CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)

Danielle B. Andrews

Project Number

S0401

Project Title

The Involvement of Small G-protein ARL-6 in the Formation of Sensory Cilia

Abstract

Objectives/Goals

Compartmentalized cilia play an essential role in mechanosensory transduction and perception of the environment. The aim is to identify and verify highly-conserved proteins required for formation of sensory cilia.

Methods/Materials

A bioinformatic approach was utilized to identify homologous small G-protein genome sequences relatively conserved among evolutionarily divergent eukaryotes with compartmentalized cilia. An identified candidate gene was characterized genetically using deletion and point mutant stocks of D. melanogaster.

Results

Two candidate small G-proteins (Arl3 and 6) were identified by comparative genomics within 37 genes tested among the four eukaryotes with compartmentalized cilia. A Drosophila deletion stock mapped to a region around Arl6 crossed to two "potential mechanosensory point mutants" on chromosome 2 yielded uncoordinated flies unable to fly or walk. This phenotype is characteristic of flies with defects in compartmentalized ciliogenesis.

Conclusions/Discussion

Arl 6 is one of two genes identified by comparative genomics to be a protein conserved among organisms with compartmentalized cilia. An uncoordinated phenotype was demonstrated in Drosophila potentially defective in Arl6, validating the bioinformatic approach used to find key genes in perception/mechanosensory transduction. Further research should be done to determine whether Arl 6 transgene insertion can rescue the phenotype. The specific role of Arl 6 in ciliogenesis warrants further study.

Summary Statement

Small G-protein Arl6 appears involved in the formation of a specific type of cilia required for mechanical sensing of the environment.

Help Received

Dr. Charles Zuker, Senior Investigator at the Howard Hughes Medical Institute, allowed me to work in his laboratory under the guidance of his post-doctoral fellow, Dr. Tomer Avidor-Reiss. Dr. Avidor-Reiss provided guidance and training in computational bioinformatics and genetic methods.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)

Peter Joseph M. Edpao; Sarthi R. Shah

Project Number

S0402

Project Title

Phase III - A Study of the Effect of pH on Mitochondria: A Practical Application with Daytona (Bush) Beans

Abstract

Objectives/Goals

To determine which solution, acidic, neutral, or basic, will affect the growth of Daytona (Bush) Beans most positively.

Methods/Materials

Daytona (Bush) Beans were exposed to different solutions: acidic, neutral, and basic. For a period of 30 days, they were grown. Once viable, germinating seeds reached the 30 day period, samples were taken from the plants and run through the #Tracking ATP Production Rate of Mitochondria Protocol,# using a Janus Green B stain and spectrometer, to track relative mitochondria activity.

Results

Half of the plants nourished with an acidic solution grew, 75% of the plants nourished with a neutral solution grew, and 0% of the plants nourished with a basic solution grew. Samples from the acidic plants had an average absorbency of 0.002 A. Samples from the neutral plants had an average absorbency of 0.035 A. There were no basic plant samples, since none germinated. Pre-test lab data shows that celery cells tested with an acidic solution continually rose in absorbency level, reaching up to 0.042 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 mitochondria. The greater amount of activity of the mitochondria signifies that it produces more ATP. The results do not directly support the conclusion that an acidic solution most positively impacts the rate of ATP production in mitochondria. While not all of the plants nourished with an acidic solution grew, the acidic plants that did grow exhibited growth qualities that were more positive than the neutral or basic plants. The results leave open avenues for testing specific levels of acidic pH on the growth of plants.

Summary Statement

The project is about using the "Tracking ATP Production Rate of Mitochondria" to assess the practical application of growing plants nourished with different pH solutions.

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
2005 PROJECT SUMMARY

Name(s)

Evangeline J. Fleischaker

Project Number

S0403

Project Title

Use of Quartz Crystal Microbalance to Study Antibody Antigen Interactions

Abstract

Objectives/Goals

The Quartz Crystal Microbalance (QCMB) is a simple device. The adsorption of a compound on the quartz surface causes a decrease in the resonance frequency that is proportional to the mass adsorbed. My goal was to use the QCMB to prepare a biosensor capable of measuring baculovirus particles.

Methods/Materials

Self assembled monolayers (SAM's) were used to modify the surface of a quartz crystal to enable the attachment of proteins such as antibodies. Antibodies against the baculovirus coat protein gp64 were immobilized on a SAM that contained an N-hydroxysuccinimide(NHS) group.

Additionally the regeneration of the bound antibody was examined by screening a series of reagents which enabled me to identify a combination of these reagents that efficiently disrupt the antibody antigen interaction with out damaging the bound antibody. This enabled the repeated reuse of the antibody.

Results

Results show that the quartz crystal microbalance successfully and rapidly detected the presence of the baculovirus via the gp64 antigen. The biosensor shows a remarkably linear response, in the range of 10E6 to 10E7 pfu per mL. On the positive side, this response is sensitive enough to be useful in cell culture, since the concentration of baculovirus produced in cell culture is typically in the range of 10E7 to 10E9 pfu per mL. On the negative side, the unit is temperature-sensitive, often requiring an hour or more to stabilize before useful measurements can be obtained.

The cleaning study showed that a mixture of acids, salts, and EDTA could completely regenerate the antibody and permitted more that 10 uses without any apparent lose in antibody capacity.

Conclusions/Discussion

The target antigen chosen for this project, the baculovirus Autographa californica, is of particular interest to many in the pharmaceutical and pesticide industries. This virus is most active against the alfalfa looper, which destroys many crops. This project has demonstrated its applicability to industry in that baculovirus titers were obtained with the quartz crystal microbalance biosensor, in less than a day, with ability to re-use it numerous times. The capability of this biosensor could expedite industry#s ability to produce the virus or products made by them.

Summary Statement

The preparation of a biosensor to baculoviruses using a quartz crystal microbalance.

Help Received

Used lab equipment at Vista Biologicals under the supervision of Robert Fleischaker; Steven Fleischaker for writing a C++ program to parse the data captured in a text file; Chisato Shiohara of Vista Biologicals for preparation of the baculovirus stock.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)

Aleksandr Gorin

Project Number

S0404

Project Title

Studying the GST-Y10 Protein: Is the C-domain Resonsible for Histone Interaction?

