CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Matthew J. Bauer

Project Number

S0401

Project Title

Preliminary siRNA Analysis of Genes Implicated in Neuronal Differentiation and Neurite Outgrowth

Abstract

Objectives/Goals

The focus of this study was to develop and validate a cell-based assay to screen previously identified candidate genes that may be essential in neuronal differentiation and neurite outgrowth.

Methods/Materials

In this study, nineteen genes which were previously shown by DNA microarray analysis to be up-regulated when mouse P19 cells were induced to differentiate into neuronal cells were selected for assay development and initial gene candidate validation. The mRNAs of candidate genes were targeted in differentiating P19 cells using Dicer-generated small interfering RNAs (siRNAs) to determine if a reduction in gene-specific mRNA levels interfered with differentiation and neurite outgrowth.

Results

Based upon statistically significant changes in cell body area and / or neurite length when differentiating P19 cells were targeted with gene-specific siRNA, three of the nineteen candidate genes evaluated appear to be important in neuronal development.

Conclusions/Discussion

As a preliminary step to test the effectiveness of Dicer generated siRNAs against specific genes, siRNAs were generated against MAP2 and b-III tubulin, two proteins found in neuronal P19 cells. When co-transfected with the NeuroD2 expression plasmid (to initiate neuronal differentiation) these gene-specific siRNAs were effective in greatly reducing the amount of MAP2 and b-III tubulin protein expressed.

To test the ability to detect phenotypic changes when genes known to be important in neuronal differentiation were targeted, siRNAs were used to inhibit the expression of AKT1 and AKT2. These experiments showed that inhibition of critical genes can result in a measurable change in cell body and neurite length.

Using this assay, nineteen genes that were previously determined to be potentially important in neuronal differentiation and neurite outgrowth were selected and siRNAs were generated for each candidate gene. The siRNAs were co-transfected with the transcription factor NeuroD2 to initiate neuronal differentiation. The knockdown of three candidate genes resulted in a statically significant change in either cell body size and / or neurite length. To further validate the importance of these candidate genes in neuronal development chemically synthesized siRNAs, which are more specific reagents for RNA inhibition, will

Summary Statement

To develop and validate a cell-based assay to screen previously identified candidate genes that may be essential in neuronal differentiation and neurite outgrowth.

Help Received

This study was conducted in the laboratory of Shelly Halpain, Ph.D. at the Scripps Research Institute in La Jolla, CA under the mentor ship of Leif Dehmelt, Ph.D.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Emmanuelle Benkoski; Todd Yecies

Project Number

S0402

Project Title

Polymorphisms in the Coding Region of Substance P

Abstract

Objectives/Goals

Complex Regional Pain Syndrome is an often undiagnosed condition that may affect millions in the United States alone. The causes of CRPS remain unknown though the syndrome has been linked to the protein Substrance P. In this experiment, we identified polymorphisms in the coding region of the protein in different strains of mouse DNA to later correlate with an increased frequency of CRPS.

Methods/Materials

In this experiment, the coding region of Substance P was isolated from genetically different strains of mice. They were then replicated, and sequenced. The resulting DNA sequences were then cross-referenced in order to detect polymorphisms.

Results

Nearly all the polymorphisms that were found were located upstream of the coding region for Substance P. This may be linked to the promoter region and over/under expression of the protein. A few polymorphisms were located in the coding region itself, and may be responsible for a different amino acid sequence.

Conclusions/Discussion

These preliminary results should be verified through further trials. Case studies should also be performed to possibly link the detected polymorphisms to an increased frequency of CRPS.

Summary Statement

We detected polymorphisms in the coding region of Substance P, which may serve to understand the causes of Complex Regional Pain Syndrome.

Help Received

Used lab equipment from Menlo School under the supervision of Dr. Strong; Applied Biosystems provided reagents and sequenced the DNA.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Christopher L. Chau

Project Number

S0403

Project Title

How Bacteria Can Keep Us Cool

Abstract

Objectives/Goals

The ice nucleating activity of the proteins coded for by the inaZ gene of Pseudomonas syringae was determined by isolating the plasmid DNA, transforming BL21 E.coli cultures, and designing a variety of primers to cut the protein into different segments to establish the minimum composition needed to assure ice-nucleating activity. The original cultures of Pseudomonas syringae obtained from Greece were grown on plates of LB agar. Freezing assays and SDS-Page were run on the transformed protein-producing BL21 E.coli cultures in order to test for the presence of the inaZ gene. These were grown on LB plates with ampicillin, as the isolated plasmid consisted of both the inaZ gene and ampicillin resistance. 1-D protein gel electrophoresis was run to compare the genetic makeup of the transformants with that of the original inaZ culture. A time trial was run on cell cultures that were incubated in LB broth overnight, in order to determine the OD600 concentration at which ice-nucleating activity is optimal. The innoculations were grown to an optical density of 0.3-0.4, the point at which cells are in late log phase and protein expression begins, before IPTG was added to activate mass protein expression. Samples were taken every 30 minutes until an OD600 of 1.1. This variety of samples was then tested in a freezing assay. There was found to be a correlation between the ice-nucleating efficiency and the amount of protein on the membrane before the point at which the cell ceases to make the protein because it is harmful to itself. The cells also proved to grow better under harsh conditions with low nutrition, while greater amounts of PI (phosphatidylinositol), which the N-terminal of inaZ binds with, is directly related to the temperature at which it actively ice-nucleates. In the freezing assay, three of the five transformed cultures ice-nucleated at -6°C, while past research only showed ice-nucleating activity at a temperature of -9°C. A mid-range OD600 of about 0.6-1.1 was found to yield optimal protein expression. Finally, agar beads were designed as a synthetic matrix upon which to anchor the proteins, thereby eliminating the need for the bacteria itself. Six pairs of primers have been designed to cut the protein into different segments to determine minimum composition to assure the most efficient ice-nucleating activity. At this point further research is required.

Summary Statement

To test the freezing capabilities of ice nucleating bacteria and the inaZ gene.

Help Received

Used lab at Cate School and Montana State University under supervision of Professor Cindy Morris, Tami Goetz, and Cheryl Powers.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Jane Chen; Susanna Shin

Project Number

S0404

Project Title

The Effect of Radiation on the Genetic Transformation of the pGLO Gene in Monera: Phase 2

Abstract

Objectives/Goals

This project was designed to test whether or not genetic transformation of the pGLO gene was possible in species of Monera after they were mutated by varying degrees of radiation exposure. By doing this experiment, one can see how mutations caused by radiation affect the creation of recombinant DNA in bacteria.

