CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Priyanka S. Adapa

Project Number
S0401

Project Title
Turn Up The Heat! Does Temperature Affect Peroxidase Catalysis?

Abstract

Objectives/Goals
The purpose of my project was to see whether the temperature of a substance affected the rate at which catalysis with the enzyme peroxidase occurred.

Methods/Materials
I used 11 pieces of meat, all of them differing in temperature by 10 degrees Celsius starting at 0 degrees Celsius and going up to 100 degrees. I then put 4 drops of the enzyme peroxidase on the meat. Once on the meat, the hydrogen peroxide reacted with another enzyme already in the meat, catalase, to form the pure form of peroxidase. I then observed the affects of the chemical reaction (bubbling and foaming on the meat#s surface).

Results
My data showed that the higher the temperature of the meat, the faster it took for the reaction to occur, until it completely dropped off at 80 degrees and the reaction did not occur. The amount of foaming also increased along with the temperature but once again, it also dropped off at 80 degrees, producing nothing but smoke when I put peroxidase on the meat.

Conclusions/Discussion
My results and data show that my hypothesis is correct since the amount of heat did affect the rate at which the enzyme was able to catalyze a chemical reaction. Once the temperature became 80 degrees, the heat was to intense for the enzyme to work with. The heat broke up the ions in the enzyme, allowing no reaction to occur.

Summary Statement
My project looks at the affects of temperature (as one factor) on the enzyme peroxidase's ability to catalyze chemical reactions.

Help Received
I had no help on my project.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Sudeep Banerjee

Project Number
S0402

Project Title
Ras Activated Tumor Suppressor Genes Nore1A and RASSF1A Have Separate Proapoptotic Effector Mechanisms

Abstract

Objectives/Goals
Induction of apoptosis by Ras may be mediated by its effector RASSF1, which appears to function as a tumor suppressor. Analysis of another Ras effector Nore1, which is structurally related to RASSF1 showed that it is frequently down-regulated in tumor cell lines. Like RASSF1, this correlates with inactivating methylation of the Nore1 promoter rather than gene deletion. Immunofluorescence microscopy indicates that the RASSF1 protein is localized in the microtubules of the cell # specifically Actin, Alpha and Beta tubulin. I predict that Nore1A is colocalized to the same microtubule structures.

Methods/Materials
Cell fixation was conducted on H23 cell culture. The fixed cells were then transfected and allowed to incubate. Then immunofluorescent antibodies were administered to tag the cytoskeleton and proteins of interest, thereby producing observable cell slides, which were viewed under an immunofluorescent microscope.

Results
Localization is based on the degree of diffusion of the layers. The immunofluorescent microscopy results were analyzed for both RassF1A and Nore1. The red layer, representative of Beta Tubulin, shows clearly defined microtubules. The green layer, representative of RassF1A, also is defined along the same distribution. Therefore we can deduce that RassF1A is localized to the microtubule. Then there is the representation of Nore1A and Beta tubulin. The Beta tubulin is still punctate or clearly defined while the Nore1A is diffused throughout the cell indicating that no localization can be determined. The same pattern was determined with all three microtubular structures: beta tubulin as well as alpha tubulin and actin filaments.

Conclusions/Discussion
The immunofluorescent images of Nore1A suggest that localization with actin filaments, alpha or beta tubulin does not exist. Although RASSF1 and Nore1 are structurally similar and the Ras gene activates both, my study shows that they do not localize to the same area in the microtubular structure of the cell. Hence if Nore1 is not localized with RassF1 then its effector mechanism for growth inhibition must be different.

Summary Statement
This project is about the intracellular localization of tumor supressor gene proteins in an effort to explain their mechanism of action

Help Received
This project was carried out at the molecular biology laboratory of Massachussetts General Hospital under the supervision of Dr. Ramnik Xavier. The compilation and presentation of the project was supervised by Mr. Wayne Garabedian

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)

Sabina Bera; Peter Joseph Edpao; Parth Shah

Project Number

S0403

Project Title

Phase II: Correlation of Thorium-232 Radiation to ATP Production Rate of Mitochondria

Abstract

Objectives/Goals

This Phase II experiment is designed to further develop a new protocol to quantify and track the ATP production rate of mitochondrion and determine the effects of thorium-232 radiation on the ATP production rate of celery cell mitochondria for possible environmental and consumer safety applications.

Methods/Materials

The following new protocol was developed: Janus Green B stains only active mitochondrion within a cell with a blue-green dye. In terms of wavelength, this is 600 nanometers. The mitochondria are stimulated by first introducing a sucrose solution. Since the stain is absorbed into the mitochondrion of the celery cells, the spectrometer can detect the amount of absorbency when set to 600 nanometers. By detecting the absorbency (A) of the 600-nanometer wavelength the spectrometer effectively measures the amount of activity of the mitochondrion based on the fact that the spectrometer absorbency reading will adjust to the absorbency of the stain, which will be based on the movements within the mitochondrion itself. A greater amount of activity in the mitochondrion means that there is a greater amount of ATP production, or energy. The theory is validated by the fact that a reflective spectrometer is designed and used to measure the intensities and wavelengths of the visible region of the electromagnetic spectrum.

Experiment: Celery mitochondria were exposed to Thorium-232 radiation from a lantern mantel and normal non-radiated conditions. ATP production rate of mitochondria of both groups was tracked by using the above-mentioned protocol.

Results

Initial starting point averages for no radiation exposure and a radiation exposure of thorium-232 were 0.000. The final average for no radiation exposure was 0.002 A. The final average for a radiation exposure of thorium-232 was #0.008 A.

Conclusions/Discussion

By staining only active mitochondria with Janus Green B, by introducing a stimulating sucrose solution, and the use of the principles of spectrometry indeed allowed us to further develop our new protocol. Data collected from the experiment did show that radioactive isotope thorium-232 greatly harmed mitochondrion ATP production rate. These data suggest possible applications to environmental safety and consumer consumption issues. Further experimentation may include the application of the protocol to radiated cancer cells.

Summary Statement

The project is about further developing a new protocol of tracking and quantifying the ATP production rate of mitochondria and determining the effects of thorium-232 radiation on the ATP production rate of celery cell mitochondria.

Help Received

Received biological stain from La Sierra University; Mr. Newton (High School Chemistry Teacher) and Webster M. Edpao (MD-candidate at Northwestern Medical School) provided validation for theory behind project design; Used lab equipment at Centennial High School.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Sara A. Bryant

Project Number
S0404

Project Title
DMSO Inhibits the Induction of Adipogenesis in 3T3L1 Cells

Abstract

Objectives/Goals
The hormone receptor, PPAR, functions to regulate the differentiation of adipocytes (fat cells). This receptor functions as part of a heterodimer with another receptor, RXR. A chemical that functions as a ligand for RXR is predicted to have similar effects as a ligand for PPAR, and thus an RXR ligand might also regulate adipocyte differentiation. Methoprene acid is a commonly used pesticide that is known to bind to the human RXR and thus might function to regulate differentiation of adipocytes. I hypothesize that methoprene acid will function as an RXR ligand and induce changes in gene expression that are similar to the changes that occur when cells are exposed to a PPAR ligand.

Methods/Materials
I am using the mouse fibroblast cell line (3T3L1) that is well characterized in its response to PPAR ligands to differentiate as adipocytes. I assay for adipogenesis with Oil Red O staining that I photograph, and for changes in target gene expression using Reverser-Transcriptase Polymerase Chain Reaction (RT-PCR). Changes in gene expression are observed as an increase or decrease in the amount of the PCR product, which is visualized by agarose gel electrophoresis.