Abstract

Objectives/Goals

The NAP1 protein is the protein that assembles nucleosomes and organizes DNA inside the nucleus of cells. The aim of this experiment is to determine whether the GST-Y10 protein has the ability to bind histones. The experiment will consist of two major steps; creating the GST-Y10 protein and a pull down assay using the produced protein and histones.

Methods/Materials

The GST-Y10 protein is made by binding the Y10 and GST protein together. The Y10 protein is a fragment of the NAP1 protein that codes for the C-Domain. The C-domain is believed to do most of the work that the protein carries out with respect to histone binding. This Y10 protein is obtained from yeast cells, whose NAP1 protein is very similar to that of humans.

After the protein is created a test will be run to see if it interacts with histones. The test will be a pull down assay. The results of the pull down assay will show whether the GST-Y10 protein can interact with histones and give the final answer to the experiment.

Conclusions/Discussion

The results of this experiment showed that the histones do interact with the GST-Y10 protein.

This science fair project was carried out with the use of the Dutnall Laboratory at the University of California, San Diego. All procedures involving undiluted ethidium bromide and unpolymerized acrylamide were carried out by a qualified supervisor. This experiment was performed with the assistance of Professor Robert Dutnall and the graduate students in his laboratory.

Summary Statement

This project is to see whether the C-Domain of the Nucleosome Assembly Protein is the section of the protein that is responsible for histone bonding.

Help Received

Professor Robert Dutnall provided facility. Joon Huh supervised experimentation. Mother helped with designing display. Mrs. Fenster edited and gave feedback on report and display.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Rachel M. Halper

Project Number
S0405

Project Title
The Efficiency of Lactase

Abstract

Objectives/Goals
The goal of this experiment is to test the efficiency of lactase enzyme, with varying concentrations and temperatures of lactose.

Methods/Materials
The materials used were: Lactose Sugar, Lactase Enzyme, Water bath, Distilled water, Glucose test strips, 10mL test tubes, 600 mL glass bottle, and dextrose. The first test done was to test the glucose test strips accuracy. The glucose test strips were able to test for concentrations of glucose at 0.005M, 0.015M, 0.030M, 0.060M, and 0.110M. So, solutions of dextrose, with these molarities, were made. Then they were tested using the glucose test strips. The test showed the strips were accurate. Then 2 litters of a 1M lactose solution was made. 3mL of the solution at room temperature were tested with 2 drops of the lactase enzyme. The a glucose test strip was placed in the solution and the concentration was read off of it. Then 80mL of the solution was heated it up in increments of 5 degrees starting at room temperature. Every five degrees the solution was tested for glucose. This was continued until the maximum amount of glucose was detected. The lactose solution was then decreased in increments of .2M. At each concentration, the solution was heated up in increments of 5 degrees and test for glucose every 5 degrees.

Results
When the concentration of the lactose is lowered, the temperature at which the maximum amount of glucose is tested becomes lower. So, at lower concentrations of lactose the lactase becomes more efficient.

Conclusions/Discussion
From the results of this experiment, the researcher can see that as the concentration is lowered and as the temperature increases of lactose, more glucose was detected. But, at lower concentrations, the temperature wasn#t as high when the maximum amount of glucose was detected. More glucose was detected as the temperature increases because the bonds in the lactose are weaker due to the heat. So, the same amount of lactase will detect more glucose because the enzyme has less work to due.

Summary Statement
This project tests lactase's ability to dissociate lactose into glucose and galactose.

Help Received

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Sandy E. Hawley

Project Number
S0406

Project Title
Size Matters: DNA Amplification

Abstract

Objectives/Goals
To optimize the PCR process and sequence my successfully amplified DNA.

Methods/Materials
PCR was performed with standard reagents and thermal cycling parameters. MgCl(2)concentration was varied, in addition to the annealing temperature, and agarose gel staining technique. DNA was sequenced and edited.

Results
In this experiment, the highest MgCl(2)concentration (8ul) and the higher annealing temperature (48 degrees C)produced the most amplified DNA. In addition, the ethidium bromide gel staining technique worked dramatically better than Ward's Quick View DNA stain. Cytochrome b gene fragment sequences were obtained for two individuals of the intertidal fish, Clinocottus analis.

Conclusions/Discussion
These results indicate that the highest MgCl(2) concentration improved the enzynmatic activity of the DNA polymerase. The increased annealing temperature allowed the primer to bind to the right complementary bases of the template DNA strand. The ethidium bromide DNA stain illuminated the amplified DNA better because the technique required ultra-violet light that made it easy to visualize the amplified DNA.

Summary Statement
I attempted to optimize the PCR process and sequence the successfully amplified DNA.

Help Received
Dr. Frank Cipriano, used lab equipment at San Francisco State University

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Logan C. Hubbard

Project Number
S0407

Project Title
Transformation of Plant Genome Using Agrobacterium

Abstract

Objectives/Goals
The goal of the project is to impliment the luc gene of the firefly into a plant using Ecoli splicing and microinjection, protoplast fursion, and the use of the plant pathogen Agrobacterium Tumefaciens.

Methods/Materials
A. Tumefaciens; E.coli bacteria, luc genes of the firefly, dicot plants for incorporartion

Results
At the moment the project is still being done, due to the fact that there are so many restrictions on the pathogen I just recently got the bacteria and could begin the experiment. As for the other projects they were partially sucessful due to the fact that the plant cell glew as well as the E.coli bacteraia.

Conclusions/Discussion
The E.coli expressed bioluminecence, but when it was incorporated intot the plant it did not succeed due to the fact that a cell can divide only so many times before it stops dividing due to biological law. The protoplast fusion experiment suceeded in making the plant cell glow, yet the same biological law came up and due to this it did not suceed either. Finally the luc gene was spliced into the A. Tumefaciens and then incorporated into the plant via incision or puncture. This is still being preformed and is waiting for results due the lengthy time for incorporation into the genome.

Summary Statement
The incorporation of the luc gene using different methods

Help Received

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Jeffrey L. Jensen

Project Number
S0408

Project Title
Isolation by Distance: Quantifying Genetic Similarity in Relation to Geographic Distance

Abstract

Objectives/Goals
1) Create an online web service for statistically calculating the impact of geographic distance on genetic distance (http://phage.sdsu.edu/~jensen/).
2) Get allelic data for multiple populations of the Jeffrey Pine Beetle and the Ponderosa Pine Beetle.
3) Analyze the allelic data and determine which species has more genetic variance.