Methods/Materials

Results from a previous experiment (Phase I) show that Escherichia coli was able to express the Green Fluorescent Protein while Lactococcus lactis was unable to express the Green Fluorescent Protein. Phase I served as the control for Phase II because Phase I was not affected by any factors during the process of genetic transformation. In this experiment, both bactiera were exposed to beta and gamma rays for fifteen minutes. Then, using a genetic transformation procedure, the pGLO gene, originally derived from the jellyfish Aequorea Victoria, was inserted into plasmid of the bacteria. The bacteria were then allowed to grow for three days. Bacteria that underwent successful genetic transformation were able to express the Green Fluorescent Protein that codes for the glowing of the bacteria colonies under an ultraviolet lamp. This bioluminescent trait, as well as the resistance to ampicillin, are two visible traits of a successful genetic transformation of the pGLO gene in the bacteria.

Results

The Escherichia coli exposed to beta and gamma rays for fifteen minutes and the Lactococcus lactis exposed to the beta rays for fifteen minutes were able to express the Gree Fluorescent Protein. The Lactococcus lactis that was exposed to gamma rays was unable to express the traits of the pGLO gene.

Conclusions/Discussion

In conclusion, radiation does affect the genetic transformation of the pGLO gene in Monera. The Escherichia coli exposed to fifteen minutes of beta and gamma radiation and the Lactococcus lactis exposed to fifteen minutes of beta radiation were able to successfully express the Green Fluorescent Protein by creating a recombinant DNA. On the other hand, the Lactococcus lactis exposed to fifteen minutes of gamma radiation was unable to express this gene. This is due to the fact that the radiation altered its DNA plasmid of the bacteria in a way that would alter the specific nucleotide sequence necessary for the precise cut into its DNA by the restriction enzymes and for the insertion of the pGLO gene.

Summary Statement

This project examines the effect of varying degrees of radiation on the genetic transformation of the pGLO gene in bacteria.

Help Received

Used lab equipment at Centennial High School

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Shiraz Ghanimian; David Saryan

Project Number

S0405

Project Title

Evolution of Species through Protein Electrophoresis

Abstract

Objectives/Goals

The purpose of this project is to analyze evolutionary links among fish, birds, reptiles, amphibians, and mammals through protein fingerprinting.

Methods/Materials

The main technique used in this project was protein electrohporesis, which includes extracting the proteins from the various samples and seperating the proteins of the different samples on polyacrylamide gels. Materials that were used include: Electrohporesis Chamber and its power supply, micropipeter, screwtop and flip top tubes, water bath, running buffer, and sample buffer.

Results

The amphibians had the most common proteins with the fish, the birds had the most common proteins with reptiles, and the mammals shared only a few proteins with the birds.

Conclusions/Discussion

The results show that fish and amphibians are closely related, and the birds and reptiles are closely related. These results coincide with the theory of evolution because according to the theory of evolution, amphibians evolved directly from fish, which explains why they are closely related and even though reptiles evolved from amphibians, according to the theory of evolution, birds are, in essence, "flying reptiles," which also shows why the reptiles and birds were found to be most closely related to reptiles. The mammals, however, only shared a few proteins with the birds, which shows how mammals evolved later than the rest of the animals. By analyzing the common proteins of the various animals, we are actually analyzing the DNA of the organism becuase proteins are reflections DNA. Thus, the smiliarities found among the various animals were actually similarties in their genetic material:DNA, which is the basis of evolution.

Summary Statement

The goal of this project is to establish and record evolutionary links among fish, birds, reptiles, mammals, and amphibians through the use of protein fingerprinting and analyze what animals are closely related.

Help Received

This project was conducted at the Ribet Academy Biology lab.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Benjamin G. Jimenez

Project Number

S0406

Project Title

Spectroscopic Analysis of Crystal Violet in Chymotrypsinogen A-Surfactant Solutions

Abstract

Objectives/Goals

The objective is to investigate the protein form-function relationship in photoresponsive surfactant and Crystal Violet solution after exposure to various light energies in order to observe possible changes in protein conformation.

Methods/Materials

10 Solutions were made with azoTAB surfactant concentrations ranging from 0-20 mM. Each solution was ~0.50 mL with 10 mg/mL chymotrypsinogen-A protein and 10 micromolar crystal violet. Solutions were analyzed with UV-Vis Spectroscopy under two conditions of visible light and UV-light. The CV peaks were curve fitted to determine the maximum wavelength and Absorbance at the max wavelength.

Results

Different light illumination causes a shift in the max wavelength and a change in Absorbance of the solutions. Upon changing Visible to UV light environments, the observed max wavelength decreased and there was an increase in Absorbance.

Conclusions/Discussion

My conclusion is that the differences in Absorbance and wavelength indicate there is a change in the form of the protein.

Summary Statement

My project is about protein misfolding and how the protein form-function relationship may be manipulated through the use of photoresponsive surfactants.

Help Received

Used lab equipment at the University of Southern California under the supervision of USC graduate student Andrea Hamill.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Swetha Kambhampati

Project Number

S0407

Project Title

Enhancing Mechanotransduction of Aging Human Dermal Fibroblasts

Abstract

Objectives/Goals

Aging is an inevitable yet complex process that has significant ramifications. Elderly people are more prone to wounds with diminished healing efficiency, diseases such as diabetes, and tissue degeneration. This study proposes to identify the cause of such problems and find a potential treatment.

Methods/Materials

1. Prepare Hydrogel Substrate
2. Measure cell proliferation by using hemocytometer to count the cells and plot in a growth curve
3. Analyze actin structure under confocal microscope after staining the cells with 3.7% Formaldehyde (FA), 3% Propidium Iodide (PI) and 3% Alexa Fluor (AF).
4. Conduct Agarose Droplet Migration Assay by preparing 0.2% agarose, fixing cells with FA, and staining cells with 0.1% Crystal Violet dye. Use SPOT 3.0 Software to analyze samples.
5. Measure the tractional forces exerted by preparing HA Hydrogel, adding a 100% sonicated bead solution, and using Digital Image Speckle Correlation (DISC).

Results

The proliferation of cells decreased as age increased, thus indicating that older cells are not as healthy as younger cells. The confocal images show that the 18 year old cells spread better and the photomicrographs in the Agarose Droplet Migration assay show that they migrate better than the 63 year old cells.

