CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Sabina Bera; Andrew Murphy

Project Number
S0401

Project Title
Ideal Deoxyribonucleic Acid Detection Through Hybridization of Complementary Strands

Abstract

Objectives/Goals
Currently, all methods of detecting anthrax have ninety-four to ninety-six percent accuracy. This four to six percent margin of error can be catastrophic when viewed on a national level. The objective was to create a DNA detection system that can identify a specific DNA strand. It operates through the use of a complementary DNA strand to bind to the foreign strand to be tested.

Methods/Materials
The sequence chosen as the foreign DNA strand is named "EM07." The testing strand is a combination of two sequences (EP08 and EP07. EP07 is the complement of EM07 (the foreign DNA) and is designed to bind together. If these two strands bind, then the foreign DNA strand has been detected. Also, biotin is attached to the test strand, to make it "EP08+EP07 5' Biotin." To demonstrate that the strands bound, the test strand is designed to push off an indicator if successful. The indicator is the fluorescent dye "TET," which is attached to the sequence "EM08+EM06" (which is designed to bind partially with EP08+EP07).

Results
The indicator was isolated through the use of magnetic, streptavidin-coated Dynabeads attached to the strand EP08+EP07 5' Biotin next to the future bonding site of the foreign strand, EM07. Using a magnet, any DNA not attached to the Dynabeads can be removed (by siphoning off any excess materials floating in the presence of a magnetic field). There was no trace of TET found in any of the trials performed; hence, no TET was attached to the strand EP08+EP07 5' Biotin. This implies that the EM07 attached to the test strand and pushed off the indicator.

Conclusions/Discussion
The incubated test strands proved able to detect the foreign strand when added to the solution. It can be deduced that it is possible to detect foreign DNA strands through the use of a complementary test strand. This can directly apply to anthrax testing, where accuracy is necessary and vital.

Summary Statement
A versatile DNA detection system was created using the idea of complimentary DNA for the identification of any type of DNA, including anthrax.

Help Received
Used lab equipment at University of California, Riverside under the supervision of Dr. Allen Mills

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Anand S. Bhagwat

Project Number
S0402

Project Title
Crystallization and X-Ray Structure Analysis of the Novel Plant Protein 279

Abstract

Objectives/Goals
The recently sequenced genome of the plant Arabidopsis has revealed five proteins homologous to Chalcone Isomerase, a critical enzyme involved in plant biosynthesis. One of these, protein 279, interacts with fibrillin, a protein pivotal in the fruit ripening process. We set out to purify, crystallize and solve the tertiary structure of 279 by X-ray crystallography in order to help unravel its mechanism of action in plant biosynthetic pathways.

Methods/Materials
Histidine-tagged 279 was expressed in E. Coli using Polymerase Chain Reactions. The cells were lysed, centrifuged and the His-tagged 279 was purified using Nickel NTA and gel-filtration columns. The His-tag was cleaved using thrombin dialysis and the pure 279 was concentrated using a Centriprep. The purified 279 was set up for crystallization in trays using the hanging drop technique under 24 different conditions of polyethylene glycol (PEG) concentration, salt and pH (5.5-8.5) per plate.

Results
Of the hundreds of conditions set up, 279 crystallized under the conditions of 21% PEG 3350 and 0.3M potassium chloride at pH 8.5, 21% PEG 10k and .05M sodium cacodylate at pH 7, 21% PEG 5k and 0.3M potassium nitrate at pH 7 and 25% PEG 20k and 0.05M ammonium formate at pH 7. Preliminary X-Ray crystallographic analysis revealed that 279 crystallizes into an orthorhombic lattice.

Conclusions/Discussion
This research revealed that 279 crystallizes and hence functions under conditions of pH 7 and above

Summary Statement
The crystallization and analysis through X-Ray diffraction of the novel plant protein 279

Help Received
Used lab equipment at the Salk Institute under the supervision of Dr. Joe Noel and Marianne Bowman.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Max R. Biessmann

Project Number

S0403

Project Title

Expression of Mosquito Odorant Binding Protein-8

Abstract

Objectives/Goals

My primary goal in this project was to successfully synthesize mosquito odorant binding protein-8 in E.coli bacteria. In order to accomplish this I had to first create a working vector which had a copy of the gene in it and to transform it into E.coli bacteria.

Methods/Materials

I amplified the coding region from the OBP-8 gene by polymerase chain reaction (PCR) and sequenced it to make sure it was correct. After purification, the PCR fragment was inserted into the pGEM-T-easy vector and grown in E.coli. From this plasmid, the insert was cut with EcoRI and XbaI and ligated into the ThioHis plasmid. This construct was transformed into E.coli cells. Two transformed lines were grown in LB/amp, and transcription from the plasmid was induced by turning on the lac promoter with IPTG. After every hour I took a 1 ml sample and separated the proteins on a polyacrylamide gel. The materials I used were: cDNA for the A. gambiae OBP-8, ThioHis A plasmid, E.coli bacteria, primers, restriction enzymes, Taq polymerase.

Results

A PCR fragment from OBP-8 was cloned into the expression vector pThioHis. I isolated several clones, checked the plasmids for size on agarose gels and verified that they had the insert by cutting with EcoRI and XbaI. Using clone #7E in SURE cells and clone #33E in XL1-blue cells, I was able to produce the mosquito OBP-8 in E.coli. This was shown by polyacrylamide gel electrophoresis, where a protein the same size as OBP-8 appeared as a band on the gel.

Conclusions/Discussion

I used various molecular biology techniques to engineer a plasmid, from which the mosquito odorant binding protein-8 could be produced in bacteria. This is a very important step for further study of the function of odorant binding proteins in insect antennae. These proteins are believed to play an important role in odor recognition in insects. Being able to produce a mosquito protein in E.coli will enable future researchers to determine which odor molecule they may bind. Mosquitoes are vectors of many diseases, for example malaria. By studying the odorant binding proteins we may be able to produce better insect repellents thus reducing the number of mosquito bites and helping prevent mosquito borne diseases.

Summary Statement

Production of odorant binding protein-8 in E.coli bacteria.