Methods/Materials
To obtain the allelic data of the beetles, the following methods are used:
1) Collect samples of Ponderosa Pine and Jeffrey Pine Beatles.
2) Isolate the DNA from the samples.
3) Do Polymerase Chain Reaction (PCR) gradients to determine the optimal amounts of added magnesium chloride, annealing temperature, and DNA concentration for each primer being used (810, 811, and 825).
4) Use PCR to replicate the DNA from the samples using the optimal configurations, determined in step 3, 3 separate times (each time using a different primer).
5) Run the PCR product on a high resolution gel.
6) Read the bands using a combination of computer software and gel analysis hardware to generate allelic data.

Results
Isolation by Distance Web Service has become a popular internet application used by biologists across the country. It stands as a unique application of statistics, genetics, and computational power to produce valuable conclusions regarding geographic distance's impact on genetic variance. The service is hosted 24/7 at http://phage.sdsu.edu/~jensen/ by the Rohwer Lab at San Diego State University.

Conclusions/Discussion
The methods used in IBDWS, including the mantel test, reduced major axis regression, and Slatkin and Rousset's measures of genetic distance combine flawlessly as a complete solution for analyzing geographic distance's impact on genetic variance.
In addition to the success of IBDWS, an article has been published detailing its operation by the scientific journal "Biomedical Central - Genetics."
At the time of this writing, the allelic data is not yet ready for analysis but it is merely supplemental to this project and may or may not be ready by the time of the fair.

Summary Statement
Isolation By Distance Web Service statistically analyzes geographic distance's impact on genetic variance.

Help Received
Dr. Bohonak and Dr. Kelley of San Diego State University Life Sciences have both played crucial roles in the creation and publication of IBDWS.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)

Xiangyi (Angela) Ji

Project Number

S0409

Project Title

In Vitro Study of Manganese Compounds as Superoxide Dismutase Mimetics: The Search for the Fountain of Youth

Abstract

Objectives/Goals

This experiment investigated the effectiveness of various manganese salts (MnCl(2) and MnSO(4)) and complexes (salen-manganese complexes, EUK-189 and EUK-8) in mimicking the catalytic function of superoxide dismutase in two different buffers.

Methods/Materials

Free radicals generated by the xanthine/xanthine oxidase system were quantified by following the oxidation of NBT, which resulted in changes in absorption property. SOD activity was then measured as the degree of inhibition of absorbance changes. Reactions were initiated with the addition of NBT and xanthine and xanthine oxidase to the manganese salts or complexes, and NBT absorption at 550 nm was measured every 10 seconds for 5 minutes using a spectrophotometer.

Results

Changes in optical densities of the various samples were plotted against time for the solutions in both buffers. Slopes of the trendlines were used to determine SOD activity per micromolar, where one unit of activity was designated as 50% of the change in absorbance per second under control conditions. EUK-189 was found to be the most efficient SOD mimetic, with a catalytic activity of 0.475 units/micromolar in Tris-HCl buffer, whereas MnCl(2) and MnSO(4) exhibited fairly low SOD activities, averaging only about 0.01 units/micromolar in both buffers. Overall, SOD activities were higher in Tris-HCl buffer than in phosphate buffer.

Conclusions/Discussion

EUK-189 was the most efficient SOD mimetic, and Tris-HCl the more efficient buffer. Perhaps both the EUK-189's structure and the nature of the buffer facilitated the binding of oxygen free radicals to the complex and the cycling of manganese between Mn(II) and Mn(III) after removing the extra electron from the superoxide. Conversely, it was likely the lack of such a surrounding complex resulted in the low catalytic activity of the manganese salts. Nevertheless, results indicated that all manganese compounds exhibit some degree of SOD activity, with simple salts demonstrating significantly lower catalytic rates than organic manganese complexes. Thus the investigation suggests the possibility of substituting SOD with a smaller, yet relatively efficient small molecule in laboratory and pharmaceutical applications.

Summary Statement

Manganese compounds, especially salen manganese complexes, are superoxide dismutase mimetics which catalyze the conversion of oxygen free radicals and thus have potentially far-reaching medical implications.

Help Received

I would like to thank Dr. Xiaoning Bi for allowing me to work in her laboratory at University of California, Irvine, Professor Michel Baudry for his insight and guidance, and Eukarion, Inc. for granting me access to its salen manganese-complexes, EUK-8 and EUK-189.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Abirami Kandasamy

Project Number
S0410

Project Title
A Study on the Molecular Evolution of Voltage-Sensitive Ion Channel Genes

Abstract

Objectives/Goals
The purpose of this experiment is to explore the molecular evolution of ion channel genes. Based upon previous studies, the hypothesis is that potassium channels were the first ion channels to arise during evolution. These channels then gave rise to sodium and calcium channels, by duplication, followed by sequence changes and then another duplication. If the hypothesis is correct, domains 1 and 3 of the sodium and calcium channels should be similar to each other and domains 2 and 4 should be similar to each other

Methods/Materials
The International Union of Pharmacology database was used to get the GenBank accession numbers of K+, Na+, and Ca++ channels. The sequences of a number of voltage-gated channel cDNAs and proteins from the NCBI database were collected. The sequence of each domain was considered a separate sequence. The MAKDAT program was used to read the sets of sequence files and create output files suitable for input to the CLUSTALW program. The sequences of each sodium channel domain and calcium channel domain were aligned with the potassium channel domains using the CLUSTALW program. An evolutionary tree was developed using the sequence alignments and the PARSIMONY program.

Results
Many phylogenetic trees were constructed using the programs throughout the course of the experiment. One tree depicted a comparison of the K+, Na+, and Ca++ channels, which showed the channels divided into two monophyletic groups, one consisting of Na+ and Ca++ channels, and the other consisting of K+ channels. Another tree displays the lineage of each of the four domains of both Na+ and Ca++ channels. It shows the corresponding domains of each type of channel as closely related. It also depicts domains I and III as sharing a common ancestor and domains II and IV as sharing a common ancestor as well.

Conclusions/Discussion
Of the many trees that were produced, one shows the phylogenetic relationships of this entire ion channel gene superfamily. The Na+ and Ca++ channels appear to have arisen as separate from the voltage-gated K+ channels. The sequence similarities of each domain of the Na+ channel to the corresponding domain of the Ca++ channel imply that there existed a common ancestral single-domain channel gene that gave rise to the Ca++ channel by two duplications and then gave rise to the Na+ channel by further divergence following gene duplication.