The DISC technique and the tractional force assay tracked the movement of beads in the substrate and mapped the deformation. The three-dimensional traction maps are obtained using FEA, which calculates the strain energy. The substrate deformation and strain energy caused by the 47 years old cell was more than that caused by the 81 years old cell.

Since the older cells are not as strong, the next step in this study was to try to increase the tractional force by adding PDGF, a growth factor absent in the environment of older cells, to the supernatant.

The deformation and strain energy caused by the control cell was lower than the cell exposed to PDGF. This indicates that the tractional force exerted by cells with PDGF in their environment increases, which may lead to an improvement in wound healing and migration into damaged tissue.

Conclusions/Discussion

This study identifies the tractional forces to be one of the most fundamental factors of mechanotransduction. The finding that PDGF can reduce the deterioration of tractional forces with aging denotes that PDGF may be a potential treatment available to elderly people.

Summary Statement

This project is about identifying the critical cause behind wound healing deficiency in older people and designing a potential solution.

Help Received

Used lab equipment at SUNY Stony Brook under the supervision of Dr. Miriam Rafailovich and Dr. Clark; Participant in Research Scholar Program

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Rebecca L. Kaspar

Project Number

S0408

Project Title

Keratins Gone Bad: A Look at the Causes of a Rare Skin Disease

Abstract

Objectives/Goals

PURPOSE
My project is to test if a plasmid expressing K16 WT (wild type or normal) protein makes filaments (chains of keratin). In addition, I will test to see if a K16 mutant plasmid, that contains a mutation identified in a Pachyonychia Congenita patient, disturbs the making of filaments (making aggregates, broken filaments, instead).
HYPOTHESIS
I hypothesize that if I transfect K16 WT (wild type) fused to Yellow Fluorescent Protein (YFP) into human tissue culture cells, the cell will express strong, normal filaments. If I transfect K16 ÄHTM (mutant) fused to YFP into human tissue culture cells, the cells will form aggregates in place of filaments.

Methods/Materials

Overall objective: make a fusion between K16 (ÄHTM or WT) and a reporter protein, YFP. Therefore, because YFP can be seen using a fluorescence microscope, the K16 ÄHTM or WT fused to YFP can be seen.
1.Using Recombinant DNA Technology, use restriction enzymes and ligase to insert the K16 PCR fragment into an expression plasmid, which contains YFP.
2.Purify the K16 WT/YFP and the K16 ÄHTM/YFP expression plasmids, and transfect them into human tissue culture cells.
3.Incubate the cells for a few days to allow the cells to make K16 WT and K16 ÄHTM proteins.
4.Look under a fluorescence microscope for filaments and aggregates.

Conclusions/Discussion

My hypothesis was correct the K16 ÄHTM/YFP plasmids that were transfected into human tissue culture cells made aggregates and the K16 WT/YFP plasmids made filaments when transfected into human tissue culture cells. According to the results, it is possible that the broken filaments, or aggregates, cause people with the deletion mutation to have the symptoms of Pachyonychia Congenita patients.
The results are not as clear as I thought they would be. Although the difference between filaments and aggregates was clear, I was surprised that there was a mix of filaments and aggregates in the cells transfected with K16 ÄHTM (mutant) protein. Furthermore, there were some variations of the K16 WT (wild type) cells which contained a few aggregates.

Summary Statement

My project was to look at the effects of a rare skin disease mutation in keratin cells.

Help Received

Transderm, Inc. helped with project plan, supplies, and equipment; Somagenics, Inc. also procided necesary equipment; Dr. Francis Smith at University of Dundee, Scotland provided the K16 mutant and wildtype constructs; Dr. Michael Dalbey at the University of California Santa Cruz allowed me to use a

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Sravya R. Keremane

Project Number

S0409

Project Title

Biochemical Analysis of Mechanisms of Cold Tolerance in Citrus

Abstract

Objectives/Goals

To study the biochemical basis of cold tolerance in citrus by analysis of gene expression of selected genes in cold sensitive and cold-tolerant citrus varieties.

Methods/Materials

1. Selection of candidate genes by review of literature on cold tolerance reseaarch in other plant species;
2. Search for similar genes in citrus and design primers;
3. Cold acclimation of selected cold sensitive and tolerant citrus plants;
4. Sampling and extraction of RNA;
5. Test the quality of RNA and useability of primers by RT-PCR and gel analysis;
6. Quantitation of gene expression before and after cold acclimation by real-time PCR analysis;
7. Analysis of results

Results

Plants cold-acclimated for 2-8 days were analyzed for expression of three different genes by real-time PCR assay. Cold-sensitive varieties did not show any appreciable increased expression of ABF3 gene. Amongst the cold tolerant varieties, trifoliate oranges did not show any change, but sour orange and Cleopatra mandarin showed an increase in the level of ABF3 gene expression in cold acclimated plants. Upon cold acclimation for two days, an increase in trancripts of ICE1 gene was observed in the cold sensitive Mexican lime, cold tolerant sour orange and trifoliate orange. The CLTA gene expression increased only in cold tolerant trifoliate orange.

Conclusions/Discussion

The present study has shown that the study differential gene expression can be used in identifying cold tolerance genes, confirming the hypothesis. Three well studied cold tolerance genes from other plant systems, dehydrin, ABF3 and ICE1 were selected; the sequences of their homologues in citrus were identified and the information was used for designing primers.

A good correlation between cold tolerance and increased ABF3 gene expression was observed in cold tolerant Citrus varieties, sour orange and Cleopatra mandarin, but not in cold tolerant Poncirus trifoliata. These preliminary results suggest that two different mechanisms of cold tolerance may be present in the two genera, Citrus and Poncirus. This observation was further supported by results of analysis of plants exposed to longer periods of cold acclimation, in which all Citrus plants showed an increase in the level of ABF3 gene expression, but not in Poncirus. The present study has suggested a major role for ABF3 gene in cold tolerance in citrus.

Summary Statement

The ABF3 gene was shown to be upregulated in cold-tolerant citrus, but not in either cold sensitive citrus varieties, or in a related cold tolerant genus, Poncirus.

Help Received

The research was carried out at the USDA Citrus Germplasm Repository, Riverside.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Thomas G. Kwong

Project Number

S0410

Project Title

"Selfish" DNA Alu Polymorphism

Abstract

Objectives/Goals

The purpose of this experiment is to identify a family tree through the chromosome 16 Alu allele of each member and understand the mechanism that passes on the gene. I predicted that after finding the length of Alu of my parent#s and my chromosome 16 that half of each of my father#s allele will form my paternal allele and half of each of my mother#s allele will form my maternal allele.