Help Received

Used lab equipment at UCI under the supervison of Dr. Daniel Woods and Dr. Harald Biessmann

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Samuel L. Chen

Project Number

S0404

Project Title

The Effect of the PI3 Kinase/AKT/GSK3 Pathway on the Regulation of GSK3beta Activity

Abstract

Objectives/Goals

The objective is to establish positive localization of phosphorylated GSK3beta (inactivated) and phosphorylated AKT1 (activated) in the mouse testes tubule, therefore accepting or rejecting the role of the PI3 Kinase/AKT/GSK3 growth signaling pathway on the regulation of GSK3beta, which is hypothesized to have a regulating effect on the initiation of meiosis.

Methods/Materials

Mouse testes tissue extracted from previously euthanized adult mouse organisms was obtained and fixed by paraffinization onto blocks. Sections were sliced and mounted onto slides, where immunohistochemical cell staining procedures were used, identifying the presence of total GSK3beta, phosphorylated (Serine-9) GSK3beta and phosphorylated (Serine-473) AKT1 through their incubation in their respective specific antibodies. Conventional immunohistochemistry and immunofluorescent immunohistochemistry was used in localization of expression of the proteins of interest.

Results

It turned out that total GSK3beta antibodies yielded expression of the protein in both Sertoli cells and germ cells, phosphorylated GSK3beta antibody immunohistochemistry had localization in Sertoli cells, but phosphorylated AKT1 antibodies only showed expression in germ cells.

Conclusions/Discussion

This information leads one to believe that the specific PI3 Kinase/pAKT1/pGSK3beta pathway is not a major pathway having relations to the initiation of meiosis, although other isoforms (AKT2,3) or other pathways entirely, may be the inhibitor of meiosis, among other possibilities. AKT1 was only chosen first because of its reputation of rapid degeneration of the testes, in AKT1-knockout mice. Understanding GSK-3b regulation might prove clinically useful, for instance a specific inhibitor for GSK-3b action can be used as a male contraceptive, or as an agent to preserve fertility by inhibiting germ-cell proliferation during cancer therapy.

Summary Statement

This project is an investigation to determine the biological and physiological pathway, by which GSK3beta is regulated, in its role in the initiation of the entry of meiosis.

Help Received

Used lab equipment at Harbor-UCLA Medical Center Research Facility under the supervision of Dr. Wael Salameh

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Peter Joseph M. Edpao; Parth R. Shah

Project Number

S0405

Project Title

pH Correlation to ATP Production Rate of Mitochondria

Abstract

Objectives/Goals

The objective of this project is to develop a new protocol to track ATP production in mitochondrion cells and use it to determine which pH level, acid-4, base-10, or neutral, will increase the ATP production rate of mitochondria most positively.

Methods/Materials

Celery mitochondria were exposed to pH conditions: acid-4, base-10 and neutral. The mitochondria were stained with Janus Green B, and a sucrose solution was added. ATP production rate of mitochondria was tracked by developing a new protocol of using a spectrometer (a device that measures and the light wavelength of Janus Green B to track mitochondrion activity.

Results

The acidic celery cells continually rose in absorbency level, reaching up to 0.042 Absorpency (A). The neutral celery cells remained stable throughout at 0.002 A. The basic celery cells had an initial jump to 0.012 A, but then eventually dropped to 0.005 A.

Conclusions/Discussion

Janus Green stains active mitochondrion cells, which allows a spectrometer to detect ATP production activity. When the spectrometer measures absorbency, it also measures the amount of activity. This means that the greater the absorbency, the greater the amount of activity of the mitochondrion. The greater amount of activity of the mitochondrion signifies that it produces more ATP. The results support the conclusion that an acidic 4 solution most positively impacts the rate of ATP production in mitochondria. The hypothesis was correct. These data suggests possible applications for low energy individuals and plant growth under acidic conditions. The results of the experiment expand our knowledge of ATP production rates of mitochondrion by demonstrating that mitochondrion are more effective in an acidic environment and a largely basic environment can eventually hinder ATP production.

Summary Statement

The project is about developing a new protocol of tracking ATP production and determining which pH level will increase the ATP production rate of mitochondria.

Help Received

Received biological stain from La Sierra University; Mr. Newton provided validation for theory behind project design; Used lab equipment at Centennial High School.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
John A. Flatter

Project Number
S0407

Project Title
DNA in a Pickle?

Abstract

Objectives/Goals
The objective is to determine if the brining process does destroy the DNA of a cucumber.

Methods/Materials
Brining cucumbers, brined pickles, and DNA extracting equipment. DNA was extracted from each of the four differently brined pickles and also from the fresh cucumber. Measure to see the amount of DNA extracted from each of the five separate samples.

Results
The fresh cucumber produced the most amount of DNA. The first brined sample did not spool any DNA. The second sample, just like sample number one, did not spool any DNA. The second sample, just like sample number one, did not spool any DNA. The fourth sample showed no signs of DNA.

Conclusions/Discussion
The results of this experiment show that the brining process does affect the DNA structure of a cucumber. I was able to extract DNA from the fresh cucumber, but not able to extract DNA from any of the brined pickles. Using this information I can say that the brining process does destroy the DNA in a cucumber. With that in mind, I begin to wonder if the integrity of DNA is compromised, will it create a health concern?

Summary Statement
This project is about the outcome of DNA in the brining process of a cucumber.

Help Received
Lori Steward helped make board and come up with idea; Dr. Joseph Landolph helped with further information.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Richard Hsu

Project Number
S0408

Project Title
Understanding CG7900, a Drosophila Gene Important for Lifespan

Abstract

Objectives/Goals
Aging is a universal process among organisms in which homeostasis decreases and chance of death increases. Trying to understand aging in a lower organism, such as Drosophila, is a possible intermediate step process in understanding aging in humans.

Methods/Materials
An overexpression screen using the gal4 upstream activation sequence binary system was performed to identify long-lived mutants. In addition, a genomic transgenic was replicated to verify that overexpression of CG7900 extends lifespan in an independent line. A recombinant was made between EP3306 and the daughterless driver to look at interactions with other long-lived lines in the lab.