Summary Statement
This purpose of this project is to explore the history of voltage-sensitive ion channels and specifically to determien the molecular evolution of K+, Na+, and Ca++ channel genes.

Help Received
Dr. G. Chandy and Dr. G. Gutman (Univeristy of California, Irvine) provided computer programs.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Trilokesh D. Kidambi

Project Number
S0411

Project Title
Underlying Mechanisms of the Lewis Negative Phenotype's Relationship with Cardiovascular Disease

Abstract

Objectives/Goals
In the Copenhagen Male Study and NHLBI Family Heart Study, the Lewis blood group Le (a-b-) phenotype was found to put patients at an increased risk for cardiovascular diseases, but the underlying mechanisms were unclear. For this study, the hypothesis was proposed that the Le (a-b-) phenotype was associated with high red blood cell aggregation, and whole blood and plasma viscosity, which are known to be risk factors for ischemic heart disease.

Methods/Materials
The Lewis blood type of 87 subjects (50 males, 37 females) from an ongoing B-Vitamin Study at the USC Institute of Genetic Medicine was determined. Hemorheological parameters such as RBC aggregation, plasma viscosity, and whole blood viscosity were measured for each subject. Data regarding conventional risk factors and drug therapy were also collected.

Results
The incidence of Le (a-b-) phenotype was 17.2% of all subjects. Statistical analysis revealed a significant difference in plasma viscosity ($p=0.004$) and RBC aggregation ($p<0.0001$ and $p=0.0002$) between patients with Lewis (a-b-) and Lewis positive phenotypes. Mean values for blood pressure, total cholesterol, low-density lipoprotein and high-density lipoprotein, fasting glucose, and homocysteine were not significantly different between Lewis (a-b-) and Lewis positive subjects.

Conclusions/Discussion
The positive association between Lewis (a-b-) phenotype and various hemorheological parameters has not been reported previously. The results showed a significant difference in RBC aggregation (plasma M: $p<0.0001$; plasma M1: $p=0.0002$) and plasma viscosity ($p=0.0004$) between patients with the Lewis negative phenotype and Lewis positive subjects. Other conventional risk factors for cardiovascular disease were also tested, but they were not found to be significantly different between the Lewis phenotypes. There was no significant difference in the RBC aggregation in 3% dextran (dextran M: $p=0.19$ and dextran M1: $p=0.90$) between the Le (a-b-) phenotype and Lewis positive phenotypes. Therefore, it seemed that plasma proteins combined with RBC surface properties in Le (a-b-) patients were more important factors in RBC aggregation than the RBC surface properties alone.
The finding that the Lewis negative phenotype had a higher plasma viscosity and RBC aggregation may provide an explanation for why the Le (a-b-) phenotype is an independent risk factor for heart diseases.

Summary Statement
Investigating the underlying mechanisms of the Lewish Negative Phenotype and its relationship with cardiovascular diseases.

Help Received
Student-Researcher in the Edmondson Summer Fellowship Program at the Univ. of Southern California.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)

June-Ho Kim

Project Number

S0412

Project Title

Investigating Multiple Sclerosis: Antibodies to CD44 and a4B1 Differentially Affect Myelin-specific T Cell Responses

Abstract

Objectives/Goals

Multiple sclerosis (MS) is a chronic autoimmune disease that affects approximately 2.6 million people around the world. Previous studies have shown that antibodies to adhesion molecules CD44 and integrin a4B1 prevent central nervous system inflammation and ameliorate the clinical symptoms of experimental autoimmune encephalomyelitis, a murine model of MS. However, the effect of these antibodies on the function of myelin-reactive T cells is still unknown. The present study investigates the molecular responses of myelin-reactive T cells to the two antibodies with the hypothesis that the antibodies will decrease secretion of harmful, pro-inflammatory cytokines and increase the secretion of beneficial, anti-inflammatory cytokines # thus, ameliorating the symptoms of MS.

Methods/Materials

T cells from the spleen and lymph nodes of MBP Ac 1-11 TCR transgenic mice were stimulated with varying concentrations of MBP peptide in the absence or presence of anti-CD44 or anti-a4B1. Thereafter, two assays were performed: Enzyme-linked Immunosorbent Assays (ELISAs) to measure cytokine secretion, and antigen-specific T cell proliferation assay.

Results

First, antibodies did not significantly inhibit T cell proliferation. Second, there was an increase in secretion of the immunosuppressive, anti-inflammatory cytokine Interleukin-10 by both antibodies. Converse from the initial hypothesis, there was an up-regulation of pro-inflammatory cytokines interferon-GAMMA, tumor necrosis factor-ALPHA, and interleukin-12 p40 in cells treated with anti-CD44. A literature search was run and it was found that the specific antibody used for CD44 (IM.7.8.1) was stimulatory. Lastly and most importantly, the pro-inflammatory cytokines were down-regulated by anti-a4B1.

Conclusions/Discussion

The results suggest that, though anti-a4B1 and anti-CD44 both prevent EAE, these antibodies differentially affect pro-inflammatory cytokine production by myelin-reactive T cells. Most importantly, the immunosuppressive role of anti-a4B1 makes it a promising therapy for MS. Anti-a4B1 may prevent CNS inflammation and ameliorate symptoms through the regulation of cytokines. Future research will include in vivo assays to confirm the regulation of inflammatory cytokines by the antibodies. Further studies into associated signal transduction pathways and the effects on myelin-reactive T cells will elucidate the antibodies# role as potential MS-specific therapies.

Summary Statement

The effects of antibodies to CD44 and integrin a4B1 on T cell cytokine secretion and proliferation were investigated; anti-a4B1 was discovered to reduce the release of pathological chemicals as a promising therapy for multiple sclerosis.

Help Received

Used lab equipment at Stanford University in the lab of Dr. Lawrence Steinman, under the supervision of Dr. Shalina Ousman; began project with the Center for Clinical Immunology at Stanford Summer Internship Program

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Meredith B. Kirchheimer

Project Number
S0413

Project Title
Does the Concentration of Pepsin Affect How Thoroughly Proteins Are Digested?

Abstract

Objectives/Goals
To determine whether or not the concentration/pH of pepsin will affect how thoroughly proteins are digested.