Methods/Materials

To do this, deoxyribonucleic acid (DNA) is extracted from each person contributing to my experiment. The Alu in the DNA sample is then consolidated by using a microcentrifuge and amplified in a polymerase chain reaction (PCR) before finally being displayed using electrophoreses.

Results

After conducting the experiment the results did show a connection between the family members, indicating the project was a success. However, the connection was different from my original hypothesis.

Conclusions/Discussion

My original hypothesis was proved incorrect, so I revised it according to my new results. I concluded the passing of Alu must be similar to the passing of sex chromosomes from parents, where one of the options was chosen at random. This new hypothesis proved true for all my results.

Summary Statement

The relationship between Alu in family members of different generations.

Help Received

Mother provided gel box and microcentrifuge

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Dylan K. Lake

Project Number

S0411

Project Title

mRNA Splicing and Transcription Are Linked: BBP's Dual Role

Abstract

Objectives/Goals

My project studies genetic interactions between the branchpoint binding protein (BBP) and transcription factors BUR2, CTK2, and CTK3. BBP facilitates binding of the U2 snRNP during spliceosome assembly. Although it is essential in the cell, its absence does not adversely affect splicing; this suggests an additional function. BUR2, CTK2, and CTK3 have been found to play a role in the phosphorylation of the C-terminal domain (CTD) of the RNA polymerase 2 during transcription. These transcription factors were selected because the phosphorylation they perform facilitates the binding of splicing factors, including BBP. Finding an interaction between these proteins would suggest a larger link between transcription and splicing as well as clarify the roles of the specific proteins.

Methods/Materials

I generated mutant strains of Saccharomyces cerevisiae in which BBP and either BUR2, CTK2, or CTK3 are deleted from the genome. A plasmid containing a mutant version of BBP was then transformed into the cells. These double mutants were grown in culture and their rate of growth compared.

Results

I found that mutations in different regions of BBP had unique effects when combined with the deletion of BUR2, CTK2, or CTK3. Surprisingly, one mutant of BBP grew slower than the mutant alone when BUR2 was deleted from the genome but better when CTK2 or CTK3 was deleted. Specific regions of interaction were pinpointed along BBP through genetic tests.

Conclusions/Discussion

These data suggest different mechanisms of interaction for each of the three proteins with BBP. This research supports a new view of splicing and transcription as two processes working simultaneously and cooperatively in the healthy functioning of the cell.

Summary Statement

mRNA splicing and transcription are commonly thought of as distinct processes; this experiment refutes that understanding by showing a direct link between proteins involved in splicing and transcription.

Help Received

I used lab equipment at University of California, San Diego under the supervision of Dr. Tracy Johnson.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Connie Lu

Project Number

S0412

Project Title

Somatic Mutations in P85 Regulatory Subunit in Phosphoinositide-3 Kinase Pathway in Human Cancer Cells

Abstract

Objectives/Goals

Cancer is one of the main diseases in our society today. With much ongoing research, there are still many unfamiliar areas to study to find a cure. Mutations play a big part in cancer development. The purpose of my project is to find somatic mutations in the regulatory subunit P85 of the Phosphoinositide-3 Kinase Pathway # an important pathway to understand because it sends survival signals for cellular functions. It is predicted that due to mutations, P85 is bound to P110 # another subunit of the PI3 Kinase Pathway # indefinitely, which causes the PI3K Pathway to be over expressed. If somatic mutations can be found in P85 within its 15 exons, it could probably explain its overexpression. If we can target this mutation and allow the PI3K Pathway to function normally again, cells can perhaps return to their regular function.

The main methods used for this project were PCR, agarose gels and electrophoresis, and sequencing. PCR was done for all 15 exons, forward and reverse ends using fourteen cell lines which consisted of breast, ovarian, and endometrial cell lines. Agarose gels of 1.5% concentration were used during electrophoresis. Sequencing was done by the ABI Company at the Norris Cancer Center CORE Facility. After receiving sequencing results, my analysis was done by using FinchTV to view sequencing results; and I used the SDSC Biology Workbench 3.2 using the tools BLAST and CLUSTALW to compare DNA sequences with my results.

Although there seemed to be a few potential areas for mutations in Exon 1 Forward, they are not significant enough to alter the role of P85. However, more analysis will be done on the other exons with hopes of finding more mutations.

Summary Statement

The purpose of my project is to find somatic mutations in P85, which may be a potential reason why cells mutate into cancer cells.

Help Received

Used lab equipment at USC Norris Cancer Center under the supervision of Dr. Melinda Epstein

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Tony A. Lu

Project Number

S0413

Project Title

Importance of Drosophila eIF4E-Binding Proteins in Lifespan Regulation

Abstract

Objectives/Goals

Aging is unclear to scientists worldwide and yet people continue to be interested in ways to live longer; in Drosophila, when insulin levels fall, due to either genetic interventions or lack of nutrients a lifespan extension is seen. My previous research suggests that longer lifespan correlates with activation of the dFoxo gene, which is regulated by insulin levels, and reduced amounts of nutrients. This project therefore tests whether or not 4E-BP, which is downstream of the dFoxo pathway, is responsible for the increase in lifespan. Thus, it is hypothesized that if there is a lack of nutrition and if 4E-BP is upregulated, then 4E-BP will stimulate an increase in lifespan.

Methods/Materials

In this study, Drosophila lifespan was studied with respect to various overexpressions of 4E-BP that differ in their composition of Gal4#PO163, or 109#drivers, which are Drosophila lines that express the yeast transcriptional activator Gal4 in a tissue specific manner. In addition, the Drosophila were fed protein in the form of yeast ranging from 0-4% yeast concentrations.

Results

I saw complex interactions between the levels of 4E-BP and the nutritional content of the food for the phenotype of lifespan. The results show overexpressed 4E-BP on low yeast has a 50% increase in lifespan while 4E-BP on high yeast, a 15% decrease in lifespan.

Conclusions/Discussion

Because the Drosophila genome shares 77% of their genes with the human genome, these findings may be applied to increasing human lifespan and allow us to study human mortality.

Summary Statement

Since my previous research suggests that longer lifespan correlates with activation of the dFoxo gene, this project therefore tests whether or not 4E-BP, which is downstream of the dFoxo pathway, is responsible for the increase in lifespan.

