmental variables, and (2) to determine if thatch ant mounds alter environmental conditions on a micro-scale, and if so, to see if these altered conditions are associated with corresponding small-scale changes in vegetation. Vegetation was sampled in three burn units (frequently burned, infrequently burned, and never burned) at ant mounds and at control points away from mounds. Soil moisture, ammonium, extractable nitrates and total nitrogen levels were sampled at mounds and control points; leaf area index, total nitrogen, burn history and elevation data were acquired for sample sites. Analyses showed that (1) along a fire gradient, vegetation in functional groups is associated with all environmental variables, (2) ant mounds act as small- scale disturbances and influence the distribution of functional groups, especially nonnative forbs and grasses, and (3) as small-scale disturbances, ant mounds influence vegetation patchiness on a small-scale, whereas large-scale patchiness is more affected by large-scale disturbances, namely fire.

### Developmental Expression of Protease Activity in Soybeans

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Soybean plants are a major source of revenue in the agriculture industry. Plants are being engineered to have high germination rates and crop yields. One major problem still facing the industry is pest control. The major pest to the soybean plant is the cyst nematode The nematodes are able to infest soybean roots, where the cysts that they form block nutrient flow from the root system to the remainder of the plant. Because the nematode has a thiol protease located in its digestive system it is possible that the damage done by the nematode could he prevented by a thiol protease inhibitor. The possibility for a TG soybean plant resistant to the nematode thus exists. If the plant were able to produce a thiol protease inhibitor in its root system the nematode would be prevented from causing any harm. If a thiol protease were needed during the normal development of the plant, however, a thiol protease inhibitor would be detrimental to the growth of the plant. The purpose of this study is to determine which types of proteases are important in the development of the soybean plant. Various tissues of the plant (roots, stems and leaves) are sampled during different stages of development. The proteins are extracted and analyzed through SDS-PAGE and protease activity is checked by using an Azo-Casein assay and zymogram gels. Tissues showing protease activity will then be further analyzed for the type of protease present. Preliminary work indicates protease activity in seedlings and the root systems of two-week old plants.

### Kinetic Properties and Regulation of Glutamate Dehydrogenase by Substrate Concentration and pH

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Eukaryotic glutamate dehydrogenases are highly regulated enzymes, which have the ability to function with either NAD+ or NADP+ as cofactors in the oxidative determination of glutamate. The enzyme is subject to both homotropic and heterotropic regulation and under some circumstances shows potent substrate inhibition by glutamate. Physical basis of the regulation is unknown, but it is thought to involve the subunit-subunit interactions. Specifically, we were interested in the pH dependence of the substrate interactions, a phenomena thought to involve not only subunit interactions, but also to the involved in the regulation by GTP and ATP. Through the series of initial rate kinetics experiments with variable glutamate concentrations and set concentrations of the NAD+ in series of pH values, we investigated the extent and mode of the substrate inhibition as a function of pH. Preliminary data at pH 6, 6.5 and 7 showed the substrate inhibition being a function of both the inflection point of the plot and the ratio of the activities at high and low glutamate concentrations. The future experiments will focus on the effects of pH ranging from 7.5 to 9. Furthermore, since the structure of the enzyme has recently been elucidated by x-ray crystallographic means, the pH dependence of these effects may give clues at to the types of amino acid side chains that mediate the interactions between the active site and the various regulatory sites on the enzyme.

### Results From a Two-Year Survey of Fungi and Lichens in the Black Hills of South Dakota

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Mushrooms and other fleshy fungi were collected four times throughout the summers of 1998 and 1999, from each of five permanent sites and less frequently from several additional sites in the Black Hills. Results (site, county, date, species, major taxonomic group, primary and secondary vegetation at the site, substrate upon which the fungi were growing, land use, edibility and collection number) were sent to the South Dakota Natural Heritage Database. Three-hundred and seventy-four specimens representing 164 different species were collected in 1999 compared to 313 specimens representing 150 different species collected in 1998. Two-hundred and thirty-six different species were collected during the 2-year study, and 38% of these were collected only in 1999. Only two species, *Collybia dryophila* and *Inocybe fastigiata,* occurred at all five permanent sites during the