Methods/Materials
1. Separate the albumin, or white of an egg, from a hardboiled egg; boil an all-beef hot dog; fry ground turkey.
2. Dice each protein into very small pieces and place equal amounts into four test tubes.
3. Number the tubes; then pour these substances into the tubes:

Tube 1: 5 ml of a pepsin solution (0.5% pepsin)
Tube 2: 5 ml of 0.4% HCl
Tube 3: 5 ml of pepsin-HCl mix (add two drops HCl to pepsin solution)
Tube 4: 5 ml of pepsin solution plus two drops of 0.5% sodium carbonate

4. Maintain all the tubes in an incubator for twenty four hours.
5. Inspect contents of each tube for visual signs of digestion.

Results
Tube 3 (5 ml pepsin- HCl mix + two drops HCl to pepsin solution) had the most consistent results. Each protein absorbed most of the solution and was fairly broken down after the 24 hour period. This is because in the presence of HCl, pepsin changes into its active form: pepsinogen (the key enzyme involved in protein digestion in the stomach).

The least effective was Tube 4 (5 ml of pepsin solution plus two drops of 0.5% sodium carbonate). Neither the egg nor the hot dog were broken down (perhaps because of their physical properties- both having a membrane type covering) while the turkey absorbed most of the solution and became greatly enlarged. This may be due to the fact that sodium carbonate can increase the stomach pH turning it from an acid to alkaline.

The results of Tube 1 (5 ml of pepsin solution) make sense since pepsin, without the presence of HCl cannot truly digest food.

The results of Tube 2 (5 ml of 0.4% HCl) can be explained in that HCl serves to break down foods as opposed to truly digesting them which is the task of pepsinogen.

Overall, the most efficient digestive solution was Tube 3.

Summary Statement
Determining the most effective concentration of pepsin in the digestion of protiens.

Help Received
Made solutions under the supervision of Dr. Kondinjari

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Sarit A. Klugman

Project Number
S0414

Project Title
pTT Influence on Genetic Change of Human Fibroblasts and Prokaryotes Exposed to UV Light

Abstract

Objectives/Goals
This project will attempt to establish cultures of human fibroblasts and example Gram negative Escherichia coli and Gram positive Staphylococcus epidermitis, subculture the above cells to maintain growth, incubate cells both in the presence and absence of pTT, expose both to varying amounts of ultraviolet B radiation, and examine cultures for subsequent chromosomal or nuclear damage and viability.

Methods/Materials
- Add 6 to 8 ml of complete growth medium to culture flasks and aspirate cells by gentle pipetting.
- Add appropriate aliquots of the cell suspension to new culture vessels.
- Introduce pTT into half of the cell cultures.
- Pour plates of tryptic soy agar.
- Put pTT in half of the agar.
- Inoculate 8 plates of each agar with Escherichia coli and 8 with Staphylococcus epidermitis.
- Incubate plates and culture flasks at 37oC and irradiate with UVB for various lengths of time
- Observe cells under an inverted microscope to observe any mutations.
- Create smears of prokaryotes to determine damage done to DNA.

Results
Cultures of both human fibroblast and representative bacteria were established. The subculturing of human fibroblast cells as well as Escherichia coli and Staphylococcus epidermitis were successful. Fibroblast cells in the control flasks both with and without pTT differentiated and divided. The flasks with pTT that had been subjected to UVB for 2 and 4 days showed some possible signs of differentiation while those flasks without pTT showed no differentiation at all. Those cells subjected to UVB for 6 days both with and without pTT showed no signs of differentiation. It is suspected that the concentration of pTT in the culture flasks was not high enough and therefore prevented those cells with pTT from completely differentiating. pTT was placed in half of the agar before being streaked with bacteria. They were then exposed to UVB for 2, 4, and 6 days time. Results indicate some damage to growing bacteria in quantity of growth rather than morphology of cells.

Conclusions/Discussion
Results suggest some damage of UVB to human fibroblast cells and prokaryotic cells in quantity of growth rather than morphology of cells. It is suspected that the concentration of the pTT was to low to allow for differentiation of human fibroblasts with pTT exposed to UVB.

Summary Statement
The purpose of this experiment is to compare the effects of pTT on the genetic changes in human fibroblasts and example prokaryotes as induced by UVB.

Help Received
Ms. Eline Preston supervised all procedures

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Dylan K. Lake

Project Number
S0415

Project Title
Analyzing Interactions between mRNA Splicing and Transcription with Proteins BBP and BUR2 in Saccharomyces cerevisiae

Abstract

Objectives/Goals
My goal was to study possible interactions between the proteins BBP, a splicing factor, and BUR2, a transcription factor, in yeast. Finding an interaction would suggest a larger relationship between the execution of transcription and splicing in the cell.

Methods/Materials
Sporulation of yeast cells was utilized to create cells deleted of both BBP and BUR2 from single mutants of the genes. These double mutants were then transformed with plasmids containing mutant versions of BBP. The growth rates of the mutated strains were compared.

Results
A particular mutant of BBP with single replacements in the zinc knuckle and in the MUD2/UAF65 binding region had no distinct phenotype by itself but showed a severe growth defect when combined with a deletion of BUR2.

Conclusions/Discussion
Because the deletion of BUR2 increases the severity of a mutation in BBP, it is likely that the two genes are involved in overlapping processes. This further suggests that the processes of mRNA splicing and transcription are closely linked and supports the theory that they occur simultaneously.

Summary Statement
I manipulated yeast genetics to determine an interaction between the proteins BBP, a splicing factor, and BUR2, a transcription factor, supporting the theory that mRNA splicing and transcription occur simultaneously.

Help Received
Used lab equipment at UCSD under the supervision of Dr. Tracy Johnson, Phd.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Alicia E. Lee

Project Number
S0416

Project Title
Manipulation of PGE(2) Levels with Various Cytokines of Thyroid Associated Ophthalmopathy

Abstract

Objectives/Goals
Graves# Disease is a form of hyperthyroidism often accompanied by Thyroid Associated Ophthalmopathy (TAO), an autoimmune-mediated inflammation of the extraocular connective tissue. Despite numerous studies to locate the specific factors that regulate the swelling and produce an effective cure, neither of these goals has been achieved. Recently, scientists discovered that Prostaglandin E(2) (PGE(2)) is present in significantly increased levels in the orbital tissues of patients with TAO, and must be an important factor in the inflammatory response around the eyes. The primary goal of this research project was to determine the mechanisms that lie behind the up-regulation of PGE(2) in patients with TAO and establish correlations among Th1 and Th2 cytokines, growth factors, and PGE(2).