Results
Results show that CG7900 is the cause of increased lifespan and the gene itself seems to play a vital role in the metabolism process.

Conclusions/Discussion
In conclusion, the CG7900 plays a crucial role in the extension of lifespan, which may be a stepping-stone for aging research.

Summary Statement
The CG7900 gene plays a vital role in the extension of Drosophila lifespan, which may be a key factor in understanding aging.

Help Received
Used lab equipments at Caltech under the supervision of Brian Zid. Special thanks to my parents for providing transportation when needed.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Arti R. Iyer

Project Number
S0409

Project Title
Could This Be the First Step to Finding a Cure for Cancer?

Abstract

Objectives/Goals

Problem/Statement: Can we identify tumor-specific proteins in a cat melanoma cell line? My objective is to see if there are proteins that are unique to the cat melanoma cells.

Hypothesis: I believe that more than one tumor-specific protein will be identified by the Mass Spectrometry machine.

Methods/Materials

Materials: 1 pipetter, 1 incubator, 12 isoelectric strips, 50 pipettes,1 mass spectrometry machine,100 needles,IEF container,10 2 gels,100 containers for proteins,12 flasks,1 isoelectric focusing machine,1 computer,Pedi-quest computer program

Procedure:
A. Thaw cat melanoma cells and normal cat cells from the freezer.
B. Let the cells grow in separate flasks in the incubator for two weeks.
C. Remove 2 X 107 cells from each flask.
D. Rehydrate the proteins.
E. Place the isoelectric focusing strips on to the solution.
F. Put the isoelectric focusing strips into the isoelectric focusing machine.
G. Place the strips on to the 2-D gel and run the 2-D gel.
H. Identify unique proteins to the cancer cells on the Pedi-Quest computer program from the 2-D gel picture.
I. Extract these specific proteins from the actual 2-D gel based on the computer program's analysis.
J. Place these extracted proteins into eppendorfs, digest the proteins, and then place it into the mass spectrometry machine.
K. The mass spectrometry machine identifies the unique proteins through the ethernet.

Results

Out of several thousand proteins, 175-200 unique proteins were identified in CT1413. Out of these 200, two unique proteins were identified in the melanoma cells that are not expressed in normal cat cells.

Conclusions/Discussion

This is how these proteins are being identified, but ten or more trials need to be done to make certain that these two proteins are actually apparent in all CT1413 cells.

Summary Statement

My project is to see if we can identify tumor-specific proteins in a cat melanoma cell lines through processes including Isoelectric Focusing, using a 2-D gel, and Mass Spectrometry.

Help Received

Dr. Suraiya Rasheed supervised my experiment. Zahrah Ali, Steven Doo, Arvan Chan, Jennifer, and Jane Chan all assisted me in various parts of my project.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Megan Li; Michael Lin

Project Number

S0410

Project Title

Novel Method for Genetic Screening for Elements of Transduction Pathways

Abstract

Objectives/Goals

Vital to our understanding of cellular responses is the identification of regulatory proteins in the transduction pathways responsible for the expression of genes. To increase the efficiency of such genetic screens, we have tested a assay using a cell sorter and a Saccharomyces cerevisiae (yeast) deletion collection.

Methods/Materials

Rapamycin and the Mep2 yeast gene are used to test this technology. The controls in this procedure are Ho1, a wildtype strain; Fpr1, a rapamycin receptor; and Gln3 and Gat1, known regulators of Mep2 pathway.

The gene expression reporter in this experiment is a plasmid made up of the Mep2 promoter and the green fluorescent protein (GFP) gene. A collection of yeast deletion strains, each with one specific gene removed and identified by two unique DNA barcodes, is transformed with the plasmid and treated with rapamycin. The cells are fed into a Fluorescence-Activated Cell Sorter (FACS), which scans and sorts individual cells by fluorescence. Polymerase chain reactions (PCR) amplify the DNA barcodes in both pools, which are hybridized to yeast genome microarray chips. The chips are scanned to measure the knockout strain concentrations. The strains that show the highest GFP- to GFP+ ratios are individually examined with rapamycin under a fluorescent microscope to confirm that they encode components of the signal pathway.

Results

Strains that do not transcribe of the vector (GFP) in the presence of rapamycin lack a gene that codes for an element of the promoter's pathway. The assay was successful in identifying the controls Gln3 and Gat1 as regulatory elements of Mep2 transcription. The individually analyzed experimental strains had partial defects in GFP induction; they were less fluorescent than the Ho1 strain, suggesting reduced transcription.

Conclusions/Discussion

This assay using the FACS and deletion strains was successful with the entire pool. It sorted out the control strains as likely candidates and also selected strains with reduced induction, indicating that the assay can effectively pinpoint potential regulators. Classical screens involve mutagenesis to few select strains; this technological development can simultaneously monitor the transcription of a gene in all knockout strains, greatly restricting the number of strains to be individually examined in genetic screens. This will make the construction of models of signal transduction pathways for genes more efficient.

Summary Statement

Our project tested the accuracy of novel method using a yeast deletion collection to efficiently identify potential pathway regulators.

Help Received

Used lab space and equipment at Stanford University under supervision of Taavi Neklesa.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
David Liang

Project Number
S0411

Project Title
Amyloid Peptide Mediates Monocyte Transmigration by Inducing Inflammatory Genes in Alzheimer's Disease

Abstract

Objectives/Goals
Amyloid-beta peptide (Aâ) is a potential cause of Alzheimer's disease, in which it accumulates in the brain, increasing monocyte migration across the blood brain barrier (BBB). Since the mechanisms of Aâ-mediated migration are not fully known, this project attempts to study some of those direct and indirect mechanisms. First, Aâ was confirmed as an augmenter of transmigration via interaction with its receptor advanced glycation end products (RAGE) and platelet endothelial cell adhesion molecule (PECAM-1). Furthermore, endothelial receptor polarity was examined. Next, Aâ was examined as an indirect regulator of migration, one that may increase the expression of chemotactic factors. Aâ regulation of placental growth factor (PlGF) mRNA expression was the specific focus, as its role in PlGF expression is unknown.