study. August was the best collecting month for both years, but in 1999 July was almost as productive as August. In 1999, 55% of the species collected were agarics compared to 1998 collections, in which 57% of the species collected were agarics. A site near Rochford, South Dakota dominated by *Pinus ponderosa and Picea glauca* had the greatest diversity of species (45 different species) in 1998. In 1999, Eleventh Hour Spring, west of Spearfish, South Dakota dominated by *Populus tremuloides* and *Pteridium aquilinum* contained the most diverse number of species (52 different species).

### Assessing the Light Activation and Dark Inactivation Kinetics of the $C_4$ Photosynthesis Enzyme, Pyruvate,Orthophosphate Dikinase, in Maize Leaves Using a Novel Phospho-Antibody Approach

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Compared to other cereal grains, maize is a remarkably productive crop species because it has a highly advanced form of photosynthesis called C4-photosynthesis. A key enzyme of C4 photosynthesis is pyruvate,orthophosphate dikinase (PPDK). Evidence is mounting that PPDK is perhaps the most rate limiting enzyme of the pathway As with several other enzymes involved in photosynthetic carbon metabolism, $C_4$PPDK is inactivated in darkness in order to prevent futile cycling of metabolic intermediates. This inactivation is due to the reversible phosphorylation of an active-site Thr residue (Thr-$^{456}$ in maize) and is mediated by PPDK regulatory protein, RP, a bifunctional regulatory protein kinase/ phosphatase. Using an immunological approach via probing soluble rapid leaf extract immunoblots with PPDK-Thr$^{456}$-P antibody, we have assessed the kinetics of the RP phosphatase and kinase functions as they occur in the leaf during light/dark transitions. When dark adapted leaves are exposed top light, dephosphorylation (activation) of PPDK is rapid and nearly complete after 5 min. When illuminated leaves are placed in the dark, phosphorylation (inactivation) is slower, requiring up to 30 min for complete phosphorylation of PPDK. Corresponding data on the in vitro activity of RP during light dark transitions will also be presented.

### Murine Strain Dependence of IgA Production Following Oral Immunization with Reovirus T1L

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The influence of genetics on immunity and health is evidenced by extensive literature. A model used to study genetic differences in immunity is the inbred mouse, as each strain is representative of one genetically unique individual. This study investigates the differential IgA production of two mouse strains following oral immunization with reovirus T1L. Reovirus is an enteric pathogen with demonstrated capability of inducing an immune response. A quantitative enzyme-linked immunosorbent assay (ELISA) is used to determine concentrations of fecal IgA in C3H and C57BL mouse strains over a 28-day time course. No significant differences were found in reovirus-specific IgA production. However, the number of deaths between days 7 and 14 post-inoculation differed significantly for the two mouse strains ($p=0.0325$). This may indicate that the differences in immunity between these strains is not related to the IgA response. A possible explanation is that C3H mice are more susceptible to viral spread. Ongoing studies will examine nonspecific fecal IgA production as well as specific and nonspecific IgG concentration in plasma.

### Potential Use of *Potentilla tridentata* and *Danthonia spicata* in Restoration Efforts at Minnesota's Tettegouche State Park

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Vegetation on thin soils such as those found on Shovel Point in Tettegouche State Park, MN is vulnerable to damage from park visitors. Park managers are concerned that damaged areas are increasing in size, therefore, they are searching for practical, biologically sound restoration methods. Possible solutions range from unassisted revegetation, to ameliorating soil conditions and revegetating with local and exotic species. Reports from literature sources suggest that unassisted revegetation in environments similar to Shovel Point can require more than 100 years, but revegetation using transplants can greatly reduce the time needed. *Potentilla tridentata* and *Danthonia spicata are* herbaceous species which are native to Shovel Point and might be useful as transplants. Since the germination requirements for these species are not well known we have tested their germination rates on moist filter paper at room temperature (rt) with and without an overnight treatment of 400 ppm gibberelic acid ($GA_3$). For *P. tridentata* we have also tested germination rates at room temperature after two and four weeks (4wk) at 4°C. The treatments of 50 seeds each were done in triplicate. For *P. tridentata* germination rates ranged from 27 to 72 percent for the rt and 4wk treatments respectively, and for *D. spicata* preliminary results suggest that germination is inhibited by 400 ppm $GA_3$, We conclude that the 4wk treatment at 4°C will be useful in germinating *P. tridentata* for the purpose of transplantation experiments on Shovel Point.