Help Received

Used lab equipment at Caltech Benzer labs under the supervision of graduate student Brian M. Zid; Participant in the Alhambra Biomed Program

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Y. John Mei

Project Number

S0414

Project Title

Tumor Suppressor Gene PTEN as a Chemosensitizer: Overexpression of PTEN in Breast Cancer Cells and Normal Cells

Abstract

Objectives/Goals

In order to assess the potential of PTEN as a chemosensitizer and its ability to reduce side effects of chemotherapy, this study was conducted to examine the effect of tumor suppressor gene PTEN on the response of breast cancer and normal cells to cisplatin treatment, a chemotherapeutic agent.

Methods/Materials

The plasmids pCMV-PTEN and pCMV-null were constructed and transiently transfected into normal (MCF 10A) and tumorigenic cells (MCF-7, MDA-MB-468, MDA-MB-231, and BT-549) in 96-well plates. After 24 hours of transfection, different concentrations of cisplatin (0.0977uM to 50uM) were added into the cell media. Cell viability was assessed at 0, 16, 24, 48, and 72 hours for the first two trials and at 0, 48, and 96 hours for the third trial. Each trial was performed in triplicate or quadricate. A western blot was performed to assess the PTEN expression level of each cell line. Transfection efficiency was monitored by co-transfection of pEGFP (enhanced green fluorescence protein).

Results

The normal cell line, MCF 10A, showed a significant increase of growth in the PTEN line over the null line ($p<0.05$) in the presence of cisplatin. The tumorigenic cell lines varied in their responses to cisplatin with PTEN overexpression. MDA-MB-231 was sensitized to the drug at concentrations higher than 0.781uM by 24 hours ($p<0.05$). In MCF-7, effects of chemosensitization were only displayed at 96 hours ($p<0.05$). BT-549 cells appeared not to be sensitized by PTEN expression within 72 hours of treatment.

Conclusions/Discussion

The hypothesis was supported to different extents. Normal MCF 10A / PTEN cells were de-sensitized to cisplatin treatment, while tumorigenic cells reacted differently, from chemosensitization to no chemosensitization. In summary, this study indicates that PTEN can enhance drug sensitivity in some breast cancer cells and decrease drug sensitivity in normal breast epithelial cells. This suggests that overexpression of PTEN along with cisplatin treatment could potentially enhance chemotherapy and lower side effects.

Summary Statement

The potential of PTEN as a chemosensitizer was determined by testing its effects in non-tumorigenic and tumorigenic cell lines.

Help Received

Mother helped in lab technique, father performed cell sorting for third trial, Peter Kretschmer and Terry Grimer allowed me to use their lab at Berlex Biosciences.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Vasilios A. Morikis

Project Number

S0415

Project Title

The Effect of Charged Amino Acids on the Formation of Alpha-helical Structures: Year 2

Abstract

Objectives/Goals

Alpha helical secondary structures in proteins are formed by backbone (i,i+4) hydrogen bonds. My goal is to evaluate the effect of salt bridges between oppositely charged amino acid side chains at positions (i,i+4) on the stability of alpha-helices. I constructed, with my computer, models of peptides with repeated pentapeptide amino acid blocks with variable charge properties. I used energy minimization computational methods to evaluate peptide stability. This project addresses protein folding at the secondary structure level, an important question in biochemistry.

Methods/Materials

Structural stability can be evaluated using minimization of the internal potential energies of proteins and peptides. I used the program Deep View to visualize the peptide models and to perform 500 steps of steepest decent followed by 500 steps of conjugate gradient energy minimizations. The peptides I examined are (DAAAK)n, (DAAAR)n, (EAAAK)n, and (EAAAR)n as well as their reverse-order sequences (KAAAD)n, (RAAAD)n, (KAAAE)n, and (RAAAE)n, where n=1-4. D and E are negatively charged acids, K and R are positively charged bases, and A is a neutral amino acid. I also used the following control peptides composed of the same type of amino acids (AAAAA)n, (KKKKK)n, (RRRRR)n, (DDDDD)n, (EEEEE)n.

Results

In all cases, I found that the peptide stability increases with n, the number of pentapeptide blocks. For direct peptides I observed the following stabilities: (DAAAR)n ~ (EAAAR)n > (DAAAK)n ~ (EAAAK)n. For the reverse peptides I found: (RAAAD)n ~ (RAAAE)n > (KAAAD)n ~ (KAAAE)n. For the control peptides I found: (RRRRR)n > (KKKKK)n > (DDDDD)n > (EEEEE)n > (AAAAA)n.

Conclusions/Discussion

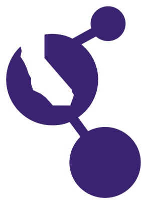
Combinations of R with D or E are more stable than combinations of K with D or E. The sequence order has a negligible effect on stability. In the control peptides, R has higher effect on stability, followed by K, D, E, A in that order. Surprisingly, the most stable peptide was the control (RRRRR)n and expectedly the least stable peptide was (AAAAA)n. Detailed examination of the data suggests that the increased stability is owed to van der Waals and electrostatic interactions. In peptides containing R electrostatic interactions are dominant. In the remaining peptides van der Waals interactions are dominant. Electrostatic interactions involve charged side chains and backbone atoms with small contributions from salt bridge interactions between side chains.

Summary Statement

My project aimed to determine the effect of charge on the stability of alpha-helical secondary structures using model peptides and energy minimization methods.

Help Received

Dr. Morikis for guidance and my mother for helping me glue the panels to the board.



CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Julia H. Nunan-Saah

Project Number

S0416

Project Title

The Effect of Alpha-Lipoic Acid on Airway Epithelial Functions

Abstract

Objectives/Goals

The purpose of this experiment was to determine how the antioxidant Alpha-Lipoic Acid affected the functions of the airway epithelium in the lungs. I hypothesized that Alpha-Lipoic Acid would improve human lung cell integrity and increase the transport of Chloride ions from the cells into the lungs.

Methods/Materials

Cell culture techniques were used to measure the resistance of lung cells when exposed to Alpha-Lipoic Acid. A Ussing Chamber was used to monitor the Chloride ion transport through the CFTR channel (Cystic Fibrosis Transmembrane Conductance Regulator) into the lungs. Alpha-Lipoic Acid was added in concentrations of 10uM, 100uM, and 1mM in the resistance tests to the Calu-3 cell line while leaving control cells, and the cell resistance was measured for 16 consecutive days. In the Ussing Chamber, Alpha-Lipoic Acid was added in 100uM, 300uM, and 1mM concentrations to the mucosal side of CFBE41o- cell filters, while the Chloride current was measured. In half of the Ussing Chamber experiments, Forskolin was added prior to the Alpha-Lipoic Acid in order to initially stimulate the Chloride ion flow.