Methods/Materials
I experimented with three types of cells: Graves# orbital fibroblasts, normal orbital fibroblasts, and dermal fibroblasts (control). I cultured each type of cell in petri dishes for two weeks. Then I treated these cells with each cytokine at different time intervals. I used the cytokine IL-1B, already proven to increase PGE(2) levels, as my positive control treatment. After treating the cells, I harvested my cultures, which included lysing and scraping the cells. I was able to solubilize the proteins and extract the supernatan to conduct a protein assay. Then I performed a western blot, using a PGE(2) Elisa Kit Protocol to test for PGE(2) levels.

Results
This research experiment led to significant insights concerning the mechanisms that lie behind TAO. Contrary to expectations, TGF-B was found to exhibit the potential to inhibit PGE(2). On the other hand, a significant relationship between the Th2 cytokine IL-4 and the up-regulation of PGE(2) was established. Both IL-4 and IL-1B shared many important similarities, including a sixteen-hour optimal time interval.

Conclusions/Discussion
The potential implications of these findings are considerable. Not only have we disproved a query that only Th1 cells are responsible for significant up-regulation of PGE2, but we have now identified another cytokine important for the progression of TAO. Although IL-4 has remained a rather obscure cytokine, these findings have revealed its important involvement with TAO. There must be some common link between IL-4 and IL-1B. Further studies concerning the mechanisms associated with IL-1B and IL-4 will indisputably be followed.

Summary Statement
The primary goal of this research project was to determine the mechanisms that lie behind the up-regulation of PGE(2) in patients with TAO and establish correlations among Th1 and Th2 cytokines, growth factors, and PGE(2).

Help Received
Mother drove me every week to the Harbor UCLA Research Insitute; Mr. Starodub helped me fill out my approval forms for working with human tissues; Used lab equipment at Harbor UCLA Medical Center under the supervision of Dr. Terry Smith.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)

Jenny Martinez

Project Number

S0417

Project Title

Investigating the Importance of Position 52 in Glycine Receptor Activation

Abstract

Objectives/Goals

Although alcohol#s effects on the body are known, its exact mechanisms of action are not. This study's goal is to better understand the glycine receptor. Position 52 in the amino acid sequence of this receptor has been suggested as a possible site of ethanol action. Previous studies found that a point mutation at position 52 caused an altered reaction to ethanol and glycine. We hypothesize that other substitutions at position 52 also affect the receptor#s characteristics.

Methods/Materials

Using two-electrode cell voltage clamp techniques, a concentration response of glycine and ethanol was performed on wild-type and mutant GlyRs as recombinantly expressed in Xenopus laevis oocytes.

Results

A reduced sensitivity for glycine resulted in the recombinant GlyR when compared to the wild-type GlyR. Potentiation of the GlyR#s response to glycine after a mixture of ethanol and glycine was applied also occurred.

Conclusions/Discussion

The results obtained support the hypothesis that different mutations at position 52 of the GlyR α1 subunit alter the receptor#s response to glycine, suggesting that more studies at position 52 are needed. These studies will also help isolate ethanol#s exact binding site and to better understand this receptor, as well as others that act similarly in the body.

Summary Statement

This study's goal is to better understand the glycine receptor and postion 52 of its amino acid sequence, which has been suggested as a possible site of ethanol action.

Help Received

Research was conducted at the University of Southern California under the supervision of Ronald L. Alkana PhD, Daryl Davies PhD, and Daniel K. Crawford

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Sierra G. Nichols

Project Number
S0418

Project Title
How Does Topically Applied Protein Affect Human Hair?

Abstract

Objectives/Goals
My project is based on the hypothesis which states that if I apply a topical protein to hair, it will thicken, strengthen and increase the heat resistance more than untreated hair under the same conditions. The experiments I chose were used to test the thickness, strength and heat resistance of each hair.

Methods/Materials
For the thickness, I compared a topically applied protein hair to a non treated hair under a microscope. Doing this I found their widths in micrometers. For the second experiment in which I was testing the strength of each hair, I placed the hairs (all individually) into a device that was used to determine how much strength it had. This was determined by how many increments of rotation it could withhold before it broke. Lastly, I tested the heat resistance of the hairs. I used a heat gun to apply heat to each individual hair. I timed how many seconds it took before each hair singed with constant heat.

Results
My results from each experiment showed that each experiment did not increase the strength and heat resistance of the hairs when the topical protein solution was applied, opposite from what my hypothesis had predicted. Generally topically applied protein solution doesn#t affect the hairs# strength or heat resistance. It only moderately affects the hairs# width / thickness.

Conclusions/Discussion
The experiments proved that the topically applied protein didn#t increase the hair#s strength and resistance to heat, only in minor and maybe even ineffective ways. It did increase the thickness. My hypothesis wasn#t correct and wasn#t supported by any of my results, except experiment number 1. I had stated that the protein treated hair would be wider than the untreated hair, while in fact its average thickness increased by 18 micrometers. Next, I had guessed that the protein treated hair would be stronger than the untreated hair. The results from experiment two showed that the hair#s strength wasn#t affected by the protein solution, and the same with experiment three. Statistical data shows no correlation between the hairs# strength and heat resistance due to topically applied proteins.

Summary Statement
The affects of topically applied protein on human hair.

Help Received
My parents, My Teacher Mr. Robinson

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Daya Raman

Project Number
S0419

Project Title
Investigation of Delayed Ripening in Climacteric Fruits

Abstract

Objectives/Goals
Good health requires that we eat high quality foods and produce. Fresh Produce and fruits are an important source of minerals, vitamins and fiber. To prevent fruits from rotting before they reach consumers, many farmers and food companies typically either pick the fruit while green and artificially ripen it. In this study, we examine the ripening in Climacteric fruits- fruits that are picked before they are ripe.

Methods/Materials
The climacteric fruits studied involved two groups of Apples, two groups of Bananas and other produe such as Pear, Tomatoes, Lime and Broccoli. In order to study the ripening process in a controlled manner, several sets of desiccators were used so that an ethylene generating station (containing apples) and a receiver station (containing the fruit whose ripening was to be studied) could be examined in a coupled manner. Fruit ripening was examined over several hundred hours using color analysis and texture, weight loss and refractometer measurements.