Results
3T3L1 cells can be induced to form adipocytes when treated with the appropriate combination of inducing hormones. The addition of several different RXR ligands does not appear to significantly effect this response, Although low concentrations of methoprene acid do not seem to affect the response, high doses appear to inhibit the transition to becoming adipocytes. Results from the control samples however indicate that DMSO (the solvent I used for methoprene acid) is responsible for the observed inhibition of adipogenesis.

Conclusions/Discussion
Since methoprene acid is an environmental hormone that humans are exposed to, the interaction of this chemical with human hormone receptors should be studied. The observed effects of DMSO on adipogenesis are of significance given the current interest in the potential therapeutic applications of this compound.

Summary Statement
The widely used solvent, DMSO, inhibits hormone-induced adipogenesis

Help Received
This project was conducted at UC Irvine as part of the Science Fair Initiative in the School of Biological Sciences

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Julio T. Chong

Project Number
S0405

Project Title
Investigating Ethanol's Effects on GABA-A Receptors Expressed in Xenopus laevis Oocytes

Abstract

Objectives/Goals
Alcohol is the most widely abused drug in the United States today and although we know what ethanol can do to the brain, we are barely beginning to understand its molecular mechanisms. Using oocytes (unfertilized frog eggs) from the Xenopus laevis frog, our lab is able to study ligand-gated ion channels that mediate ethanol activity and the intitial binding sites of ethanol. The objective of this experiment was to see if pressure is an ethanol antagonist on GABA-A Receptors.

Methods/Materials
The frogs used are Xenopus laevis frogs, more commonly known as the South African clawed frog. The oocytes are surgically removed from the Xenopus laevis frogs by our lab technician twice every week. The proposed experiment will utilize cDNAs expressed in Xenopus oocytes. Female Xenopus laevis frogs will be used as the donor source of oocytes for electrophysiological assays. This approach minimizes the use of animals by conducting experiments in unfertilized oocytes. The frogs that undergo surgery are allowed to recover for one week after surgery before being reintroduced into their normal tank. The frog that undergoes surgery is only allowed to have surgery 5 times, with each surgery capped at once every 4 months. After the 5 surgeries, the frog is euthanized according to USC biological safety guidelines approved by the State of California. After oocytes are removed from the Xenopus laevis frog, the follicular layer is removed which allows easy injection of the cDNA and is important for accurate two-electrode voltage clamp readings. The oocytes are injected with GABA-A cDNA and left in an incubation medium for 48-72 hours. Afterwards, these oocytes are individually voltage clamped and exposed to different drugs. When finished, the oocytes are safely disposed of according to the University of Southern California's regulations.

Results
Ethanol potentiation under normal conditions shows a 60% effect, while under pressure, ethanol potentiation is around 35%.

Conclusions/Discussion
Pressure does not physiologically modify the GABA-A receptor in any way and can be seen as a direct agent. Pressure is a direct ethanol antagonism on GABA-A receptors.

Summary Statement
The synthesis of GABA-A receptors on Xenopus laevis oocytes allow me to test pressure#s role in ethanol antagonism.

Help Received
I used lab equipment at University of Southern California#s School of Pharmacy under the supervision of Dr. Daryl L. Davies.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Jenny Y. Chow

Project Number
S0406

Project Title
Screening for Transgenic Lines for Chromatin Immunoprecipitation (ChIP) Analysis of AG Targets in the Arabidopsis

Abstract

Objectives/Goals
AGAMOUS (AG) is a C-function floral identity gene, which is responsible for stamen and carpal development in the Arabidopsis floral meristem. In addition, AG is also required for meristem determinacy such that ag mutants are indeterminate forming endless numbers of whorls in a repeating pattern: (sepal, petal, petal)n. The main focus of this project is to identify lines that contain the T-DNA.

Methods/Materials
To determine and identify target genes of the AG, a special genetic construct with epitope tag is amplified and is inserted in the Arabidopsis through transgenesis. The offspring of these transgenic plants are observed and genotyped for heterozygous lines, which are isolated and self crossed. The offspring is treated with basta to determine if the parental lines are heterozygous for T-DNA

Results
Offspring of about 30 of 60 lines planted showed that the parental lines are heterozygous for the T-DNA. Thus, these 30 lines will be used for the ChIP analysis.

Conclusions/Discussion
By choosing these lines for the ChIP analysis, AG targeted sites can be more accurately identified and its function in organ identity can be understood.

Summary Statement
My project is about identifying the lines that contain the T-DNA construct, in that these lines will be used for ChIP process.

Help Received
Used lab equipment at Caltech, Worked on project under the supervision of Dr. Toshiro Ito.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Krystina R. Daniels

Project Number
S0407

Project Title
Protein Sequences and the Testudines' Tree of Life

Abstract

Objectives/Goals
Determine if evolutionary trees for turtles, based on morphological characteristics, are as accurate as evolutionary trees made using DNA. Research the DNA similarities of squamates, mammals, birds, crocodiles, and testudines. Re-categorize these animals based on their protein sequences and determine if they share a common ancestry.

Methods/Materials
Research which animals are directly related to turtles using a morphological evolutionary tree. Select five genetic diseases afflicting humans. Identify mutated nuclear genes and chromosome locus then identify protein deficiency associated with each disease. Using taxonomy reports, identify various species of animals located on the evolutionary tree, selecting ~40. Capture related amino acid sequences using the NCBI/Genbank website. Using the BLAST program, identify the protein sequences of genetic diseases in humans, and then locate similar protein sequences in other organisms to determine if the same gene can be found. Organize/compile the amino acid sequences of the proteins from the various species in a FASTA format. Using a multiple sequencing alignment program, CLUSTAL W, line up the proteins from different sequences in a Distance Matrix. Build a dendrogram animal tree based on the specific proteins entered repeating steps for each disease.

Results
Studied five genetic diseases found throughout the animal kingdom: Thalassemia, Albinism, Sickle Cell Anemia, Myoglobinuria and Pituitary Gland disorders, from which their proteins were identified: alphaglobin, tyrosinase, betaglobin, myoglobin and prolactin, respectively. Distance matrixes were developed representing paired evaluations of species and how close they were related to each other. Some matrixes yielded very consistent data such as displayed with the betaglobin matrix. Contrary, myoglobin had extremely wide variability even within species. Resulting dendrograms were different from the ancestral dendrogram based on morphological characteristics.

Conclusions/Discussion
I successfully proved my hypothesis that a different animal tree would result from the analysis of amino acids/proteins. Combining all data from all proteins, yielded the following summary: Testudines and Birds are the closest relatives at 77% similarity by DNA; Testudines and Crocodiles have 75% in common; Testudines and Squamates have 73% in common; and Testudines and Mammals are the furthest relatives at 67% similarity by DNA.

Summary Statement
Evolutionary trees for testudines, based on morphological characteristics, are not as accurate as evolutionary trees made using DNA.

Help Received
Professor A. Islas for proving a tutorial on the use of NCBI's websites; Science Buddies on-line website for additional tutorial materials; Mrs. D. Watkins for encouragement; and finally my mother for giving me so much encouragement and assistance in understanding the websites, and focusing my research.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)

Joshua DuBois; Danielle Williams

Project Number

S0408

Project Title

Killing Cancer: The Effect of Galectin-7 Overexpression in Oral Cancer Cell Line

Abstract

Objectives/Goals

To examine the effect of galectin-7 overexpression in oral cancer cell line.
Aim 1. Clone galectin-7/pcDNA3.1 expression vector
Aim 2. Transfect into galectin-7/pcDNA3.1 or pcDNA3.1 into SCC4 and SCC9
Aim 3. Serum-starvation and assay for apoptosis (examine morphology, nuclear condensation and DNA fragmentation)

Methods/Materials

Our study has examined our hypothesis that exogenous expression of galectin-7 in oral cancer cell line will facilitate apoptosis. We tested this by constructing galectin-7 expression vector in pcDNA3.1 and transfecting it into SCC4 and SCC9 oral squamous cell carcinoma cell line.