Results

After measuring the resistance of lung cells and monitoring the Chloride ion transport from the cells into the lungs, it was found that concentrations of Alpha-Lipoic Acid up to 100uM improved cell resistance when compared to the control cells over the 16 day period. It was also found that Alpha-Lipoic Acid, up to a 1mM concentration, stimulated the transport of Chloride ions through the CFTR channel, with and without the prior addition of Forskolin.

Conclusions/Discussion

This study suggests that Alpha-Lipoic Acid improves lung cell integrity and stimulates Chloride ion transport through the CFTR channel. The resistance test also confirmed the safety of Alpha-Lipoic Acid when exposed to lung cells up to a 100uM concentration. These results are significant in that they show that Alpha-Lipoic Acid is a beneficial antioxidant in improving cell health in human lungs. Most importantly, Alpha-Lipoic Acid has now been shown to stimulate Chloride ion transport in the lungs, an effect that would be greatly helpful in the treatment of Cystic Fibrosis, which results from lower levels of Chloride ion transport into the lungs.

Summary Statement

This project examines how Alpha-Lipoic Acid affects lung cell integrity and the Chloride ion transport into the lungs through the CFTR channel.

Help Received

Dr. Beate Illek and Dr. Horst Fischer allowed me to use their lab equipment and answered any specific questions that I asked. However, I individually designed, conducted, and analyzed the results for this project.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Sapna Y. Patel

Project Number

S0417

Project Title

Snake Venom and Cancer: Production of Functional Disintegrin through Metalloproteinase

Abstract

Objectives/Goals

Contortrostatin (CN) is a protein found in the Southern Copperehad snake and it is known to contain disintergins that inhibit angiogenesis, thus blocking the cancerous cells from spreading and growing due to no food or transportation. The interesting things we noticed was when we purified the venom to separate the proteins, we also found a couple of fractions that reacted to the CN antibodies, so we hypothesize that there is a pro-protein containing a metalloproteinse and an disintegrin (CN). But the disintegrin is tested and found to be inactive, thus the goal of my experiement is to try to activate the metalloproteinase, in return it will cleave itself and release a functional disintegrin, doubling the yield of our protein (CN).

Methods/Materials

First, we purified our protein using HPLC. Then all fractions were tested for CN using AN anitbodies in Western Blots and ELISAs. Then a Platelet Aggregation Assay was performed to test for platelet inhibition, a function of CN. Then many more ELISA'a and Westerns followed in order to prove that the protein does contain CN because it is responsive to the CN antibodies. Lastly, we activated the pro-protein using APMA(Amino-phenyl mercuric acetate), and did a Fibrin Plate Assay to see if the metalloproteinase released a dunctional disintegrin by measuring the amount of lysis through an spectrophotometer.

Results

Our results indicate that the metalloproteinase did not release a functional disintegrin because the lysis in the wells was inconsistent, and our negative control failed because it was not suppose to have any fibrin eaten since it didn't have any activated protein, but our spectrophotometer showed lysis, thus making our results invalid.

Conclusions/Discussion

We conclude that the disintegrin is inactive and attached to the metalloproteinase, thus not doubling the yield. Possible errors include making the fibrin plates incorrectly or improper purification of the protein. Other types of method we are thinking about are zymograms to evaluate the activity of the disintegrin.

Summary Statement

My project is about the production of a disintegrin through activation of a metalloproteinase

Help Received

I recieved tremendous help from Fritz Costa and Dr. Steve Swenson at Cancer Research Laboratories located at USC Keck School of Medicine.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Jeffrey C. Peterson

Project Number

S0418

Project Title

The Effect of FeS on Yeast Metabolism

Abstract

Objectives/Goals

The objective of this experiment is to see whether sulfide ions can be oxidized fast enough by S. Pombe and brewer's yeast sulfide oxidizing proteins, or if their rate of oxidation is too slow and the cytochrome c oxidase lipoprotein complex in the mitochondria and inhibited by the ions and the aerobic metabolism pathway of the yeast is shut down and it is forced into aerobic respiration.

Methods/Materials

I used 18 food solutions for each yeast sample: 10 for brewer's yeast and 8 for S. Pombe yeast. 2 samples of each brewer's yeast and S. Pombe sets were simple control, only having yeast and food. 2 of each set contained FeS, which ionizes in water to form HS^-. 2 of only the brewer's yeast set contained FeS and vinegar, which was used to makes the sulfide ions form faster. 2 of each set contained Na(2)SO(4), because it is a reducer of oxygen in the water. This was used because FeS is also a reducer of O(2) and Na(2)SO(4) was used as a control for this. 2 of each set also had NaHSO(3), and this was also used to the same purpose. The density of the water was measured every 12 hours to see if alcohol was being produced. The yeast was also aerated at this time. The experiment took 22 days.

Results

The brewer's yeast exposed to FeS showed the most dramatic decrease in water density. The control brewer's yeast went down slightly in density. The rest of the controls in the brewer's yeast set showed no change in water density. The S. Pombe set showed little change.

Conclusions/Discussion

The experiment showed that at least the brewer's yeast, were forced to switch to the anaerobic pathway because of the exposure to sulfide ions, inhibiting the production of ATP by means of the electron transport chain. The S. Pombe yeast didn't grow fast enough in order to see a change. The Na(2)SO(4) and NaHSO(3) stopped the yeast growth. The vinegar and FeS did the same. The controls went down in density slightly because the sugar in the water was still being converted to water and CO(2), which caused the density to go down slightly. This means that yeast sulfide oxidizing proteins can't handle the HS^- because the rate of oxidation for these proteins was too slow. This means that sulfide ions will have an effect on human mitochondria and won't simply be snuffed out by the defense system against these ions. That means that using H(2)S to induce suspended animation may be a possibility.

Summary Statement

My project is used to see whether yeast can handle a saturation of sulfide ions using the same sulfide ions oxidizing proteins which are found in the human genome or if their metabolism will be forced into the anaerobic pathway.