### Does the Tick, *Rhipicephalus sanguineus*, Have an IgG Binding Protein?

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Recent research shows that the African tick, *Rhipicephalus appendiculatus,* produces a protein that binds the mammalian antibody Immunoglobin G (IgG). This protein is secreted by male ticks feeding directly adjacent to females and provides improved feeding efficiency. The goal of my project is to determine whether a homologous protein is produced by the related species *Rhipicephalus sanguineus.* A DNA fragment of approximately the correct size has been isolated by using PCR and is now being sequenced. The future path of this research will be to determine whether IgG binding proteins are present in other tick species. This research may also have implications in the understanding of tick feeding behavior and in the development of anti-tick vaccines.

### Cytogenetic Analysis of Deformed Frogs

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The increased incidence of deformed frogs in North America has become a concern since amphibians are seen as indicators of environmental quality. The deformed frogs which have been reported in numerous Minnesota counties are frequently missing hind limbs or have extra limbs. While possible explanations for such developmental abnormalities include parasite infestations, ultraviolet radiation and pesticide usage, there has been insufficient evidence supporting any single causative agent. It is possible that environmental agents have damaged the genetic material of the afflicted frogs. In order to begin to address that possibility, metaphase chromosome spreads were prepared from the peripheral blood of adult northern leopard frogs *(Rana pipiens)* and mink frogs *(Rana septentrionalis)* collected from several Minnesota counties. Blood was collected lay cardiac puncture and cultured in the presence of mitogen and colcemid. After fixation, good quality metaphase plates were analyzed. All metaphase plates had the normal chromosome number (2N = 26) and morphology, and selectively stained metaphase plates had the normal number of nucleolar organizer regions (NOR = 2). It is possible that the deformed frogs that survive to adulthood have normal chromosomes, while the animals with severe chromosomal defects die as embryos or larvae. Thus, chromosomes were prepared from embryonic *R. pipiens* collected at Minnesota sites known to have a high incidence of adult frog deformities. All analyzable embryonic chromosome preparations were normal in number and

morphology. While some finer-resolution banding procedures have been attempted on these animals, a more detailed analysis of the frog chromosomes is technically difficult.

### Cloning and Sequencing the Gene for the C4 Photosynthesis Enzyme, Pyruvate,Orthophosphate Dikinase, from the Polyploid Tall Grass Prairie species, *Andropogon gerardii* (Big Bluestem)

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The Tall Grass Prairie ecosystem represents a rich source of floristic biodiversity. A dominant fraction of the ecosystems flora are grasses that use the C4 photosynthesis. An unusual number of these "C4" grasses are polyploid (6N, 8N). In order to explore the theory that polyploidy is related to adaptive value via genesis of gene diversity in a prairie ecosystem, we have cloned the gene for pyruvate orthophosphate dikinase, PPDK, a key $C_4$ photosynthesis gene from the $C_4$ grass Big Bluestem *(Andropogon gerardii)*. This full length cDNA clone was then used to probe Big Bluestem genomic Southern blots. Analysis of these blots will be used to determine the effect of polyploidy on PPDK gene copy number and hence gene potential diversification due to polypoidy. corresponding comparative analysis of the complete coding sequence for the PPDK gene will also be presented.