Methods/Materials
Human brain endothelial cells cultured in Transwell chambers were used to model the BBB in vitro. Monocytes were added to the top compartment medium, representative of the luminal side, and allowed to migrate across the monolayer to the abluminal side, where medium was removed for cell counting. In gene expression experiments, THP-1 monocytic cells were cultured in medium containing Aâ. RNA was subsequently collected and RT-PCR procedures were performed to analyze mRNA expression.

Results
Aâ was shown to increase HL-60 monocytic cell migration across the endothelial monolayer, and RAGE and PECAM-1 were shown to be involved. Aâ on both sides of Alzheimer's endothelium was able to induce migration, but only on the luminal side in normal endothelium. The peptide was shown in RT-PCR results to increase the mRNA expression of PlGF as well as its receptor Flt-1, but not vascular endothelial growth factor (VEGF), a sister molecule of PlGF.

Conclusions/Discussion
Results suggest that Aâ binds to RAGE, initiating possible signaling leading to increased membrane permeability involving PECAM-1 and increased monocyte migration. The absence of this effect in normal endothelium exposed to abluminal Aâ suggests that Aâ receptor RAGE is not present on that side, while present on both sides of Alzheimer's cells. RT-PCR experiments suggest that Aâ interaction with THP-1 monocytes increases expression of PlGF and its receptor Flt-1. Since expression of VEGF was unchanged, the induction of the two chemokines may take place through separate pathways.

Summary Statement
Amyloid peptide, a potential cause of Alzheimer's disease, increases the migration of monocytes into the brain, leading to the destruction of brain tissue by the cells after differentiation.

Help Received
Used lab equipment at USC under the supervision of Dr. Vijay Kalra

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Gloria Lin

Project Number
S0412

Project Title
Variability of MHC Class I Alpha 1 Gene in Selected Mammals

Abstract

Objectives/Goals
MHC proteins play a significant role in the immune response as the recognition factor of all foreign antigens. This study is directed to show that because of their similar habitats and exposure to similar pathogens, the sequences of the variant segments of MHC Class I alpha 1 gene of marine carnivores will be more similar to other aquatic carnivores. As part of this study, I will sequence the MHC Class I alpha 1 gene in Enhydra lutris (sea otter), which has never been recorded and published by any scientist in the world yet. The terrestrial carnivore genome will also be more closely related to that of other terrestrial carnivores.

Methods/Materials
I sequenced the MHC Class I alpha 1 gene in three different individuals of both the Enhydra lutris (sea otter) and Felis catus (cat) by performing polymerase chain reaction (PCR), gel electrophoresis, gel extraction, cloning, restriction digest, and sequencing. Then I edited and analyzed my sequences using powerful Vector NTI software. I used the worldwide genomic database, BLAST, to search for the most closely related gene sequences, and constructed phylogenic trees based on the similarity and absolute complexities of the sequences.

Results
I found in the DNA phylogenic tree a closely related feliform group consisting of the cat, cheetah, ocelot, and cow. Another family that emerged was the caniform group, including the dog, sea otter, and harbor seal. Yet in the amino acid phylogenic tree the only close relations left in the feliform group were among cat, cheetah, and ocelot, whereas the cow had been omitted. In the caniform group, the dog had been omitted in the amino acid phylogenic tree.

Conclusions/Discussion
My results indicated that while DNA sequencing revealed certain groups of close relation, the functional amino acid groups had branched off differently, perhaps because of habitat and the exposure to similar pathogens. Amino acids construct polypeptides and in turn proteins, and are thus directly involved in protein structure and function, as opposed to DNA. Species of the same habitat showed considerable similarity in amino acid phylogeny. This study is only a small segment of a broad sector of genetic research that could bring huge implications to the health of animals across the globe. I recommend that more research on the factors influencing MHC proteins and their significance in the immune response be done.

Summary Statement
This study is directed to show that because of their similar habitats and exposure to similar pathogens, the sequences of the variant segments of MHC Class I alpha 1 gene will be closely related in animals of similar habitat.

Help Received
Dr. Brian Aldridge of UC Davis Immunology supplied cDNA and primers.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
John P. Mahajan

Project Number
S0413

Project Title
MicroRNA-Oncogene Proximity and Observed Rates of Cancer: A Statistical Study

Abstract

Objectives/Goals
The objective is to determine if a correlation exists between the proximity of microRNAs (miRNAs) to oncogenes related to specific cancers, and incidence rates for each specific cancer.

Methods/Materials
For this project I needed the list of known miRNAs and their loci, or positions on their respective chromosomes. I obtained the list and sequences of the known miRNAs from the National Center for Bioinformatics (NCBI). I then entered each miRNA into UC Santa Cruz's Genome Browser v17 to find the exact position of each miRNA, of which only a few are known. The list of oncogenes with corresponding cancers was located at the Cancer Gen website. Some oncogenes were specific for one type of cancer, but most are associated with more than one cancer, which complicated the analysis that followed. To obtain the oncogene loci, I also used UC Santa Cruz Genome Browser v17. I did find some discrepancies between the data collected from various web sites. I subsequently had to throw out that data. Next I collected the incidence rates for each cancer that I had an associated oncogene for, from the National Cancer Registry SEER search engine. The analysis of the usable data was done using excel.

Results
The correlation coefficient for the average distance between all miRNAs and overlapping oncogenes against each type of cancer was 0.3365. The correlation coefficient after removal of overlapping oncogenes was 0.05178. After redistributing the data between high and low incidence rate cancers, the correlation coefficients were 0.5368 and 0.2338, respectively. Again, removing overlapping oncogenes and redistributing the data between high and low incidence rate cancers, the correlation coefficients were 0.9988 and 0.125, respectively.

Conclusions/Discussion
A correlation between the proximity of miRNAs to oncogenes related to specific cancers, and incidence rates for each specific cancer does not exist; each analysis resulted in low correlation coefficients. Even though there was one strong correlation coefficient of 0.9988, it is not statistically significant because there are only three data points for that category. These findings suggest that the distance between miRNA and oncogenes is not correlated to rates of cancer.