Help Received

I did part of my experiment under the supervision of a lab technician at the Placentia Linda Laboratory.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Sean Pi

Project Number

S0419

Project Title

The Electrical Transport of DNA across Periodic Cross-Pattern Surfaces

Abstract

Objectives/Goals

We have propsed a new method of electrophoresis and the seperation of biomolecules such as DNA and proteins. This proposed method is dubbed "surface electrophoresis". In this method, we have vastly increased the efficiency of the conventional method of gel electrophoresis and increased its applicability to extremely wide range. We propose a cheap, fast, and efficient way for biomolecule (or in this case DNA) seperation and analysis.

Methods/Materials

We used silicon surfaces that were printed through soft lithography with alternating Au strips. After doing so, we would dilute the concentration of our DNA with a concentration of 1-250ìg/ml in a solution of 0.1M Tris-EDTA. A 1ìl drop of the DNA solution was deposited onto the surface and allowed to air dry, thus "loading" the DNA. We then set a laser approximately 2mm away from the load point and turn on the electric field. Mobility was then measure by the excitation of photons in the laser as the DNA gets closer to it/hits it. The photons are measured using a photomultiplier tube which is connected to a computer.

Results

We found that this method is far superior from past electrophoretic methods. First, we saw that there was a dependence of mobility on pattern spacing. With different pattern spacings, there was different mobility and this allowed for the segregation of different DNA sizes. We also discovered that the intrisic rigidity, or persistence length, affects the time it spends in the "traps" (interfaces between gold and silicon). Time spent at the traps directly correlates to persistence length which correlates with the intrisic structure of the DNA as well (linear, supercoiled, etc.)

Conclusions/Discussion

Through our new proposed method, we have created a new method for DNA seperation and analysis as originally discussed. It uses far less voltage than current methods (5V instead of 1500V), small run times (5 minutes instead of hours), and extreme portability. Our further investigation proved that mobility is completely dependent on pattern spacing, providing for a huge range of DNA seperation, unlike gels. It can also differentiate between DNA of different structures (which gels cannot do). Overall, the proposed method far improves the current method for biomolecule (DNA) analysis.

Summary Statement

A novel, more efficient and more portable way to conduct DNA electrophoresis and analysis

Help Received

SUNY Stony Brook; GARCIA MRSEC

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Kavita Renduchintala

Project Number

S0420

Project Title

The Insulin-like Growth Factor Pathway and Breast Cancer Risk in African-American Women

Abstract

Objectives/Goals

This study investigated the association between single nucleotide polymorphisms (SNPs) in the Insulin-like Growth Factor Pathway and the increased susceptibility to breast cancer in African-American women. The Insulin-like Growth Factor pathway was chosen specifically because it is known to be associated with cancer risk due to two important phosphorylation cascades it contains: cell proliferation and apoptosis. Single Nucleotide Polymorphisms are variations that occur in the DNA sequence when there is a change of one single nucleotide. These SNPs must occur in at least 1% of an epidemiological population to be considered significant.

Methods/Materials

DNA Samples were obtained from the Northern CA Breast Cancer Family Registry. TaqMan Assay Plates of 171 DNA samples were created using a Beckman Coulter Robot. ABI and Epoch Probe Cocktails were created and used for the Polymerase Chain Reaction Process. The SNPs in the DNA were then genotyped using the ABI Prism 7900 HT Plate Reader. Gel Electrophoresis was used in cases where the ABI and Epoch probes didn't produce proper results. Linear regression analysis was then performed to determine if the data was statistically significant.

Results

The SNPs IGFBP5 12239, IGFBP2 33312, and IGFBP2 31341 in a DNA sequence are extremely significant in the increased susceptibility to breast cancer because of the high odds ratios with which they are associated. Odds Ratios represent the increased risk of developing the disease as compared to an African-American women without the SNP. There is a clear correlation between certain SNPs in the IGF Pathway and the development of breast cancer in African-American women.

Conclusions/Discussion

This study has established the fact that the percentage chance of an African-American woman's susceptibility to breast cancer can be determined by looking at the genotype of certain SNPs in their DNA. There are still questions as to whether certain SNPs are dependent on other SNPs in a gene and whether or not the combination of certain genotypes creates a higher odds ratio of developing breast cancer. Future research will involve the investigation of the full function of the SNPs and genes that are significantly associated with cancer risk.These results can provide clues for strategies to aid in the prevention and treatment of breast cancer.

Summary Statement

Single Nucleotide Polymorphisms in the Insulin-like Growth Factor Pathway are associated with the increased susceptibility to breast cancer in African-American women.

Help Received

Used lab equipment at UCI under the supervision of Dr. Neuhausen and Dr. Ding; Participant in the American Cancer Society High School Research Program

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Amy Shipley; Andrea Trader

Project Number

S0421

Project Title

SNPs and Their Correlation with Alzheimer's

Abstract

Objectives/Goals

We are investigating a successful protocol to screen DNA for SNPs. We chose two SNPS of interest using the University of California Santa Cruz (UCSC) Genome Browser and then applying our protocol to analyze their correlation with Alzheimer#s. Our belief is that it is possible to screen for SNPs and research suggests that certain SNPs do contribute to the chance of developing Alzheimer#s.

Methods/Materials

We will be using ZR Genomic DNA II Kit# and using resources in the UCSC labs to analyze the DNA. Our procedures include collecting the DNA from the human subjects by having them swish Saline Solution in their mouths for 30 seconds and then spitting the solution back in a test tube. We will continue by following the Rinse Method for Buccal Cell extraction. After we have isolated the DNA, we will design a PCR (polymerase chain reaction) to amplify the DNA. If we successfully amplify the DNA, we will add restriction enzymes to cut the DNA at the SNP sites of interest. Finally, we will analyze DNA for SNPs using electrophoresis and see if results correlate with the genetic history of each sample.

Results

Our protocol is still underway and not completed at this time.

Conclusions/Discussion

Analyzation is not complete at this time but it is taking place.

Summary Statement

The purpose of this experiment is to show that it is possible to screen for Single Nucleotide Polymorphisms (SNPs) that could be associated with Alzheimer#s disease.

Help Received

Science Teacher layout project outlines; Rachel Heart helped teach us how to use UCSC Genome Browser; Used Lab equipment at UCSC under the supervision of Sofie Salama; Mothers helped drive us to UCSC; Linda Rogers helped organize project deadlines; San Lorenzo Valley High School Staff helped

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Kanan K. Sindhu

Project Number

S0422

Project Title

The Effect of Testosterone Depletion on Superoxide Radical Production in Gastrocnemius Muscles

Abstract

Objectives/Goals

It is now known that oxidative stress is one of the major causes of the initiation, progression, and consequences of diabetes mellitus, including heart disease, diabetic neuropathy, and nephropathy. People with several chronic diseases including diabetes are known to have lower levels of testosterone. My objective was to test the hypothesis that testosterone depletion will lead to oxidative stress.