### Post Conflict Color Change Losing *Anolis anolis* Tactic for Camouflage or Gesture of Submission?

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Animals, that have the ability to change their skin color rapidly, can use this ability as camouflage and to signal social status. Much has been documented about the green angle *(Anolis carolinensis)* and its social status communication with skin color. Little is known about social signaling in its close relative, the brown *anole (Anolis sagrei)*. I investigated whether *A. sagrei* turned a different value of brown after losing fignts with conspecifics. I also determined whether these losers chose backgrounds that either camouflage them from predators or contrasted with their color to signal their losing status to other conspecifics. I observed fights between adult male angles and compared the skin color of the losers before and after each fight. I also observed the color of the perch losing angles 'escaped to after each fight. In my first experiment, I paired lizards into eight separated dyads, so that each lizard would lose and win a fight to a different angle. In a second experiment I housed lizards in a communal terrarium where they interacted freely Terrariums, in which the lizards were housed, were furnished with perches painted in two values of brown representing the two extreme color variations of *A. sugrei* skin color. In both experiments, losers were darker after then before fights. In the communal tank losers preferred light perches over dark whereas in the dyadic encounters perch selection was random. Losers in the communal tank may have been maximizing a submissive status signal to other anoles to avoid additional conflicts.

### Movements and Space Utilization by Gunnison's Prairie Dogs *(Cynomys gunnisoni)* Living Multiple Years in a Colorado Colony

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Movements and space utilization were studied in Gunnison's prairie dogs *(Cynomys gunnisoni)* living for multiple years on our study site in Archuleta County, Colorado. A 3-ha portion of a larger prairie dog colony was live-trapped during the summers of 1991 through 1997. Each captured prairie dog was weighed, aged, sexed, ear-tagged, and marked with a unique design using Nyanzol D dye. Reproductive condition and capture location were recorded before the animal was released. The 3-ha area was divided into 24 blocks (each 25 m by 50 m); all burrows were then mapped. Approximately 350 prairie dogs were ear-tagged between 1991-1997. Of these, only 30 females And 10 males were captured for more than 1 year. Capture locations indicated that these 40 animals concentrated most of their activities within 8 of the 24 blocks. Of the 10 males, 7 were captured both as pups and as adults. As adults, 3 were caught at least once in the block(s) in which they had been caught as pups, while the other 4 moved to more distant locations. It was more common for females than males to remain in or near their "natal block(s)" over several years. By looking at common burrow use, we could sometimes reconstruct possible family units. It appeared that some of the longer-lived animals also had a longer-lived mother and/or sisters. We will further investigate possible reasons why longer-lived animals seemed to be concentrated into certain areas of the study area, including access to optimal burrow sites and social stability.

### Potential Involvement of a Protein Component in Fish Alarm in Fathead Minnow

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Fishes in the superorder Ostariophysi represent about 64% of all freshwater fish species (minnows, catfish, suckers, tetras, etc). One trait common to members of this group is specialized skin cells that contain an alarm pheromone. A pheromone is a chemical signal. When a predator attacks and ruptures the skin cells, the pheromone is released, warns nearby minnows of the predator and induces an antipredator behavior. The chemical nature of this pheromone is not clearly understood. Early reports suggested hypoxonthine- 3(N)-oxide (H3NO) is the signaling molecule. However, independent tests using Pure H3NO do not elicit behavioral or physiological responses. In this study, the presence of a protein component of the alarm substance in fathead minnows *Pimephales promelas* was tested. An alarm pheromone solution was prepared from the skins of fresh minnows. The solution was then heated or unheated and denatured protein removed by centrifugation. The resulting supernatant pheromone solution was used to test aquaria containing individual fathead minnows for antipredator behavior. If protein is a component of the pheromone response, then denaturing the protein with heat should render the solution ineffective. Preliminary data indicate that heat denaturation of the sample resulted in a loss of alarm response. Additionally, the total protein concentration in these samples caused by heating was significantly decreased. No significant changes in pH was determined after heat treatment. Finally, the related compound, hypoxanthine, was used to model the effect of heat on alarm pheromone. These studies should further elucidate the chemical nature of an important biological alarm signal in fishes.