Summary Statement
My project tests whether a correlation between the distance of microRNAs from oncogenes, and incidence rates of cancer exists.

Help Received
My mother and brother helped me glue and mat my board, and my father proofread my writing. My biology teacher helped clarify some of my questions on gene regulation.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Ranjeet Minocha

Project Number

S0414

Project Title

The Urea Transporter (UT) Family: A Bioinformatic and Phylogenetic Analysis

Abstract

Objectives/Goals

This experiment was undertaken in order to determine whether proteins of the Urea Transporter (UT) family are similar amongst different species, both eukaryotes and prokaryotes, and in addition, to trace evolutionary pathway of the protein.

Methods/Materials

This experiment was conducted using the following programs which analyze and look at the phlogeny and structure of the sequences:AveHAS,WHAT,IntraCompare (IC),TMS_ALIGN,BLAST

Results

Sequence, structural, and phylogenetic analyses reveal conserved regions and amino acid residues, suggesting that a primordial 5 trans-membrane helical segment (TMS)-encoding genetic element duplicated to give rise to a 10 TMS-encoding element early during evolutionary history, at about the time when eukaryotes diverged from prokaryotes. Two well conserved, strongly amphipathic, putative alpha-helices that precede both 5 TMS repeat elements are predicted to be of structural, functional, or biogenic significance. Further, a second duplication event (or a gene fusion event) occurred during development of the vertebrate lineage, giving rise to 20 TMS proteins in some mammals. These results suggest that vertebrates acquired UT genetic information from bacteria only once and that all current orthologues and paralogues in the animal kingdom arose from this one primordial system.

Conclusions/Discussion

The objective was met in that an evolutionary pathway was found and similarites of the UT protein family in different species was established. Using this same approach in a disease model can possibly help correct the malfunctioning part/s of the protein involved.

Summary Statement

This project is concerned with the evolutionary pathway and similarities of the UT protein in the different species that it is expressed.

Help Received

Used lab equipment at UCSD under Dr. Milton Saier; brother gave advice; students in lab helped with learning techniques; mom and dad for help with display

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Ashwin Mudaliar

Project Number
S0415

Project Title
Detection of Decreased Renal Expression of Organic Anion Transporter-2 in Aging Mice

Abstract

Objectives/Goals
Organic anion transporters (OATs), transmembrane proteins found in the kidney, are important in the excretion of many commonly used drugs and environmental toxins, and it has been hypothesized that decreased drug excretion in older individuals might in part be due to decreased expression of OATs. In this study I assayed the expression of four members of the organic anion transporter (OAT) family: NKT/OAT1 (novel kidney transporter), ROCT/OAT3 (reduced in osteosclerosis transporter), RST (renal specific transporter), and NLT/OAT2 (novel liver transporter) in renal tissue of geriatric and juvenile mice.
Further, I investigated the evolution of OATs and OCTs (organic cation transporters) through searches of genome sequence databases and generated dendrograms ("family trees").

Methods/Materials
Normal PCR procedures, in non-saturated conditions, were followed to test the expression of the genes being tested. Renal cells where harvested from three distinct juvenile (11 week) BALB/cJ mice and three separate geriatric (44 week) mice of the same strain.
To investigate the evolution of the organic ion transporter family, genome sequence databases were searched, using the BLAST search engine (available at http://www.ncbi.nlm.nih.gov/BLAST). Using known sequences as queries, the genomes of two organisms, D. Melanogaster (fruit fly) and C. Elegans (worm), were searched for putative transporters. With the isolated sequences, a dendrogram of the entire evolution of the organic transporter family was created.

Results
All four known mouse members of the OAT family (NKT/OAT1, NLT/OAT2, ROCT/OAT3 and RST) were tested. Of these, only OAT2 showed reduced expression in aged mice, while the expression of the other three genes remained unchanged.

Conclusions/Discussion
Adverse drug reactions (ADRs) are common in the elderly, in part because of reduced renal function. Specific proteins, called organic ion transporters are responsible for the transportation of drugs and waste in various organs. If the expression of a transporter is reduced by age, as we have found for OAT2, it only makes sense that renal function will itself diminish. Moreover, since each transporter is specific for a drug, if a transporter's expression is reduced, the ability to handle that drug will also be reduced. In the future, one might be able to test patients for transporter expression and by avoiding specific drugs, it may be possible to decrease ADRs.

Summary Statement
My project tests the expression of members of the organic anion transporter family, transmembrane proteins expressed in excretory organs that are responsible for the excretion of drugs and environmental toxins.

Help Received
Foremost I must thank Dr. Satish Eraly and Dr. Sanjay Nigam at the Stein Clinical Research Facility at UCSD. I must also thank my mother, father, and chemistry teacher Ms. Victoria Coordt for their help and support throughout my entire project.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Khang D. Nguyen

Project Number
S0416

Project Title
Mutations in Nod2 Affect NF-kB Activation

Abstract

Objectives/Goals
The purpose of this project is to determine if mutations in Nod2, a gene thought to be involved in Crohn's disease, affects the activation of the inflammatory response due to induction of NF-kB.

Methods/Materials
Dr. Laurie Bankston made two deletion mutations in the mouse gene Nod2. I transfected these mutations along with control constructs into 293 cells (human embryo fibroblast continuously maintained cell line). The total protein of the 293 cells was extracted and a luciferase assay was done to determine the amount of NF-kB activation. The relative amounts that each mutation activated NF-kB was compared.

Results
Overexpression of wild type (N2F) and the two mutations (N2M, N2M2) caused activation of NF-kB. Wild type and N2M activated NF-kB to about the same level while the N2M2 induced higher activation. The amount of activation by N2M2 was about three-quarters of IKKbEE (constitutively active, positive control). These arbitrary numerical values were compared to the b-actin (empty vector), the negative control.

Conclusions/Discussion
A high percentage of patients with Crohn's disease have the N2M mutation. It is not certain whether this mutation is really the cause of the disease. From the results of this project, we can conclude that N2M activates NF-kB at least as well as wild type in overexpression experiments. Thus, it is a potential candidate for causing Crohn's disease.