A 550-bp restriction fragment containing full-length galectin-7 was ligated into pcDNA3.1 mammalian expression vector. The recombinant construct was transformed into Escherichia coli DH5a strain and ampicillin resistant colonies were selected. Gal-7/pcDNA3.1 was digested with HindIII/BamHI and the restriction fragment fractionated on a 1% agarose gel. Transient transfection of gal-7/pcDNA3.1 resulted in expression of 700 bp gal-7 mRNA in SCC9 cells. Under serum starvation exogenous expression of gal-7 in SCC9 resulted in apoptosis sensitivity compared to cells with or without pcDNA3.1 vector.

Results

Plasmid isolation and subsequent restriction digest showed that some of the ampicillin resistant colonies contained the galectin-7/pcDNA3.1 construct.

Analysis of transiently transfected cells showed that galectin-7 mRNA and galectin-7 protein were expressed in SCC4 and SCC9 that was transfected with galectin-7/pcDNA3.1.

Serum starvation of SCC4 and SCC9 overexpressing galectin-7 resulted in cells with increased membrane blebbing along with cytosolic shrinkage.

Conclusions/Discussion

We conclude that overexpression of galectin-7 in oral cancer cells resulted in sensitivity to apoptosis. We plan to:

·Obtain results from cells transfected with pcDNA3.1 control that did not express neither mRNA or protein for galectin-7

·Overexpress Galectin-7 in HPV16-Immortalized Human Oral Keratinocytes

Summary Statement

Overexpression of galectin-7 in cancer cell line resulted in induction of apoptosis under serum-starvation.

Help Received

Advisor assisted in designing and carrying out experiment. Used lab equipment at King/Drew Medical Center under the supervision of Dr. Nishitani.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Amreeta K. Gill

Project Number
S0409

Project Title
A Structural Genomic Approach to Mycobacterial Drug Design Targeting Replicative Synthesis: Structure & Function of dnaQ

Abstract

Objectives/Goals
Multidrug resistant tuberculosis has emerged as a major public health threat; thus, new strategies are needed for mycobacterial drug development. The ultimate goal of this ongoing study is to design a drug to inhibit the function of a protein important for survival of the tuberculosis bacterium from the M. tuberculosis H37Rv genome. The present study focuses on determining and validating the structure of a protein target and conducting a preliminary virtual ligand screening of potential compounds for the design of a putative drug.

Methods/Materials
Genes from the H37Rv genome were selected as encoding potential protein drug targets, based upon annotation, sequence and fold similarity, and literature search, and then prioritized. Valid homology models were produced for high priority targets and functionally analyzed. A preliminary library of compounds was screened in silico for binding to the targets.
Materials included the sequenced genomes for laboratory and clinical strains of Mycobacterium tuberculosis, H37Rv and CDC1551, Swiss Institute for Bioinformatics proteomics tools; homology modeling, structure validation, and virtual ligand screening software including LOOPP, 3D-PSSM, Domain Fishing, RAMPAGE, Swiss Model, Deep view, ICM-Pro.

Results
Thousands of genes were examined, and dnaQ (Rv3711c) was given the highest priority among these genes for modeling and virtual ligand screening. dnaQ, encoding the epsilon subunit of the DNA polymerase III core, serves as the proofreader in the replicative synthesis of the bacterial DNA, affecting organism viability. The model of dnaQ is comprised of two domains: an N-terminal catalytic exonuclease domain, and a previously unknown C-terminal BRCT-like domain. Virtual ligand screening results suggest that carbonyldiphosphonate derivatives are promising drug lead compounds.

Conclusions/Discussion
dnaQ is a valid and promising antimycobacterial drug target. Because it is responsible for proofreading base-pair mismatches in the replicative synthesis of bacterial DNA, inhibition of dnaQ catalytic activity will affect organism viability. A valid homology model of dnaQ has been produced and a family of promising lead compounds was discovered for drug development. Future research will include the validation of native and complexed structures of the target by crystallography, followed by in vitro and in vivo assays of inhibition by proposed drug lead compounds.

Summary Statement
The two-domain structure of dnaQ was modeled in silico, validated, and analyzed; a family of potential lead compounds was discovered for the design of a drug for dnaQ.

Help Received
Used lab equipment at California State University, Fullerton under my mentor, Dr. Katherine Kantardjieff; my school counselor, Ms. Cheney, provided encouragement and support

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Georgina E. Hartzell

Project Number
S0410

Project Title
What Is the Effect of Protein Source on the Amount of Digestion that Occurs?

Abstract

Objectives/Goals
The goal of this project was to determine what type of protein is broken down the most. The categories were: poultry, red meat, tofu, wheat gluten, and beans.

Methods/Materials
I used 4 oz. samples of each protein source, and cooked them all in a similar manner. The process of digestion was simulted by grinding the protein in a food processor and adding digestive enzymes. These included Pepsin, artificial gastric juice, and pancreatic enzymes. The pH was taked before and after a two hour period of digestion, and the change in pH showed how much the protein had broken down. (three trials)

Results
I found that the greatest change in pH occured in the poultry samples, where the mean pH dropped from 6.84 to 6.64. The tofu and wheat gluten samples also experienced a statistically significant drop in pH, while the red meat and beans did not.

Conclusions/Discussion
Poultry appears to be the best digested source of protein, and it also contains the most protein: 35 grams. While red meat offer more iron, beans offer more fiber, poultry stands out for it's digestibility.

Summary Statement
The amount that different types of protein cn be digestion.

Help Received
Dr. Morey of Cal Poly University helped me come up with a method of quantifying digestion with pH.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Richard Hsu

Project Number
S0411

Project Title
Investigating a Novel Lifespan Gene in Drosophila melanogaster

Abstract

Objectives/Goals
The goal is to isolate a long live strain by affecting gene expression with the use of the enhancer promoters (EP) and driver P-element. Then identified the function and find the significance of this gene. If possible, find useful applications of this gene.

Methods/Materials
A lifespan analysis was perform to identify if EP3306, a fly line which has an overexpression of an unknown gene region, was long-lived. After EP3306 was shown to be long-lived, the next step was to find the overexpressed gene responsible for the extension of lifespan by conducting a plasmid rescue protocol. This resulted in the discovery of a putative gene, CG7900. A transgenic (clone) was made to verify that the gene, CG7900, was actually the gene responsible for longevity. Lastly, RT-PCR was perform to measure the amount of RNA levels for the EP3306 lines and the transgenic line to verify that the gene, CG7900, is actually be overexpressed.

Results
A screen for lifespan extension was performed using a driver line and a collection of EP lines. A driver line has a P-element which expresses the yeast transcriptional activator, gal4, while an EP line has a complimentary P-element which contains the DNA binding sequence of gal4. When a driver is crossed to an EP, the region downstream of the EP will be overexpressed. The mutant EP3306 was identified and found to have a reproducible lifespan extension. The EP3306 insertion site was found, and the putative gene overexpressed appeared to be an uncharacterized gene, CG7900. A genomic transgenic was constructed to verify that overexpression of CG7900 extends lifespan in an independent line. A recombinant was made between EP3306 and the daughterless-gal4 driver to look at interactions with other long-lived lines in the lab. Results show that CG7900 is the cause of increased lifespan and the gene itself seems to play a vital role in metabolism.