### Characterization of Adh Gene in Transformed Lines of *Drosophila melanogaster*

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The alcohol dehydrogenase (Adh) gene of *Drosophila melanogaster is* an excellent model system that has allowed researchers to combine genetic and molecular approaches to questions of development and gene expression. The Adh gene is extensively studied and provides a model for the mechanism of developmental biology because it exhibits expression in a tissue specific manner. The purpose of this study was to characterize the Adh expression regulated by tissue-specific position effects in larvae of genetically transformed *Drosophila melanogaster*. Transformed lines of *Drosophila* had been isolated by the insertion of an Adh gene fragment into an Adh negative strain and nine lines were generated. To determine if the Adh gene had integrated into the genome, adults were ethanol selected A histochemical staining assay was used for detection of Adh expression in larvae and the dissected larvae were mounted on slides and scored. The results of this experiment show a range of possible patterns and levels of Adh expression. By comparing the stained tissues of the transformed lines with stained somatic tissue of a normally expressing larva, the results were that all nine transformed

lines expressed some Adh activity; however, none of the transformed lines exhibited normal patterns of Adh expression. A significant feature of the results was that a restricted pattern of expression was observed. Five lines exhibited patterns not before observed in previous research. Understanding the mechanism of these position effects is an important step in developing, practical uses for transferred genes in genetically engineered organism and particularly in using gene transfer for human gene therapy.

### AN ANALYSIS OF STABLE CARBON ISOTOPE RATIOS FROM FOSSIL *CELTIS* FRUITS

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Stable carbon isotopes have been shown to be sensitive environmental indicators. We have studied stable carbon isotope ratios in fossil *Celtis* (hackberry) fruits. Isotope ratios have shown an environmental gradient which can be traced over the last 15 million years. The evidence from the fossil hackberry fruits shows an increasing amount of water stress on plants in the Great Plains over the time period studied.

### OBESITY AND FERTILITY IN THE LETHAL YELLOW MOUSE

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The lethal yellow mouse possesses a mutation at the agouti locus on chromosome two. This mutation is lethal in the homozygous condition, (Ay/ay). In the heterozygous condition (Ay/a), mice live but experience altered coat color, decreased immunocompetence, increased obesity with age, and reduced fertility. These characteristics are collectively referred to as the lethal yellow syndrome (LYS). Many details of the effects of the gene's mutation are still being investigated, including how the mutation causes or contributes to a decrease in fertility.

Based on knowledge of how obesity affects fertility, it was hypothesized that the obesity the lethal yellow mouse experiences contributes to, or is even responsible for, the high frequency of infertility in this mutant. Decreased estrous cycling has been observed in obese yellow females. In this study, forty female mice were split into two equal groups. One group of ten yellow, mutant, female mice and ten black, control, female mice were raised on a high (10%) fat diet. The second group comprised of ten yellow, mutant, female mice and ten black, control, female mice were raised on a low (4%) fat diet. Once the females were 100 days old, each individual female was mated with a black male. All mated mice were placed on a high fat diet to make pregnancy and lactation possible.

Analysis of the data revealed that weight restriction provided by the low fat diet improved the fertility of lethal yellow mice. Restricting their weight allowed for improved fertility, increasing the production of litters. The data suggest that obesity rather than some other effect of the mutated gene causes infertility in the lethal yellow mouse.

### EFFECTIVENESS OF TRITERPENOID COMPOUNDS AND POLYETHYLENEIMINE DERIVATIVES OF BETULIN AGAINST HUMAN PATHOGENIC *CANDIDA* SPECIES.

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### RITANSERIN ALLEVIATES TICS IN A TRANSGENIC MODEL OF TS+OCD

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### NHE1 ACTIVATION BY LYSOPHOSPHATIDIC ACID AND PHENYLEPHRINE IN CHINESE HAMSTER LUNG (CCL39) CELLS.