Summary Statement
To determine if mutations in Nod2, a gene thought to be involved in Crohn's disease, affects the activation of the inflammatory response due to induction of NF-kB.

Help Received
Used lab equipment at the Basic Science Building on the campus of the University of California, San Diego, under the supervision of Greg Cadwell, Staff Research Associate III

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Shirley Phan

Project Number
S0417

Project Title
Analysis of Dauer Formation in Various Caenorhabditis Species

Abstract

Objectives/Goals
The objective of the project is to discover the molecular components underlying the evolutionary changes in development and physiology by comparing the dauer pathway(a developmentally arrested third larvae stage that allows worms to survive when environmental conditions become adverse), in five Caenorhabditis roundworm species: C. elegans, C. briggsae, C. remane, CB5161, and PS1010.

Methods/Materials
An EMS (ethylmethanesulfonate) mutagenesis was conducted to obtain Daf-d, worms that cannot form dauers under any circumstances and Daf-c mutants, worms that forms dauers even when conditions are favorable to them are two classes of mutations that affect dauer formation. After Daf-d and Daf-c mutants are obtained, analysis such as, dye-filling assay to categorize the dauer mutants obtained, X-linkage tests to test for X-linked traits in dauer mutants, temperature assays and comparison of the level of Daf-7 (promoter gene that plays a role in either inducing or preventing dauer formation) production were conducted to analyze how the dauer pathway has evolved in diverging Caenorhabditis species.

Results
A totl of 24 Daf-d mutants were obtained through the EMS mutagenesis in the C. briggsae strain of which 8 out of the 1 was categorize as Daf-d cilium structure mutants. Two Daf-c mutants that are capable of suppessing dauer formation were also obtained in the C. briggsae train. A significant difference discovered is that a C. briggsae SDS (Sodiumdodecylsulfonate) sensitive mutant is not X-linked whereas the ancestral specie C. elegans exhibits both SDS sensitivity and X-linked characteristic. Temperature assay demonstrated that C. briggsae, C. remanei, CB5161 and PS1010 are more heat tolerant and hyperinductive to increasing temperature than the ancestral specie C. elegans. Results also concluded that the level of Daf-7 production is higher in C. briggsae, C. remanei, CB5161 and PS1010 than in C. elegans.

Conclusions/Discussion
In conclusion, the various comparison experiments conducted validatd that varying Caenorhabditis species such as C. briggsae, C. remanei, CB5161 and PS1010 exhibit both differences and similarities in gene function and gene locus thathave been brought forth by evolution which is significantly different in C. elegans, the ancestor specie.

Summary Statement
My project is to study and to better understand the molecular components of evolution by comparing a shared pathway, the dauer pathway in various Caenorhabditis species.

Help Received
Professor Paul Sternberg provided the equipment for my projet; Dr. Takao Inoue supervised my experiments and helped me with technical difficulties.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Oksana A. Sergeeva

Project Number

S0418

Project Title

Effect of Natural and Synthetic Substances on Transformation of dUMP into dTMP

Abstract

Objectives/Goals

Thymidylate synthase (TS) is the enzyme that catalyzes the transformation of deoxyuridine monophosphate (dUMP) into deoxythymidine monophosphate (dTMP) in cells. In cancer cells, TS is overexpressed. While usual drugs treat cancer by inhibiting TS, the New-Biotics' drug is a substrate of TS that produces toxic products upon TS activation. When the drug enters the cell it is transformed into bromovinyl deoxyuridine monophosphate (BVdUMP), a substrate for TS. Both dUMP and BVdUMP reactions require certain thiols, although other thiols inactivate TS, as researched in previous project. The natural reaction requires a cofactor, methylene-tetrahydrofolate (CH(2)-THF), but the BVdUMP reaction does not. The effect of different natural compounds on TS while it catalyses dUMP transformation to mimic its intracellular BVdUMP activation during chemotherapy was studied.

Methods/Materials

Different concentrations of CH(2)-THF were used while changing the concentrations of BVdUMP and dUMP, to find the effect of CH(2)-THF on BVdUMP inhibition of TS. Then irriversible inactivation of TS by BVdUMP was studied by preincubation the BVdUMP and enzyme and adding it to the reaction mixture.

Results

It was found that BVdUMP and dUMP are competitive substrates, so BVdUMP appears to act as an inhibitor for the natural reaction. It was also discovered that the higher the concentration of CH(2)-THF, the less the effect of the BVdUMP on the dUMP reaction. The irreversibility of TS inactivation by BVdUMP was also studied and it was discovered that for the first 30 minutes the inactivation is reversible and does not harm the enzyme, TS. The inactivation becomes irreversible over time. In the presense of CH(2)-THF the irreversible inactivation of TS by BVdUMP was less extreme than its absense.

Conclusions/Discussion

Since BVdUMP and dUMP are competitve substrates, they bind at the From this it was concluded that at higher CH(2)-THF concentration, BVdUMP is bound less tightly to the enzyme, meaning that they have mutually exclusive binding sites on the enzyme. CH(2)-THF also acts as a protector against irreversible inactivation of TS by BVdUMP.

Summary Statement

New Biotics' drug, an alternative substrate of TS that competes with the natural substrate, is affected by CH(2)-THF and irreversibly inactivates TS only at long incubation times.

Help Received

Used lab equipment at New Biotics, Inc. under the supervision of Dr. Maria Sergeeva.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

Zhuo Sun

Project Number

S0419

Project Title

Effects of Vitamin D3 on Osteocalcin mRNA Expression in MG63 Cells

Abstract

Objectives/Goals

To find out how Vitamin D3 will benefit bone formation by testing the effects of Vitamin D3 on osteocalcin mRNA expression in MG63 cells

Methods/Materials

Methods:

1). Grow MG63 cells
2). Seed the cells into dishes.
3). Add vitamin D3 and its solvent into dishes, respectively.
4). Isolate total RNA from using TRIZOL reagent.
5). Measure total RNA concentration with spectrometer.
6). Perform electrophoresis to test isolated total RNA.
7). cDNA synthesis by reverse transcription reaction of total RNA, which is used for PCR and real-time quantitative RT-PCR.
8). Amplify OC gene by RT-PCR to get its fragment from gel for probe preparation of Northern blot.
9). Perform real-time RT-PCR to quantify OC mRNA expression in control and Vitamin D3-treated cells, respectively.
10). Perform Northern blot.