Results
The experimental observations in this investigation focuses on the post-processing methods used for climacteric fruits and is summarized in the following points: (a) Examination of ripening in climacteric fruits and other produce show that there are changes in the color, weight, Refractometer Brix values and appearance with time (b) the weight loss depends on the type of fruits and typically varies in the range from 5-80% - the weight loss likely due to loss of water as a result of respiration, (c) Ripening is accelerated in the presence of Apples # an ethylene generator. d) Ripening in Bananas and Tomato, two climacteric fruits, is accelerated in the presence of Ethylene (e) Environmental conditions of temperature and humidity influence ripening. (f) #White Mold# formation and #Degreening: is observed in Citrus fruits in the presence of Ethylene (g) Survey of tomatoes shows that they are of less than average quality in terms of Total solube solids (Refractometer Brix) and sugar content. .

Conclusions/Discussion
The conclusion from this study is that fruit ripening is accelerated in the presence of ethylene. Some concern is raised regarding the post-harvest ripening methods used currently regarding nutritional value. Global distribution of produce and post harvest treatments in climacteric fruits may need reassessment taking into account nutritional aspects of produce.

Summary Statement
Fruit ripening is accelerated in the presence of ethylene but current ripening methods needs re-examination taking public health factors into account.

Help Received
Guidance and direction of Dr. John Howe is gratefully acknowledged.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
David Saryan

Project Number
S0420

Project Title
Protein Electrophoresis on Flying and Non-Flying Birds

Abstract

Objectives/Goals
The objective of my project is to use electrophoresis to see if there is an evolutionary relationship between birds that fly, fly short distances, and birds that do not fly.

Methods/Materials
Turkey wing, Duck wing, Pheasant wing, Goose wing, Partridge wing, Quail wing, Fowl wing, Chicken wing, Water bath, Actin and Myosin standard, Laemmli sample buffer, Kaleidoscope prestained standard, Gel staining trays, Ready gel precast gels, 15%, Mini-Protean 3 cell, PowerPac junior power supply, Flip to tubes, Screw cap tubes, 2-20 microliter micropipet, distilled water, 10x Tris/glycine/SDS, Floating microtube rack, Safety goggles, Gloves, Bio-Safe coomassie stain, Micro centrifuge.

Results
The results showed that flying birds were related to flying birds and that partially flying birds were also related to flying birds.

Conclusions/Discussion
In conclusion the results showed that my hypothesis was partially correct because flying birds were related to flying birds, but partially flying birds were not related to non flying birds, they were related to flying birds also.

Summary Statement
The point of my project is to use electrophoresis to see if there is an evolutionary relationship between different types of flying, non flying, and partially flying birds.

Help Received
Ribet Academy Biology Lab

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Susanna M. Shin

Project Number
S0421

Project Title
Is Genetic Transformation of the pGLO Gene Possible Between any Species of Monera?

Abstract

Objectives/Goals
The objective of this project was to determine if all species of Monera were able to express the pGLO gene(originally derived from the jellyfish, Aequora victoria) with the means of genetic transformation.

Methods/Materials
Using the process of genetic transformation, recombinant DNAs were attempted to be created with five different strands of bacteria including: Bacillus megaterium, Escherichia coli, Lactococcus lacti, Micrococcus luteus, and Serratia marcescens. The genetically transformed bacteria were cultured for full growth, then later observed under the ultra violet lamp. In the bacteria strands that had successful transformations/creations of recombinant DNAs, the Green Fluorescent Proteins(GFP) were switched on to glow bright green under the UV lamp. This bioluminescent trait, as well as the resistance to ampicillin, were two visible traits of a successful genetic transformation in the bacteria.

Results
The Bacillus megaterium, Escherichia coli, and the Lactococcus lactis species of Monera were able to successfully express the Green Fluorescent Protein by genetically transforming the pGLO plasmid into their own DNA strand. On the other hand, the Micrococcus luteus and Serratia marcescens were unable to express this gene.

Conclusions/Discussion
The pGLO gene was unable to be expressed in just any species of Monera. This is due to the fact that not all the strands of bacteria has the correct restriction sites/complementary sticky ends for the "new" gene to be inserted into its DNA strand successfully. Bacteria such as, Bacillus megaterium, Escherichia coli, and Lactococcus lactis were able to express the GFP because each of them had the correct nucleotide sequences required for a specific cut into its own DNA and for the insertion of the new DNA, in order to create the recombinant DNA.

Summary Statement
The genetic transformation of the pGLO gene into several species of Monera was tested.

Help Received
Used lab equipment at Centennial High School under the supervision of Mrs. Houseman.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Kunal K. Sindhu

Project Number
S0422

Project Title
The Effect of the Superoxide Anion on Cytochrome C

Abstract

Objectives/Goals
My goal in this experiment is to determine whether O2- causes damage to proteins, which are essential to all forms of life. Hopefully, this can lead to further research on the specific results of the damage that O2- causes to proteins. I hypothesize that upon exposure to the superoxide anion, cytochrome C will indeed be damaged, and hopefully this will be evident in my data.

Methods/Materials
Xanthine; Xanthine Oxidase; Spectrophotometer; Pipettes; Test Tubes; Cytochrome C; Superoxide Dismutase; Potassium Phosphate Buffer (pH 7.8).

Results
Cytochrome C was damaged in the presence of the superoxide anion, which was produced in a reaction consisting of xanthine, oxygen gas, water, and xanthine oxidase. Using the data from the spectrophotometer, I calculated the amount of cytochrome c that was damaged by the superoxide radical. After five minutes, approximately 16.7% of the protein had been damaged. With the addition of SOD(superoxide dismutase), the damage to cytochrome c slowed down drastically. I calculated the amount of cytochrome c that was damaged in the solution included SOD (which removes the superoxide radical). After five minutes, only 6.7% of the protein had been damaged. As you can see, the presence of SOD slows the damage to cytochrome c significantly. Therefore, the superoxide radical must be responsible for the damage to cytochrome c (because SOD removes it).