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The $Na^+$-$H^+$ exchanger is a $Na^+$-dependent, amiloride-sensitive, proton extrusion mechanism found in virtually all eukrayotic cells. Six isoforms of the exchanger have been identified to date, NHE1 - NHE6. The NHE1 isoform of the $Na^+$-$H^+$ exchanger is the only isoform expressed in Chinese hamster lung cells (CCL39). In these cells, the exchanger's primary function is the regulation of intracellular pH (pHi). The exchanger can be activated by an increase in intracellular $H^+$ or through stimulation by a variety of agonists. We have demonstrated that both Iysophosphatidic acid (LPA) and the α-adrenergic agonist phenylephrine (PE) activate the $Na^+$-$H^+$exchanger at normal steady-state pHi. Experiments have shown that LPA increased steady- state pHi by 0.2-0.3 pH units. Likewise, PE stimulation of NHE1 induced a 0.15-0.2 pH unit increase in steady-state pHi. To determine whether the LPA induced change in steady-state pHi was due to a shift in the pHi-dependence of the exchanger, cells were allowed to recover from an intracellular acid load in the presence and absence of LPA. These data show that LPA activation of NHE1 caused a shift in the pHi-dependence of transport between 0.15 and 0.2 pH units in the alkaline direction. We hypothesize that LPA activation of NHE1 occurs through a $G_{13}$-dependent mechanism that involves the activation of RhoA, Phospholipase D and MAP Kinase. Additionally, we hypothesize that PE activation of NHE1 occurs through a $G_q$-mediated pathway that involves the activation of Phopholipase Cβ, Protein Kinase C and MAP Kinase.

### DETERMINING WHETHER THE DEER TICK, *IXODES SCAPULARIS*, HAS IgG BINDING PROTEINS

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Parasites need a way to protect themselves from their host's immune system. To prevent internal damage from host IgG the African tick, *Rhipicephalus appendiculatus,* uses immunoglobulin-binding proteins (IGBP) to bind and excrete host antibodies. Male *R. appendiculatus* transfer IGBP to females when feeding. In the Midwest, the vector for Lyme disease is the deer tick, *Ixodes scapularis.* Based on mating and feeding behavior observations, we investigated whether *I. scapularis* uses IGBP. We used PCR with primers specific for the *R. appendiculatus* IGBP gene to try amplifying the related gene in *I. scapularis.* DNA sequencing was used to verify the PCR product's identity.

### ANION ANALYSIS OF LAKE BEMIDJI USING ION CHROMATOGRAPHY

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Nutrients in the form of anions can be a significant cause of lake eutrophication. Anions such as phosphates and nitrates are often growth limiters in aquatic systems, Ion chromatography can be used to monitor levels of various anions down to parts per million levels. Water samples were taken from surface and subsurface depths from various locations over a period of time. The samples were analyzed on a Dionex ion chromatograph with a Dionex IonPac AS 1 1 anion column. Qualitative and quantitative analysis of lake water samples were performed to detect the anions present to the detection limits of the instrument.

### ROLE OF SEX HORMONES IN IMMUNITY TO REOVIRUS

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It was found in an earlier study by a colleague, Wendell Patton, that sex hormones seem to play a role in the fostering of a general IgA response in mice. The purpose of my current

study is to further explore this relationslip between sex hormones and an IgA and IgG response through a more comprehensive experimental design. In my ongoing sex study, male and female mice have had gonadectomies and supplementation with opposite hormone, i.e. males receive estradiol and females receive testosterone. 5 male and 5 female ND4 Swiss mice have had their gonads removed and are receiving daily injections of methyl testosterone at 1µg/g or estradiol benzoate at 0.1 ʊg/g. The hormones have been dissolved into a cyclodextrin/saline solution. Mice are used as their own control, and have had baseline IgA and IgG levels taken for 28 days prior to oral immunization with T1Lang virus. Further samples will be taken for 25 days after the oral immunization, and levels of IgA and murine IgG will be observed using ELISA and a standard curve.

### The Effect of Creatine on Amateur Athletes

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Creatine (Cr) is a nutritional supplement that is widely used to promote strength gain and increase athletic performance. Increasing the Cr pool in the body leads to an increased level of the high energy intermediate creatine phosphate (PCr). During short bursts of anaerobic exercise. PCr supplies energy through the breaking of the high energy phosphate bond. The phosphate is donated to adenosine diphosphate (ADP) to regenerate adenosine triphosphate (ATP) which is used by the muscles for energy. The aim of this study was to see whether Cr supplementation would help college football players maintain their strength during an intense two week training period. Eight consenting division three football players were randomly assigned to either a Cr treatment or a placebo treatment. Identical physical tests of maximal bench press were conducted before and after the supplementation protocol. It was found that the mean difference in repetitions was different for those subjects in the Cr group compared to the placebo group (P=.0253). The results of this study show that Cr can help maintain and even increase strength during a two week period of intense exercise.