Conclusions/Discussion
In conclusion, the EP is located 300 base pairs upstream of the CG7900 gene, which has a high sequence similarity to a human protein. Hence, the understanding of how overexpression of CG7900 extends lifespan could be important to not only the understanding of fly aging, but also the aging process in humans.

Summary Statement
The gene, CG7900, is found to increase lifespan in the fruit fly when being overexpressed and that there is a high possibility that this gene can be integrated into the human genome.

Help Received
Brain Zid- my mentor for guiding me throughout this project; Pankaj Kapahi- for encouraging me to continue to work hard by rewarding me with indian food; Duane Nichols- my teacher who commented on my work and making sure I make the deadlines; Mickey Hsu- my sister who helped me find a laboratory

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Jan M. Humphrey

Project Number
S0412

Project Title
Inebriation as Seen Through the TV

Abstract

Objectives/Goals
At the conclusion of last year#s study, it was found that radiation did have an effect on the genetics of Drosophila, and did cause certain visible genetic mutations. After extensive research, a gene was found in Drosophila known as the amnesiac gene, which controls the Drosophila#s reaction to alcohol. The purpose of this year#s project is to determine if radiation has an effect on the gene that controls Drosophila#s sensitivity to the effects of alcohol, and therefore, effects the amount of time needed for a wild type fruit fly to become unconscious after being exposed to ethanol vapor.

Methods/Materials
Crosses were made between Wild type Drosophila at varying distances in front of a television. The F(1) progeny were exposed to ethanol vapor until unconscious. Data was recorded, calculations were made, and conclusions were stated.

Results
Initial examination of the control group, placed randomly at distances of
6 # 24 inches from the television, which was left off, and then exposed to ethanol vapor in an inebriometer, exhibited unconsciousness in 24-32 minutes.
Drosophila located six inches from the source of radiation for 48 hours of exposure demonstrated 100% fatality.
Drosophila located 12 inches from the source of radiation for 48 hours of exposure demonstrated 100% sterility, there were no eggs or viable offspring produced.
Drosophila located 18 inches from the source of radiation for 48 hours of exposure and then exposed to ethanol vapor in an inebriometer, took 15-21 minutes for all the Drosophila to become completely unconscious.
Drosophila located 24 inches from the source of radiation for 48 hours of exposure and then exposed to ethanol vapor in an inebriometer, took 22-29 minutes for all the Drosophila to become completely unconscious.

Conclusions/Discussion
Through experimentation and observation, my data and conclusions disprove my hypothesis. Varying amounts of radiation from a household television did have an affect on the amount of time needed for wild type fruit flies to become unconscious from ethanol vapors, and therefore, did have an effect on the amnesiac gene.

Summary Statement
Radiation has an effect on the gene that controls Drosophila#s sensitivity to the effects of alcohol, and therefore, effects the amount of time needed for a wild type fruit fly to become unconscious after exposure to ethanol vapor.

Help Received

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Serena A. Lee

Project Number
S0413

Project Title
Analysis of Ferritin Expression in Various Cell Lines with Flow Cytometry

Abstract

Objectives/Goals
The purpose of this study was to analyze the expression of the iron-storage protein heavy-chain ferritin in various cancer cell lines. A correlation was predicted to be the higher amount of ferritin, the higher amount the regulatory T cells will be activated. Previous studies showed that melanoma cell lines that expressed ferritin had higher percentages of T-regulatory cells.

Methods/Materials
The cell lines analyzed include: Raji, MT-1, YT-2C2, TPH-1-0, DEV, L540, Colo 667, Holm-3, Su-DHL-4, Su-DHL-6, Su-DHL-10, and Su-DHL-16. Each cell line had 3 sample tubes: control, cells with the secondary antibody only, and stained. The reason for the secondary antibody control is to make sure that the secondary antibody isn't randomly binding to any part of the cell. The cells were first washed and then a primary antibody was added and then the cells were incubated. The cells were then again washed and a secondary antibody with a FITC conjungate was added to the sample. After incubation, the cells were washed a final time and immediately analyzed by flow cytometry.

Results
The TPH-1-0 cell line showed the highest percentage of ferritin expression (98.13%) and the Su-DHL-16 came in second highest with a ferritin expression percentage of 80.28%. The DEV cell line had the lowest expression of ferritin (2.74%) with its secondary antibody control expressing a ferritin percentage of 3.85%.

Conclusions/Discussion
This means that the DEV cell line does not contain ferritin at all. Future studies will involve injecting ferritin-positive cell lines into separate mice. The cells will be collected and stained to determine whether there is a correlation between the amount of ferritin and the regulatory T cells or not. The TPH-1-0 mice are expected to contain a high level of T-regulatory cells in the blood while the DEV mice are expected to contain the least amount of T-regulatory cells in the blood.

Summary Statement
Various cancer cell lines were analyzed by flow cytometry to detect the amount of ferritin expressed in each cell line.

Help Received
Robyn Arias for her advice, support, and instruction; Dr. Alan Epstein for his guidance and support; Sam Kim for placing orders for needed supplies; Hal Soucier for his assistance with analyzing the samples with the flow cytometer; Mr. Nichols for his guidance; my parents and friends for providing me transportation

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)

Michael Y. Lin

Project Number

S0414

Project Title

Context-Dependent Binding by the MYC Oncoprotein: The Cellular Basis for Sustainable Tumor Regression

Abstract

Objectives/Goals

Research has shown that even temporary inactivation of the oncogene c-MYC can induce osteosarcoma tumors to regress. Surprisingly, when MYC is reactivated in these differentiated cells, the oncogene lacks the ability to reinduce tumorigenesis. Once this phenomenon is better understood, oncogene inactivation will hold much promise in the clinic.

The hypothesis tested here is that the MYC oncoprotein, a transcription factor, binds to target loci differently upon reactivation, depending on cellular context (differentiated or cancerous state), which produces the behavioral change in response to MYC.

Methods/Materials

Murine cancer cell lines containing a conditionally regulated MYC transgene were compared across three conditions: #MYC-activated# (MYC on for 48 hours; cancerous); #MYC-inactivated# (MYC off 48 hours; differentiated); and #MYC-reactivated# (MYC off 48, then on 20 hours; differentiated).

Results

First, a cDNA microarray assay showed global changes in gene expression. Genes were selected for further study by a short, custom-written Perl program that searched for MYC binding loci in promoter regions, as well as confirmation of microarray results by RT-PCR. A chromatin immunoprecipitation (ChIP) assay showed up to 5-fold changes in the binding of MYC to target loci.

Conclusions/Discussion

The results suggest that MYC#s inability to reinduce tumorigenesis may stem from compromised regulation of target genes, which in turn may come from a change in MYC#s binding to target loci. Therefore, cellular context affects a cell#s vulnerability to the oncogene through binding and regulation of target genes. Future work will identify factors that affect MYC#s binding and bring oncogene inactivation therapy one step closer to the clinic.

Summary Statement

Oncogene inactivation leads to sustained tumor regression because upon reactivation the oncoprotein binds to target genes differently.