Materials:

1. MG63 cells from ATCC, DMEM, FBS; 1á, 25(OH)2 Vitamin D3, TRIZOL Reagent
2. Electrophoresis Apparatus, Superscript II reverse transcriptase kit, SYBR Green PCR kit; Real-time PCR machine
3. Hybond-XL nylon membrane Random Primer Labeling Kit; X-ray film; primers ;spectrometer
4. test tubes, pipettors, Eppendoff tubes, centrifuge, X-ray film, Milli Q H2O

Results

It was found that vitamin D3 up-regulated osteocalcin mRNA expression in MG63 cells with a fold induction of 91.66. Northern blot also showed the similar result.

Conclusions/Discussion

Osteocalcin is the human bone formation marker of osteoblast cells, an increase in its expression means an increase in bone formation by the osteoblast cells. Vitamin D3 does benefit bone formation.

Summary Statement

Through real-time RT-PCR and Northern Blot analysis it was found that vitamin D3 up-regulated osteocalcin mRNA expression in MG63 cells.

Help Received

the experient was done in my father's lab.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Hong L. Truong

Project Number
S0420

Project Title
Transgene Expression of Wild Type and Mutant Myosin Binding Protein C (MyBP-C) in the Hearts of MyBP-C Knock Out Mice

Abstract

Methods/Materials
Familial Hypertrophic Myopathy (FHM) is a disease in which the heart is enlarged to compensate for mutations in muscle proteins. Twenty to thirty percent of cases of FHM were found to have mutations in MyBP-C. To determine the significance of MyBP-C, the MyBP-C gene was "knocked out" through gene targeting and deficits in phenotype were determined. To test specific hypotheses regarding MyBP-C, transgenes encoding for normal and mutant MyBP-C are inserted into the genome of knockout mice. To test the effectiveness of the transgene to restore or modify function, homozygous (knockout gene) mice testing positive for the transgene are tested for the presence of transgene MyBP-C.

Results
From CTW mice lines 90, 984, and 982, only line 90 has showed strong expression for transgene MyBP-C and none of the CTP mice showed expression.

Conclusions/Discussion
This low expression rate may be due to low copy numbers of the transgene in the other lines.

Summary Statement
To determine whether the phosphorylation site plays a role in the function of MyBP-C in the heart

Help Received
Used lab equipment at the University of Wisconsin, Madison under the supervision of Dr. Samantha Harris and Dr. Richard Moss; Participant in the NASA SHARP Plus

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Brannon T. Weeks

Project Number
S0421

Project Title
The Inhibition of Lipid Raft-Mediated Platelet Plug Formation by Methyl-beta-cyclodextrin in an in vitro System

Abstract

Objectives/Goals
The purpose of the study is to prolong coagulation through the introduction of cholesterol-binding methyl-beta-cyclodextrin (MBCD). It is thought that MBCD, through its known prevention of the formation of lipid rafts, will inhibit platelet aggregation and plug formation.

Methods/Materials
Healthy, medication free human donors supplied the blood, which was drawn into a one-ninth volume of acid-citrate-dextrose (ACD) at a pH of 8.8. After centrifugation to separate the platelet-rich plasma (PRP), platelets were isolated from the PRP through sepharose gel filtration. A desired concentration of 3.0*10^8 platelets per ml was achieved through dilution of the platelet suspension, and 2.5 mM Thrombin Receptor-Activating peptide 1 (TRAP1) was added in a 1:1000 volume dilution to activate the platelets. To determine a standard curve for the clotting times, against which the data could be compared, 20 trials of a clotting assay were run. Likewise, to determine the curve for the clotting times, in which MBCD was introduced, 20 trials of a different chemical (drug) assay were run.

Results
The standard curve assay yielded platelet plug formation times that spanned from .533 minutes for the PNP-rich group to 6.017 minutes for the PNP-deficient group. The factor XI-deficient plasma increased the clotting times at regular intervals. At the same platelet concentration as the standard curve assay, the chemical assay yielded expected results. While the average times for the control were initially relatively disparate, the trends became similar as the concentration of MBCD increased. The greatest increase measured was from 1.983 minutes for the MBCD-free group to 9.915 minutes for the group with the highest concentration of MBCD.

Conclusions/Discussion
Results show that when MBCD is introduced into an in vitro model of platelet plug formation, coagulation times increase by as much as five-fold; on average, however, the time change for all groups was a 126% increase. The data suggests that lipid rafts are related to platelet activity, specifically aggregation, because it has been shown that inhibition of the rafts prolongs coagulation times. Further experimentation will probably determine (a) at what concentration MBCD is most effective in prolonging coagulation times, (b) how MBCD affects binding of proteins to lipid rafts, and (c) other roles lipid rafts may play in protein reception and hemostasis as a whole.

Summary Statement
The effects of the decrease in concentration of lipid rafts on the period of platelet plug formation are studied by decreasing the rafts' assembly through the introduction of the cholesterol-binding chemical methyl-beta-cyclodextrin.

Help Received
Grant funding and equipment used at the Temple University School of Medicine; advisement and consultation from Drs. Frank Baglia and Fredda London

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Harvey H. Xiao

Project Number
S0422

Project Title
Actin and Actin Associated Proteins: A Developmental Study

Abstract

Objectives/Goals
Actin and actin associated proteins are involved in thin filament regulation which is important in the regulation of vessel contraction. The goal of this project is to find out how the expression of actin and the major actin associated protein (actinin, vinculin, talin, and paxillin) changes with development from immature fetus, term fetus, newborn, to adult. This study will help us understand more details about cerebral vessel contraction mechanisms and the regulation of blood flow.