### Molecular Modeling of Phosphofructokinase-1 for *Thermus thermophilus*

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Phosphofructokinase-1 (PFK-1), which catalyzes the conversion of fructose-6-phosphate to fructose 1,6-bisphosphate, is perhaps the most important enzyme in carbohydrate metabolism. The activity of PFK-1 is enhanced by the presence of fructose 2,6-bisphosphate, high levels of ADP, and substrate. The three-dimensional structure of PFK-1 from *Escherichia coli (E. coli)* has a unique, dumbbell-shaped alpha helical fold. Using computer technology, a molecular model of PFK-1 from the bacteria *Thermus thermophilus* was constructed using the E. *cold* structure as a template. *Thermus thermophilus is* a bacterium that lives in a thermophilic environment. The modeling of PFK-1 will allow us to compare similar proteins from organisms that live in different environments. We hope to answer questions about adaptations on a molecular level. The three-dimensional model for the PFK-1 of *T. thermophilus* may provide structural explanations for the regulatory mechanisms associated with the enzyme along with possible commercial applications of this enzyme.

### Effect of Bead Size on Thermal Diffusivity of Porous Media

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This study experimentally determines the thermal diffusivity of porous media of 200mm and 2mm spherical glass beads. A temperature gradient was created by heating a copper plate to 50°C and placing it on the glass beads in an insulated Dewar. Temperature recordings of the beads as a function of depth and time allowed modeling of the thermal diffusivity of the media. Comparison of these results to those obtained in similar studies shows that these data support previous conclusions that bead size does not affect thermal diffusivity

### Quantitation of L-Citrulline from Mitochondrial Nitric Oxide Synthase

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Nitric oxide measurements in biological systems have become important due to the diverse role nitric oxide has in neurotransmission, vasodilation, and immune response Mitochondrial nitric oxide synthase produces nitric oxide and L-citrulline from L-arginine (1). L-citrulline has been reported to be a product in the formation of nitric oxide in a 1:1 mole ratio. Rates of nitric oxide formation have previously been measured using the oxymyoglobin oxidation assay. This method measured nitric xide formation indirectly from a coupled reaction, however no direct measurements were performed of either nitric oxide or L-citrulline. The goal of this study was to measure L-citrulline produced from mitochondria! nitric oxide synthase L-citrulline concentration was determined from a modified, colorimetac carbamyl aspartate assay from Prescott and Jones (2). This test was designed to compare citrulline measurements with those obtained with the oxymyoglobin assay. The citrulline production in permeabilized mitochondria was found to be 6.8 +/- 0.2 nmol/min/mg protein), a value 1.4-times higher than the rate of nitric oxide production. These results indicate that either the yield of nitric oxide measurement was underestimated by about a 30% or that there is another source of L-citrulline that contributes to these values. Future studies will address these possibilities.

[1] Giulivi, C., Poderoso, J.J., and Boveris, A. (1998) J. *Biol. Chem.* 273, 11038- 11043. [2] Prescott, L. M and Jones, M. E. *(1969) Anal. Biochem. 32,* 408-419.