Help Received

Used lab equipment at Stanford University in lab of Dr. Dean Felsher, under the supervision of Dr. Natalie Wu; began project as a summer intern under the Center for Clinincal Immunology at Stanford Summer Internship program

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)

Daphne D. Lo

Project Number

S0415

Project Title

Effects of PUFA on Oxidative Stress in Aged Rats

Abstract

Objectives/Goals

It is commonly known that fish oil, rich in n-3 PUFA (polyunsaturated fatty acids), is beneficial to the living body, and that safflower oil, rich in n-6 PUFA, is not. The higher the oxidative stress, the higher the risk of cancer. Oxidative stress levels are highest in the brain and heart organs. The objective is to see whether n-3 PUFA or n-6 PUFA will increase or lower oxidative stress in rat heart and brain.

Methods/Materials

Rats were categorized into four groups: basal group; Menhaden oil diet (M-group); Safflower oil diet (S-group); and a 1:5 ratio (Menhaden/Safflower) oil diet (M/S-group). Rat heart and brain tissues extracted from a previous research experiment funded by the Texas Tech University Seed Grant 2002-2003 were isolated of its DNA. After DNA was tested of its purity using a Spectro Photometer, it underwent DNA digestion and preparation for DNA 8-OHdG adducts. Using HPLC system, 8-OHdG was detected and was analyzed using one-way ANOVA.

Results

It turned out that the rats fed with Menhaden oil diet tends to decrease the oxidative stress levels in the heart but tends to increase the oxidative stress levels in the brain. On the other hand, rats fed with the Safflower oil diet tends to increase the oxidative stress levels in the heart yet tends to decrease the oxidative stress levels in the brain.

Conclusions/Discussion

Although the findings are not statistically significant, this experiment demonstrates that the Menhaden oil diet (or fish oil diet), 19% fish oil + 1% corn oil (rich in n-3 poly-unsaturated fatty acids, PUFA) has a tendency to reduce DNA damage in rat heart. And safflower oil diet has a tendency to lower levels of DNA adduct formation in brain of middle-aged rats. Because of budget consideration, the Basal group was 5 months younger than the other two groups. Proven in a previous research, the older the subjects, the higher the oxidative stress levels. This 20 week difference would have increased the statistical significance between the Basal group and the other groups.

Summary Statement

This project is an investigation to determine the effects of n-3 and n-6 polyunsaturated fatty acids, PUFA, on oxidative stress in rat heart and brain tissues.

Help Received

Used tissue samples, chemicals, and lab equipment at Texas Tech University Health Sciences Center, Department of Pathology under the supervision of Dr. Chwan-Li Shen.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)

Phung V. Luong

Project Number

S0416

Project Title

The Study of A3174 G and G3877A Double Mutation in the 3' UTR of the SRD5A2 Gene

Abstract

Objectives/Goals

The A3174G and G3877A are double mutation, which is in the SRD5A2 gene of the 3#untranslated region (UTR). The double mutant has been found to be common in males. This has become an interest in whether the double mutation will become a significant factor for developing prostate cancer by determining if the enzyme activity of the 5alpha-reductase does increase. To determine the enzymatic activity of the 5aplha-reductase my overall goal is to reconstruct my SNP through site directed mutagensis. Then the DNA will be transfected into African green monkey kidney cells to amplify the copy of DNA
and Vector. Later a Thin Layer Chromatography protocol is used to determine the enzymatic activity of the double mutation.

Methods/Materials

To determine the enzymatic ativity of the SRD5A2 gene with the A3174G and G3877A double mutation a Site Directed Mutagensis protocol is used to reconstruct the SNP. The reason why we need to reconstruct the plasmid is because the A3174G comes as a mutant and the G3877A comes as a Wild Type so I will need to reconstruct the G3877A to a mutant. Therefore I will have a double mutant. Second I transform the SNP to amplify the copy of the plasmid with the gene. Third a Miniprep is performed to purify the plasmid DNA. Fourth a digestion and electrophoresis is performed to make sure I have a correct fragment size DNA. Fifth a sequence is used to make sure I actually have the correct sequence. Then a Maxi prep is used to obtain an larger amount of purify plasmid DNA to be used for transfection. After Maxi prep a sequence, Digestion and Electrophoresis is performed once again. Next a B- glactosidase assay is used to test the efficiency of the transfection. Finally my mentor will perform a Thin Layer Chromatography(TLC) because in this protocol the samples will have to be radioactive and so as a student I am not allow to work with radioactive materials. The reason why TLC is important for this study is because TLC allows us to analyze the enzymatic activity of the 5 alpha #reductase.

Results

The double mutation did not show a significant increase of the 5 alpha reductase activity.

Conclusions/Discussion

The double mutation may not be a possible factor in males developing prostate cancer.

Summary Statement

The A3174G and G3877A double have not show a significant increase of the 5 alpha reductase activity based on my data

Help Received

Dr. Juergen Reichardt provided guidance and the chance to work under the USC at the Institute for Genetic Medicine (IGM).: Frank Luh provided tremendous guidance help performing the TLC experiments.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Matthew F. McArdle

Project Number
S0418

Project Title
Identification of Africanized Bees in Orange County using Mitochondrial Genetics

Abstract

Objectives/Goals
Africanized bees have been spreading throughout the United States, and in recent years have arrived in the southwest. These bees are a problem because of their hyper-aggressive behavior My goal is to identify the possible Africanization of bee colonies in the county using mitochondrial genetics.

Methods/Materials
I am using the polymerase chain reaction (PCR) to amplify a fragment of mitochondrial DNA that is polymorphic for a restriction enzyme. This polymorphism segregates between Africanized and European bees. I am using PCR to amplify the cytochrome B gene from bee genomic DNA, and then digesting the PCR fragment with the restriction enzyme, Bgl II. The Africanized bee DNA fragment is not cut by the enzyme; whereas, the European bee DNA fragment is cut. I use agarose gel electrophoresis to determine if the fragment is cut or not.
A. I first extract genomic DNA, then use cytochrome B specific PCR primers. I digest the DNA with the restriction enzyme, after which I run and gel and take a photograph.
B. I test bees collected from multiple hives that have been found in Orange County.
C. I test DNA from multiple bees from each hive.
D. I determine whether or not the restriction enzyme cuts the PCR fragment, and in turn whether or not the bee is maternally Africanized.

Results
I can successfully PCR amplify the mitochondrial genes and carry out the restriction digest. Based upon the gel results, 8 out of 12 samples tested were Africanized.

Conclusions/Discussion
Africanized bees are spreading throughout Orange County and the majority of hives in the county are Africanized. Because of my work with mitochondrial DNA, which is inherited solely from the mother, the hives that tested positive for being Africanized are the result of Africanized queen bees.

Summary Statement
I have examined samples from many different beehives from around Orange County and used PCR and restriction enzymes to cut the DNA I extracted from the bees in order to determine whether or not the bees were maternally Africanized.

Help Received
Worked under the guidance of David Gardiner and Felix Grun at the University of California, Irvine. I worked with Bee Busters, a bee removal service located in Laguna Beach, to obtain samples from various hives around the county.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
David F. McCleary

Project Number
S0419

Project Title
Efficiency of ATP Production of Mitochondria in Different Body Segments of Drosophila melanogaster

Abstract

Objectives/Goals
The goal of my experiment was to determine the relative efficiencies of ATP production of mitochondria in different body segments of Drosophila melanogaster.