Conclusions/Discussion
My hypothesis was indeed correct. After observing that the spectrum of the cytochrome c solution experienced a marked change when exposed to the superoxide anion, I have concluded that cytochrome c was indeed transformed, and in fact damaged, by this reactive oxygen species. Upon further research, I discovered the exact reaction for cytochrome c and the superoxide anion:

O2- + cytochrome c[Fe(III)]--> O2 + cytochrome c [Fe(II)}

Hence, in the presence of the superoxide anion, cytochrome C was reduced, and thus damaged. The addition of an extra electron to cytochrome c causes the protein to change its inherent shape, and therefore lose its function. In the absence of antioxidant enzymes, I have shown that the superoxide anion can damage proteins, and as a result, it can impair essential cell processes. It is no wonder that oxidative stress can play a major role in Parkinson's disease, diabetes, Alzheimer's disease, and many other significant

Summary Statement
I discovered that the superoxide anion damages proteins significantly, but this damage can be reduced if superoxide dismutase is injected into the solution because this enzyme removes the destructive anions.

Help Received
I used lab equipment at the Charles R. Drew Medical School under the supervision of my father, Dr. Ram K. Sindhu.

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Brittany M. Weems

Project Number
S0423

Project Title
Common Roots?

Abstract

Objectives/Goals
My objective was to observe similarities and differences in the DNA of various fruits and vegetables by extracting the DNA and performing gel electrophoresis.I believe that both fruits and vegetables will have common traits, considering they are all plants, but I hypothesize that the fruits and vegetables will each have distinct DNA patterns that may be attributed to different qualities such as duration of shelf life, coloration, and other traits that are unique to each fruit or vegetable.

Methods/Materials
I used a blender,measuring cup,strainer,wire,glass rods,test tubes,strawberries,banana,blueberries,kiwis,pear,grapes,broccoli,cauliflower,onion,Brussels sprouts,carrots,raspberries,and the lab at California State University Bakersfield.I extracted the DNA at my home by blending the fruits and vegetables,straining the mixture,adding detergent,adding contact solution,and using rubbing alcohol to separate the DNA.I then had the assistance of Dr.Szick-Miranda at CSUB and performed gel electrophoresis to separate the DNA of the fruits and vegetables.After running the gels for 45 minutes each, we put the gels on a UV light and took pictures to compare.

Results
In the first trial I was only able to extract DNA from strawberries,banana,and blueberries.The gel electrophoresis did not work properly,and not much DNA was visible.There are some factors that may have caused this, too much water mixed with the fruits and vegetables,not using cold alcohol,adding too much water to extracted DNA,not letting mixture sit with rubbing alcohol long enough,and other variables that I changed.The second trial had better results;I was able to extract DNA from strawberries,banana,blueberries,as well as broccoli,cauliflower,onion and Brussels sprouts.

Conclusions/Discussion
The initial results that I achieved were not enough to form a conclusion,because the DNA did not show up properly in the electrophoresis,and I was only able to extract fruit DNA.I learned from my first trial and attempted to extract the DNA again.The second trial yielded better results in DNA extraction,but I was still unable to get an efficient electrophoresis reading.I have come to the conclusion that I must try a more complex procedure in order to get the results I am looking for.I have acquired a new procedure and plan to work in my school lab to achieve better results.

Summary Statement
My project was conducted to compare the DNA of various fruits and vegetables through gel electrophoresis.

Help Received
I used lab equipment at California State University Bakersfield under the supervision of Dr. Kathy Szick-Miranda, and I plan on getting help from my teacher Mr. Matt Day at Ridgeview High School on my new procedure

CALIFORNIA STATE SCIENCE FAIR
2005 PROJECT SUMMARY

Name(s)
Joanne Y. Zhang

Project Number
S0424

Project Title
Proliferation and Extracellular Matrix Remodeling in Tumor Cells HT-lo/diss and HT-hi/diss

Abstract

Objectives/Goals
There are two objectives: 1. To determine if the HT-lo/diss (45a) and the HT-hi/diss (47a) tumor cells are in fact different in extracellular matrix (ECM) remodeling and 2. if the cell lines are different in proliferation in 3-D.

Methods/Materials
HT-Proliferation in 3D-Collagen: Same numbers of cells were seeded in 7 sets of 3 wells for each cell line. The cells were suspended in collagen for a 3-D environment effect. Each set represented a day (Day 0 to Day 6). Each day the corresponding set was used and cells were isolated from the collagen, counted, and the numbers recorded.
Collagen Contraction Assay: Same numbers of cells were seeded in 2 sets of 3 wells for each cell line. Cells were suspended in collagen. 1 set per cell line had solutions of dimethylsulphoxide overlaid on the collagen; the other contained solutions of control ilomastat. After 5 days, the collagen was cut from the sides of the well and the amount of contraction was measured on the 8th day.
Materials: HT-lo/diss (45a) and HT-hi/diss (47a) from Scripps Research Institute; Control Ilomastat, Dimethylsulphoxide, Dispase (Calbiobchem); Invitrogen Dulbecco Modified Eagle Medium with 10% Fetal Bovine Serum; Sigma-Aldrich Collagen (3.0 mg/ml density); incubator (37¡aC in 5% CO2/95% air and passaged at confluence); 24-well plates

Results
Results from HT-Proliferation in 3-D Collagen, demonstrate that 45a cells have a longer doubling time than 47a cells when placed in 3-D collagen. Because the cells lines grow at the same rates in 2-D collagen, it is the 3-D collagen that slows the proliferation of 45a.
Results from Collagen Contraction show 45a contracts collagen less than 47a in 3-D collagen, meaning it is less able to modify its environment. Thus, 45a is less able in ECM remodeling when compared to 47a.

Conclusions/Discussion
My conclusion is that in vitro 3-D collagen proliferation is impaired for 45a and that the proliferation difference between the cell lines is not inherent but due to the 3-D collagen environment. Also, 45a is found to be less able in ECM remodeling than 47a. Although the result of this research is only a small part of the overall project, when pieced together, it will offer a more comprehensive understanding of the mechanics of tumor growth and metastasization. Such an insight will pave the road to discover the means to inhibit tumor growth and metastasization and the eventual treatment of cancer.

Summary Statement
I investigated the differences in the proliferation and extracellular matrix remodeling abilities of two similar tumor cell lines.

Help Received
Used the equipment of Dr. James Quigley's lab at the Scripps Research Institute under the supervision of Dr. Elena Deryugina