### S-Nitrosoglutathione Metabolism in Mitochondria

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Nitric Oxide (NO) is produced by nitric oxide synthase (NOS) by converting L-arginine to L-citrulline and NO. This free radical affects various functions including neurotransmission, vasodilation, and immune response. Under certain conditions NO is stored as a bioactive pool in the form of S-nitrosothiols such as S-nitrosohemoglobin. We propose that since glutathione (GSH) is present in such high concentrations in the mitochondria that NO could combine with GSH to form S-nitrosoglutathione (GSNO). NO could then be released from GSNO by combining with GSH to yield oxidized glutathione (GSSG) and NO. This GSNO could have important biological consequences such as facilitating NO transport, prolonging its half-life, targeting delivery, and mitigating its biologically adverse effects. In order to investigate this hypothesis the formation and degradation of GSH, GSSG, and GSNO in intact rat liver mitochondria were measured by HPLC. Mitochondria were isolated from rat liver and incubated in either L-arginine (a substrate of NOS) or $N^G$-monomethyl-L-arginine (NMMA, an inhibitor of NOS). Aliquots from these incubations were taken at various time points and treated using four methods. Samples from three of the treatments were separated using HPLC and the GSNO was identified using electrochemical detection. GSH and GSSG quantification was accomplished by the fourth treatment followed by HPLC separation and detection at 254nm. Thus for three conclusions have been drawn fi:om our research: 1) there is a GSNO pool present in the mitochondria, 2) production of GSNO is dependent on L-arginine, and 3) GSNO production is inhibited by NMMA.

## Differential Activation of Mitogen Activated Protein Kinase Isozymes by Lysophosphatidic Acid and Phenylephrine

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The mitogen activated protein kineses (MAPK) are an important family of signaling molecule responsible for mediating the actions of many hormones. There ore three closely related MAPK isoforms: extracellular signal regulated kinase (ERK1/2), c-Jun N-terminal kinase (NK), and p38. The classical means of MAPK activation involve growth factors, however G proteins are also able to activate MAPK. Regulation of MAPK activity by G proteins is likely to be responsible for mediating the activities of several intracellular functions including pH homeostasis. The actions of two such agonists, lysophosphatidic acid (LPA) and phenylephrine (PE) are investigated in Chinese hamster lung (CCL39) cells and neonatal cardiac myocytes.

Both LPA and PE dramatically increased the phosphorylated state (and presumably the activity) of ERK 1/2 in a dose dependent manner. The activity of p38 was mildly activated by LPA while addition of PE showed a diminished response. Phosphorylated JNK was not detected after treatment by either agonist. LPA stimulation of ERK1/2 was 3-fold greater than that observed for PE stimulation in CCL39 cells. The stimulation of ERK 1/2 in myocytes was determined at maximal agonist concentration to illustrate the divergence of signaling pathways in various tissues types.

These data suggest that there is a distinctly different mechanism of MAPK activation by LPA and PK. The determination of which agonist provokes a specific MAPK response is a pertinent step in comprehension of cell function. This understanding will be essential in defining the links to several diseases, such as hypertension, ischemia, and neoplastic transformation.

## Serum Dependence of Intracellular pH Regulation in Cultured Neonatal Cardiac Myocytes.

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The $Na^+$-$H^+$ exchanger is a Na+-dependent, amiloride-sensitive, proton extrusion mechanism found in all eukrayotic cells. The NHE1 isoform of the exchanger is the only isoform expressed in neonatal heart cells. In these cells, the exchanger functions to regulate intracellular pH (pHi) and cellular volume. As part of its role in pHi regulation the exchanger is involved in establishing resting pHi levels. Since the exchanger can be activated by a variety of hormones and growth factors, cells that have been grown in serum containing media have higher resting pHi values than those that have been serum deprived for 12 to 16 hours. Steady-state pHi for cells in the presence of serum was 7.34 + /- 0.07. In contrast, cells which had been serum deprived overnight had a steady-state pHi of 7.04 +/- 0.06. This difference is due to changes in the pHi-dependence of transport for NHE1 in the presence and absence of serum. We have demonstrated that some of the changes in transport activity can be mimicked through exchanger stimulation by the α-adrenergic agonist phenylephrine (PE) and the purinergic agonist adenosine triphosphate (ATP). Addition of PE or ATP to heart cells that had been serum deprived overnight lead to an increase in pHi. PE stimulated an increase in pHi of 0.13 +/- 0.03 pH units, while ATP caused an increase of 0.12 +/0.03 pH units. We hypothesize that both ATP and PE activate NHE1 through a G-protein linked mechanism that involves the activation of MAP Kinase and P90rsk.