Methods/Materials
I extracted and purified by centrifugation mitochondria from the head, thorax, and abdomen of 200 one week-old Drosophila. The samples were first standardized for protein concentration and thus mitochondria concentration using the Bio-Rad protein concentration assay protocol (Bradford assay). I inserted an oxygen sensor connected to a biological oxygen monitor and rolling grapher into a fluid chamber to measure the change in oxygen concentration in the fluid. I measured the rate of oxygen consumption of the samples in this chamber both when all substrates of respiration were present and when substrates were exhausted in four different trials. By dividing the former value by the latter value I constructed a Respiration Control Ratio (RCR) for all trials of the three samples, which gives the relative efficiency of oxygen consumption directly as a result of oxidative phosphorylation in mitochondria.

Results
I found the average RCR of head mitochondria to be 1.532, the average RCR of thorax mitochondria to be 6.986, and the average RCR of abdomen mitochondria to be 1.348. A high RCR means that large amounts of oxygen were consumed when substrates were present and low amounts of oxygen were consumed when substrates had already been used up, which is a sign of efficient ATP production.

Conclusions/Discussion
My data seems to show that my hypothesis was correct and thorax mitochondria are much more efficient in the production of ATP. Head and abdomen mitochondria RCR values are too close to show which is more efficient. If after further research I determine the cause of these differing values to truly be variable efficiency of mitochondria in different tissues, there are two hypotheses I wish to explore. The first being that mitochondria with different features function as egg polarity factors (cytoplasmic determinants) in the early embryo, and the second being that different nuclear genes that control mitochondria function are transcribed in different tissues causing different efficiencies of mitochondria in these tissues.

Summary Statement
I determined the relative efficiencies of ATP production of mitochondria in the head, thorax, and abdomen of Drosophila melanogaster by measuring oxygen consumed by oxidative phosphorylation in the mitochondria of these segments.

Help Received
I used lab equipment at the University of California, Irvine in the laboratory of Dr. Douglas Wallace under the supervision of Dr. James Tong.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Edith A. Pierre-Jerome

Project Number
S0421

Project Title
The Role of Brassinosteroids in Root-Cell Differentiation in Arabidopsis thaliana

Abstract

Objectives/Goals
The objective of this project was to answer two questions: 1) Do brassinosteroids play a role in cell differentiation in the root of Arabidopsis? 2) Is this role due to the biological connection between brassinosteroids and WEREWOLF?

Methods/Materials
Homozygous werewolf mutant seedlings were obtained and grown on five different concentrations of brassinolide (the most active form of brassinosteroids) and the hypocotyls were measured. Brassinosteroid mutants were then grown and compared to wildtype(control) on plates containing either 1mM brassinolide or no brassinolide. The length of the meristematic zones and root hairs were measured and a count of root hairs were taken for comparison between the mutants and wildtype on both plates. This experiment was then attempted again using seeds from what were thought to be adult homozygous werewolf plants.

Results
A comparison of the hypocotyl lengths of the werewolf mutant and wildtype found that the mutants were less sensitive to the brassinolide at higher concentrations suggesting that the WEREWOLF protein is neccessary for a normal brassinosteroid response. The measurements of the brassinosteroid mutants and wildtype showed that the addition of brassinolide affected all three factors of cell differentiation being studied through a decrease in root hair length, meristematic zone length, and root hair count. When the experiment was tried again on the werewolf seeds, the phenotype was unconvincing so a PCR was done to confirm the genotypes of the plants that were grown. All were found to have a wildtype gene suggesting that the mutant seedlings had not lived into adulthood to set seed; most likely due to the allele used which is possibly seedling lethal for the homozygous mutants.

Conclusions/Discussion
A strong biological connection between brassinosteroids and WEREWOLF was confirmed when it was found that WEREWOLF was neccessary for a normal brassinosteroid response. The results from the experiment done on the brassinosteroid mutants supported the idea that brassinosteroids play a role in cell differentiation. The possibility that this role is due to the connection with WEREWOLF is highly likely but has yet to be tested.

Summary Statement
The role of brassinosteroids in cell fate in the root of Arabidopsis thaliana.

Help Received
Used lab equipment at the Salk Institute under the supervision of Dr. Jennifer Nemhauser

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)

Brian Sa; Leslie Sheu; Jennifer Wan

Project Number

S0422

Project Title

The Effectiveness of RNA Interference at Inhibiting the Proliferation of HSV-1 by Silencing ICP27

Abstract

Objectives/Goals

Determine what sequence of siRNA is most effective in targeting ICP27.
Compare the amount of viral proteins between cells with and without RNAi.
Determine whether RNAi can inhibit HSV-1 infection.

Methods/Materials

1) Synthesize siRNA corresponding to desired genomic sequence of ICP27 of Herpes Simplex Virus Type 1 (HSV-1).
2) Determine which strands of siRNA are most effective via gel electrophoresis (SDS PAGE).
3) Transfect HeLa cells with siRNA and Oligofectamine Reagent.
4) Infect HeLa cells with HSV-1, harvest after 5-6 hrs.
5) Collect samples and run SDS PAGE; continue with a Western Blot.
6) Proceed with antibody staining and develop onto film.
7) For HSV-1 infected cells, label in vivo with S35-methionine to measure protein production and run SDS PAGE.
8) Stain gel with Coomassie blue for visualization.
9) Collect infected cells for a plaque assay count.

Results

Results confirm that siRNA is extremely effective in knocking down ICP27, with knockdowns upwards of 84% and statistical significance at the P=0.00446 level. Differences among siRNA sequences were not statistically significant (P=0.2112). The silencing of ICP27 shown by Western blots resulted only in minor differences in the amount of viral proteins between normal cells infected by HSV-1 and those with siRNA, shown by autoradiography and Coomassie blue staining. The plaque assays show siRNA#2 is more effective than siRNA#3 in inhibiting the proliferation of HSV-1. The number of plaque forming units (pfu) shows that HSV-1 cannot reproduce without ICP27. The difference in number of pfu in the mock and siRNA wells is statistically significant at the P=0.08987 level. A comparison of the siRNA wells shows that siRNA#2 worked better than siRNA#3 at bringing down the infection rate, statistically significant at the a=0.05 level with a p-value of 0.023.

Conclusions/Discussion

Our hypothesis was partially correct. Reduction of the viral protein ICP27 was successful, but a complete knockdown may be necessary to stop the proliferation of the virus. Further research includes using multiple siRNAs simultaneously or targeting several important proteins at once. Our research serves as a

Summary Statement

In this study, RNAi is used to silence ICP27, a crucial protein that exports intronless viral mRNA in Herpes Simplex Virus (HSV-1), in hopes of inhibiting the proliferation of HSV-1 in HeLa cell culture.

Help Received

Used lab equipment at the University of California, Irvine under the supervision of Dr. Rozanne Sandri-Golden and Santos Rojas; Santos Rojas did infections for us.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Shamsher S. Samra

Project Number
S0423

Project Title
Characterization of Mus81/Mms4's Role in Homologous DNA Repair during S-Phase of the Mitotic Cell Cycle

Abstract

Objectives/Goals
The Mus81/Mms4 heterodimer is a highly conserved member of the XPF endonuclease superfamily and part of the Rad52 epistasis group, which is known to serve a large role in recombinational DNA repair. Previous extraction of Mus81/Mms4 for in vitro experimentation has varied based on the proteins' host and has differed in conclusions about the proteins' function. The objective of my research was to directly isolate and characterize the Mus81/Mms4 heterodimer, along with its respective mutant, from a native eukaryotic host, Saccharomyces cerevisiae; ultimately providing a tool necessary for comprehending the Rad52 epistasis group of proteins as well as the mechanisms of DNA repair synthesis.