Methods/Materials
We will use common carotid artery from the 96-day, 140-day gestation age fetuses, newborn lamb, and adult sheep for this study. Immediately following sacrifice we will remove the common carotid arteries (CCA), clean them in the Krebs buffer and rapidly freeze them in liquid nitrogen. Frozen samples will be homogenized in the lysing buffer. Nuclei and debris will pellet with centrifugation at 14,000 g for 20 min. The whole cell lysate will be stored at ¡V80#aC. We will use the Western Immunobloting technique to quantify the expressions of actin, actinin, vincullin, talin and paxillin.

Results
1. In sheep common carotid artery, ƒÑ-actin expression did not change with development from 96-, 140-gestation day fetus, to newborn, and to adult.
2. In the four experimental age groups, the expression of actin associated proteins vinculin and actinin had no significant difference.
3. The expression level of talin was low in both fetal groups and dramatically increased in newborn sheep then decreased to fetal level in adult common carotid.
4. Although the paxillin expression did not change with development, phosphorylated paxillin (the active form of paxillin) showed significant differences between the four age groups. 95-day and the 140-gestation day fetuses had similar low level of phosphorylated paxillin expression.

Conclusions/Discussion
In this study, we found that expression levels of several actin associated proteins changed with developmental age. In a previous study it was shown that for a giving potasium concentration, vessels from two fetal groups had smaller contraction response than the newborn and adult vessels. This might, in part be due to the differences in actin associated protein expressions. This project provided us with a strong foundation which we will use to further understand the mechanisms involved in blood vessel contraction and blood flow regulation.

Summary Statement
In this opening study of maturation affects on actin and actin associated proteins, we found that expression levels of several actin associated proteins changed, which is significant in our growing understanding of blood vessel contractions

Help Received
Used Lab equipment at Loma Linda University under the supervision of Dr. Lawrence D. Longo; Wen Long helped me with a immunocytochemistry technique.

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Yibo Yang

Project Number
S0423

Project Title
Different Caspases Mediate Age-Related Apoptosis in Neurons Compared with Astrocytes

Abstract

Objectives/Goals
Apoptosis is a cell suicide program; however, effects of age and cell type on apoptosis in the brain are poorly understood. The age at which different brain cells are most susceptible to apoptosis is unclear.

Methods/Materials
Neuron and astrocyte cultures were deprived of serum. To characterize DNA fragmentation, Terminal deoxynucleotidyl transferase-mediated dUTP labeling (TUNEL) staining was performed. To identify apoptotic or necrotic cells in cultured cell population, Hoechst and Propidium Iodide (PI) staining were performed. Cell injury was quantified through lactate dehydrogenase (LDH) assay. Caspase and MAP kinase phosphorylation were measured using immuno-blotting. Caspase 3, 8 and 9 were also inhibited in both cultures to determine its role in determining age-related differences. All data were analyzed by one-way analysis of variance (ANOVA), followed by two-tailed Student's t-test.

Results
Young neurons showed more apoptosis and greater caspase (essential protease for apoptosis) expressions compared to mature neurons. Mature neurons and astrocytes were found to be less vulnerable to serum deprivation and to undergo necrosis rather than apoptosis, which correlates with a lack of caspase activity and TUNEL-positive cells. Astrocytes showed condensed but non-fragmented nuclei and a different caspase expression.

Conclusions/Discussion
Caspase 3, 8, and 9 contribute to neuronal apoptosis while caspase 11 seems important to astrocytes. Our findings suggest that neurons and astrocytes utilize different apoptotic pathways as they develop and anti-apoptotic interventions against brain injury should be developmentally targeted and specific to cell type

Summary Statement
Caspases differentiate neurons and astrocytes; they contribute to the different apoptotic pathways.

Help Received
This study was supported by grants (NIH GM49831) to Dr. R.G. Giffard at the Department of Anesthesia, Stanford University School of Medicine. I wholeheartedly thank Drs. Giffard, Xu, and Chock for help on the supervision for all methodology, discussions of the experiment designs and results, and critical

CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
Michelle Rengarajan

Project Number
S0499

Project Title
Induction of Drosophila melanogaster Immune Response by a Parasitic Nematode and Its Bacterial Symbiont

Abstract

Objectives/Goals
H. bacteriophora, P. luminescens, and Drosophila constitute a useful system for studying bacterial-nematode symbiosis, bacterial-insect pathogenesis, and nematode-insect parasitism. Because of close correlation between human and Drosophila immune response elements, we may use Drosophila as a model organism to shed light on the mechanisms of immune system activation and suppression in humans. We also hope to provide information with implications in human diseases caused by parasitic nematodes, such as lymphatic filariasis.

Methods/Materials
We have investigated the induction of the insect immune system by assaying GFP and lacZ reporters for diptericin, cecropin, and metchnikowin, genes known to be induced in antimicrobial immune response.

Results
This research has shown that H. bacteriophora is capable of infecting Drosophila melanogaster. By infecting fly strains with bacteria, we have determined that P. luminescens does not induce cecropin response whereas E. coli does, suggesting that P. luminescens is able to either evade or suppress Drosophila immune response.

Conclusions/Discussion
Our research shows that P. luminescens is capable of suppressing or evading the cecropin antimicrobial peptide of the Drosophila immune response. We hypothesize this lack of activation occurs either because upstream receptors in the Toll pathway cannot detect P. luminescens or because P. luminescens is able to suppress elements in the Toll pathway upstream of Dif. With this result, in conjunction with results on antimicrobial elements that P. luminescens does activate, we have established a model system with H. bacteriophora, P. luminescens and Drosophila. Our model has applications in immunology, parasitology, and microbiology studies. We can apply our results to studies of bacterial infection, because of the many homologous elements that exist between Drosophila and mammalian immune response. Additionally, our model may be used to investigate diseases such as lymphatic filariasis that are caused by human parasitic nematodes. It is possible that our model may be applied to complex systems of immune suppression, such as the parasitic Venturia wasps and human immunodeficiency virus.

Summary Statement
By infecting fruit flies with a parasitic nematode and its symbiotic bacterium, I established that the bacterium evades or suppresses the flies immune response; this system can be used as a model for human immune response.

Help Received
Used lab equipment at Caltech under the supervision of Dr. Paul Sternberg and Dr. Takao Inoue; Dr. David Schneider and Dr. Dan Tracey provided fly strains.