Methods/Materials
An overexpression plasmid was created and propagated within E. coli cells prior to being transformed into S. cerevisiae cells for protein overexpression. Following Comassie staining and Western Analysis to verify overexpression, the endonuclease was purified through affinity chromatography. The applicability of the protein was verified through DNA sequencing and complementation testing, prior to conducting nuclease assays using P-32 labeled DNA substrates.

Results
DNA sequencing of the purified protein revealed the endonucleases was identical to the accepted sequence. Complementation tests verified that the addition of protein tags in no way inhibited or altered the in vivo functions of Mus81/Mms4. Mus81/Mms4 nuclease assays reveal the structure specific endonuclease displays a penchant for cleaving the 3' Flap of ssDNA-dsDNA junctions. The findings suggest Mus81/Mms4 serves to maintain stability in stressed replication fork elongation, and not to resolve the four-way Holliday Junction resulting from homologous DNA fork repair, as is suggested by the current model of DNA repair synthesis.

Conclusions/Discussion
Comprehending Mus81/Mms4's in vivo substrate preference assists in developing a strong understanding of the endonuclease's function during S-Phase cellular division. The fact that cells lacking the Mus81/Mms4 heterodimer are highly sensitive to carcinogens, suggests the possibility of a homologous understanding of failures in cancer averting mechanisms. By better comprehending the components and processes of DNA repair synthesis during cellular division, we may be in a better position to pinpoint the failed mechanisms attributed to tumor formation and cancer development.

Summary Statement
Purification and biochemical characterization the Mus81/Mms4 protein to determine its role in DNA repair synthesis during the S-Phase of mitotic cellular division.

Help Received
Use of Equipment at the University of California Davis Microbiology Department under the supervision of Kirk Ehmsen.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Rohini Shantharam

Project Number
S0424

Project Title
Searching for Mechanisms for Activating 2,4-D Degradation in Soil Bacteria TFT5, TFT39, K19: A Comparative Promoter Stud

Abstract

Objectives/Goals
2,4-Dichlorophenoxyacetic acid is a recalcitrant xenobiotic herbicide widely used as broad-leaf weed control for cereal crop production. The toxin has been accumulating in our soil thus increasing likelihood of health risks associated with its exposure. Studies have shown that the plasmid pJP4 from Ralstonia eutropha and Burkholderia spp. have evolved metabolic pathways to use 2,4-D as its soul carbon and energy source.
The main objective of this new approach is to test the promoters from JMP134 and RASC in various 2,4-D degrading bacterial soil strains to identify which promoter influences most enzyme activity. Prior to beginning research it was hypothesized that each promoter in each soil strain would show similar enzyme activity.

Methods/Materials
The experiment used E. coli containing plasmids with a variety of tfd promoters for the lacZ gene coding for beta-galactosidase. A quantifiable beta-galactosidase expression would be helpful for a better determination of promoter suitability. The experiment included mating, through conjugation, the E. coli containing cloned promoter regions from strain JMP134 and RASC with three different soil bacteria. Following this Beta-Galactosidase Assays were performed to quantify gene expression. Finally, DNA extraction and gel electrophoresis were run for further assurance of the plasmid in the soil strain.

Results
Results indicated that promoter pMD96 showed comparatively higher enzyme activity in both of the tested soil strains. Beta-Galactosidase Assays were terminated due to continuous growth of E coli. Gel electrophoresis indicated that promoter insert pMM7700 was deleted.

Conclusions/Discussion
This suggests that perhaps promoter pMD96 contains regulatory elements superior to the others. Understanding these gene regulatory mechanisms now will permit upcoming attempts at engineering toxin degrading regulatory and structural genes.

Summary Statement
The project tests various promoters from 2,4-D degrading soil strains TFT5, TFT39, and K19 to see which one results in more enzyme activity.

Help Received
The project was conducted at California State University of Fresno

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Ryan M. Short

Project Number
S0425

Project Title
Pyrotex-Optimization of Protein Polymerization

Abstract

Objectives/Goals
By recreating the salt conditions, pH, and temperature of the original environment of the organism, Pyrodictium abyssi, the canA encoded polymerizing protein, will produce the helix forming proteins more efficiently than with the common laboratory salts magnesium chloride, and calcium chloride used in the original experimental studies.

Methods/Materials
Each cation in the experiments was combined in separate reactions with either calcium, or magnesium. The results of these reactions were collected and further experiments were performed, based on the results of which cations were optimal for polymerization. Each reaction occurred at 80°C along with a primer reactant, consisting of polymerized proteins, to initialize the reaction.

Results
What was discovered is that with these salt variables, the nanotubes had over tripled in length compared to the original controls of the experiment, calcium chloride and magnesium chloride, combined without additional cations.

Conclusions/Discussion
This progress could potentially lead to new breakthroughs in the field of nanotechnology, with countless applications, from areas such as cardiovascular health, to optical fibers in computers.

Summary Statement
The optimization of polymerization conditions of a protein monomer obtained from the isolated Pyrodictium abyssi microorganism, by recreating the salt conditions, pH, and temperature of the original deep sea enviornment.

Help Received
Used laboratory chemicals, centrifuge, and confocal microscope at Diversa Corporation, San Diego, CA., under the supervision of Dr. Eileen O'Donoghue.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Arjun A. Suri

Project Number
S0426

Project Title
Modeling of Tyrosine Sulfation Sites in 7TM Receptors: A Novel Approach to Pharmaceutical Drug Design

Abstract

Objectives/Goals
Sulfation as a post-translational modification of tyrosine has been shown to influence the physiology of proteins and play a role in protein-protein interactions. Determination of the location of sulfation sites in three-dimensional structures of proteins may help elucidate the precise function of tyrosine sulfation in drug-cell interactions. The purpose of this study is to suggest the role of tyrosine sulfation in ligand-binding. This understanding will allow us to create pharmaceutical drug designs that more effectively target cells involved in diseases such as Alzheimers, Parkinsons, and AIDS.

Methods/Materials
21 Seven-transmembrane receptors (7TMs) were selected based on their scores in the Position-Specific-Scoring-Matrix for tyrosine sulfation. The Position-Specific-Scoring-Matrix assigned a score denoting the probability that tyrosine sulfation occurred at a specific site. The three-dimensional model of Rhodopsin was used to align various receptors. Amino acid sequence alignments, enhanced by the use of helical wheels, were used to align the equivalent amino acids of the known Rhodopsin structure with the predicted tyrosine sulfated sites.

Results
The three-dimensional locations of all predicted sulfated tyrosines existed in a ring-like formation within 10 angstroms of the ligand-binding site, suggesting that sulfation plays a role in ligand-binding affinity and specificity. The helical and extracellular alignments showed regions with predicted sulfation sites that were conserved throughout all receptors, suggesting the common function of tyrosine sulfation. The concentration of putative sites of sulfation in the extracellular regions suggests the role of sulfation in the binding process of drugs.

Conclusions/Discussion
As the predicted sulfation sites are located within 10 angstroms of the ligand-binding site, they are accessible to the ligand and may interact with it to regulate binding affinity and specificity. Sulfation has been proven to increase binding of the HIV-1 virus, and understanding its role in drug-cell interactions may lead to improved pharmaceutical therapies, including small-inhibitor drugs. As 7TM receptors are currently known to play a role in up to seventy percent of drug-cell interactions, a model for the role of tyrosine sulfation in these receptors would benefit pharmaceutical designs for compounds that more effectively target the binding sites of 7TM receptors.

Summary Statement
The purpose of this study is to suggest the role of tyrosine sulfation in drug-cell interactions and to create pharmaceutical drug designs that more effectively target these receptors.

Help Received
I would like to thank Dr. Grace Rosenquist for her invaluable support throughout my research. I would also like to thank the UC Davis Young Scholars Program for giving me the opportunity to perform this research.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Tara K. Tran

Project Number
S0427

Project Title
The Living Liquid: A Study in Integrating d-Glucose in Thrombocyte Preservation

Abstract

Objectives/Goals
The biochemical complexes of thrombocyte currently require FDA regulations that shorten the number of days thrombocytes can be stored. The objective is to determine if a solution of the simple sugar monosaccharide aldohexose d-glucose will significantly affect thrombocyte preservation in mammalian blood.

Methods/Materials
5 different samples of blood were collected from mammal Canis familiaris. Half of each sample was separated into another test tube. Samples in EDTA test tubes were labeled "A" and samples in non-EDTA test tubes were labeled "B". Slides were made for all 10 test tubes, where a microscope was used to observe for thrombocyte count and condition. A d-glucose solution was added to "B" samples and all samples were left in a controlled environment for 3 days. After 3 days, slides were made for all 10 test tubes, where a microscope was used to observe for thrombocyte count and condition.

Results
In 4 out of the 5 samples, the percent decrease of thrombocyte count levels of samples that contained d-glucose was slightly higher than samples that did not contain d-glucose. The average thrombocyte count of samples that contained d-glucose decreased by 6.42 % on day 3, while the average thrombocyte count of samples that did not contain d-glucose decreased by 10.90 %.

Conclusions/Discussion
Data analysis establishes that d-glucose does not significantly affect thrombocyte count levels in mammalian blood. Although 4 of the 5 samples demonstrated signs of lengthened thrombocyte preservation in d-glucose samples, statistical data did not demonstrate a significant difference between non-d-glucose and d-glucose samples, but did demonstrate a positive change. To expand on results, d-glucose had a minor effect on thrombocyte receptor beta-N-acetylglucosamine, screening it from macrophage alpha-M-beta-2 integrin receptors which would have consumed the thrombocytes sooner had d-glucose not been added. Additional experimentation may lead to further understanding of these thrombocyte complexes, and contribute to the greatly needed effort towards thrombocyte preservation.

Summary Statement
To determine if simple sugar monosaccharide aldohexose d-glucose screens thrombocyte receptors from macrophage integrin receptors and significantly affects thrombocyte preservation in mammalian blood.

Help Received
Used lab equipment and facility at AAA Animal Hospital. Dr. Richard Yamaguchi and medical staff provided instruction in specific procedures.

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Stephanie A. Tse

Project Number
S0428

Project Title
SNP Detection of a 410bp Region of MTRR Promoter in Colorectal Adenoma Patients

Abstract

Objectives/Goals
Studies have shown low folate is associated with colorectal cancer precursor lesion (adenoma) development. The one-carbon cycle is a folate-dependent biochemical process important for DNA synthesis and methylation. Methionine synthase reductase (MTRR) is an enzyme responsible for restoring cobalamin activity, an important process in the one-carbon cycle. Defects in the MTRR gene are hypothesized to lower folate availability because of potential unwanted effects on MTRR enzyme efficiency. The purpose of this project is to analyze a 410bp region of the promoter for single nucleotide polymorphisms (SNPs), a single base pair change not yet considered a mutation, in samples with colorectal adenomas.

Methods/Materials
Denaturing High-Performance Liquid Chromatography (DHPLC) was used. Computer screening and direct sequencing protocols of 28 randomly-selected samples specialized for SNP detection were used.

Results
Results show a heterozygous 2479T##C/T SNP in the targeted region in the positive controls. In actual cases, 27% of the case showed similar peak formation as that of the positive control.

Conclusions/Discussion
Results suggesting that problems may occur on a transcriptional level, but the effect of the SNP will be studied in future research.

Summary Statement
Detecting single nucleotide polymorphisms in the promoter region of patients with colorectal adenomas, precursor cancer lesions, to elucidate possible genetic factors to be studied for affect on colorectal cancer.

Help Received
Dr. Robert Haile's Laboratory & Staff for equipment and guidance (USC/Norris); Dr. Yong Liu & Joan Levine, mentors; Daan Ren, DNA sequencing technician

CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)
Shantal N. Villalobos

Project Number
S0429

Project Title
The Effects of Calcium Regulation on Flower Timing in Arabidopsis

Abstract

Objectives/Goals
THERE HAS BEEN PREVIOUS ANALYSIS OF AN ACA2 MUTANT IN ARABIDOPSIS THALIANA, WHICH IS A MUSTARD PLANT WITH A CALCIUM-PUMP KNOCKED OUT. SINCE THE ACA2 MUTANT SHOWED A DELAY IN THE FLOWER TIMING, THERE IS A POSSIBILITY THAT THE REASON FOR THE DELAY WAS THE ABSENCE OF THE CALCIUM-PUMP. IN THIS PROJECT, A DIFFERENT ALLELE (ACA2-3), WAS CHOSEN AND TESTED FOR ITS ROLE IN CALCIUM REGULATION. THE PURPOSE OF THE PROJECT IS TO SHOW THAT THE ABSENCE OF THE PUMP IN THE ACA2-3 GENE CAUSES A DELAY IN THE FLOWER TIMING AS WELL. IF EXPERIMENTS SHOW THE PHENOTYPE OF DELAY IN FLOWERING IN BOTH CONDITIONS, THEN WE CAN BE REASONABLY SURE THAT THE CAUSE OF THE DELAY IS THE ABSENCE OF THE CALCIUM-PUMP.

Methods/Materials
PLANTS WERE GROWN UNDER SPECIAL CONDITIONS, SPECIFICALLY IN A CONVIRON PRODUCING 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS. AS WELL AS A SEPARATE PLANT ROOM UNDER CONSTANT CONITIONS OF LIGHT (24 HOURS OF LIGHT). THE PLANTS WERE SEPARATED ACORDING TO THE LIGHT CYCLE. 4 FLATS OF SEED WENT TO THE 12/12 CONVIRON, AND 4 FLATS WENT TO THE 24-HOUR PLANT ROOM. THE DATA SHOWS THAT THE ACA2-2 GENE DID INFACT SHOW A DELAYED PHENOTYPE UNDER 24 HOUR LIGHT CONDITIONS.

Results
THE ACA2-3 GENE SHOWED A DELAYED PHENOTYPE . UNFORTUNATELY, THE ACA2-2 GENE DID NOT SHOW THE SAME PHENOTYPE UNDER THE 12/12 HOUR CONDITIONS WHEN THE ACA2-3 GENE DID SHOW A DELAY.

Conclusions/Discussion
THESE RESULTS CONCLUDE THAT IT IS NOT THE MISSING CALCIUM PUMP THAT CAUSES A DIFFERENT PHENOTYPE. THEREFORE, I CAN SAY THAT IT IS BECAUSE OF ANOTHER MUTATION IN THE GENE THAT THE FLOWER TIMING IS DELAYED, AND THAT PREVIOUS BELIEFS OF THE ACA2 PUMP ARE INACCURATE IN THE SENSE THAT THE ACA2 PUMP ALWAYS SHOWS A DELAYING PHENOTYPE. FURTHER RESEARCH CAN DEVELOP TO FIND THE EXACT MUTATION CAUSING THE PHENOTYPES FOUND.

Summary Statement
To see if the deletion of the calcium pump, aca2, is the cause of delayed flowering in Arabidopsis thaliana plants.

Help Received
Used some planting equipment at The Scripps Research Institute