Methods/Materials

To check for oxidative stress, aconitase activity was measured; aconitase is an iron and sulfur containing enzyme that is inactivated by the superoxide radical. Fumarase activity was measured to serve as a negative control; fumarase is a sulfur containing enzyme that is not inactivated by the superoxide radical. Aconitase activity was measured by monitoring the formation of cis-aconitic acid from isocitric acid by following the absorbance at 240 nm in 50 mM Tris-HCl, pH 7.4 containing 600 uM manganese chloride and 20 mM isocitrate at room temperature (RT). Fumarase activity was measured by following the increase in absorbance at 240 nm at RT using 30 mM potassium phosphate buffer, pH 7.4, and 100 uM L-malic acid. To express fumarase and aconitase activity as a function of protein (specific activity), the protein content of the muscle samples was determined using the Biorad Assay Kit.

Results

In the castrated samples, aconitase activity was significantly lower compared to the control group. The enzyme activity returned to the control levels in the muscle samples of the castrated animals that were supplemented with testosterone. Fumarase activity was not affected. Therefore, testosterone depletion does cause oxidative stress as demonstrated by the loss of aconitase activity in the testosterone depleted group.

Conclusions/Discussion

The results supported my hypothesis. My conclusion is that testosterone depletion indeed causes oxidative stress. Supplementation with this androgen in testosterone depleted animals ameliorated the production of the superoxide radical, and thus oxidative stress.

Summary Statement

Testosterone depletion causes oxidative stress.

Help Received

Used lab equipment at Charles Drew University under the supervision of Dr. Sindhu

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Pratyaksh K. Srivastava

Project Number

S0423

Project Title

Synthesis and Biological Evaluation of a Glycoprotein as Antibacterial Agent

Abstract

Objectives/Goals

The purpose of this project was to synthesize cell surface carbohydrates found on both bacterial and human cell walls as modified glycoconjugates to assist in the inhibition of bacterial binding to human cells and eliminating bundle forming pili. They will serve as receptors, meant to inhibit localized adherence of the bundle forming pili of bacteria such as mutant Enteropathogenic Escherichia Coli.

Methods/Materials

The synthesis included the incorporation of the N-benzoyl lactosamine with 8-methoxycarbonyloctyl linker for its attachment to the protein (BSA). The synthesis of the glycoprotein involved the preparation of the monosaccharide components followed by their attachment to each other and finally to the carrier protein. Two key building block intermediates given the names acceptor and donor monosaccharide respectively were synthesized and joined together via a glycosylation reaction to obtain the protected disaccharide. The glycoprotein that was synthesized was then put through the first of a two part biological testing. The first of which, included testing whether the formed glycoprotein would disrupt an already colonized group of E-Coli. This was done through a MIC (Minimum Inhibitory Concentration) test. The second part of the biological testing is currently underway and tests the binding efficiencies of the glycoprotein to the E-Coli (EPEC)and if the glycoprotein prevents EPEC from colonizing and damaging the host cell.

Results

The accuracy with respect to structure of the product obtained was confirmed with NMR results. Furthermore, the accuracy of the product achieved with respect to molecular weight was achieved through mass spectrum analysis. The mass spectrum of the desired compound was estimated to be around 638 mass units. Mass spectrum of the compound synthesized showed the final official mass of 638.37 mass units confirming the great accuracy of the product. The MIC test showed that the glycoprotein does not disrupt already colonized bacteria. This, although, was expected for the glycoprotein was designed to inhibit the binding of the EPEC to the host cell (currently under testing), essentially to prevent colonization from ever occurring, not to disrupt already colonized bacteria.

Conclusions/Discussion

In conclusion, the desired glycoprotein was synthesized with great accuracy. The first of the two part biological testing has been completed and the second part is currently underway.

Summary Statement

The goal of the project was to synthesize a modified cell surface carbohydrate as a glycoprotein to act as a means by which to prevent EPEC (Enteropathogenic Escherichia Coli) from attaching to a host cell.

Help Received

Supervision of Optimer Pharmaceuticals Chemistry and Biology Departments.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Carol Y. Suh

Project Number

S0424

Project Title

Micropathology Lab Device for Detecting Molecular Lesions of Glioblastoma

Abstract

Objectives/Goals

Current techniques for cancer diagnosis such as imaging (MRI) and tissue examinations are inadequate because they can detect distinguishing morphological patterns, but lack the capability to accurately identify specific intercellular interactions causing a particular cancer. Generalized models for diagnosis lead to ineffective therapies and high mortality. The objective of this project is to develop a system capable of detecting and analyzing cancer signaling pathways for specific diagnoses and targeted treatments.

Methods/Materials

Glioblastoma, the most lethal form of brain cancer, was the model used to integrate a systems biology approach with microfluidics. The device was fabricated by soft-lithography. Antibodies were immobilized onto the channels of the device through surface modification processes. Transfected and untransfected U87 lysates were labeled with fluorescent probes and passed through the device. Expression levels of EGFRvIII, PTEN, mTOR, p-Akt, and p-S6 from the PI3K pathway of Glioblastoma were analyzed under a fluorescent microscope.

Results

PTEN was detected and showed to be 19.7 fold greater in intensity than in lysates without PTEN. EGFRvIII was also identified and showed detection efficiency two times greater than the controls. Phosphorylated downstream signaling proteins such as mTOR, Akt, and S6 were efficiently analyzed as well. The results were validated with western blots.

Conclusions/Discussion

This newly developed device can efficiently analyze protein interactions from cancer signaling pathways. Proteins such as PTEN, EGFRvIII, p-Akt, p-mTOR, and p-S6 from the most malignant form of brain cancer were efficiently detected on chip. The Micro Pathology Lab Device will ultimately improve cancer diagnostics, accelerate drug screening clinical trials, and aid in the development of targeted therapies.

Summary Statement

A microfluidic device for detecting and analyzing cancer cell-signaling pathways was developed to improve the specificity of diagnostics and treatment.

Help Received

Used lab equipment at the University of California, Los Angeles under the supervision of Dr. Tseng and Dr. Sui.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Ryan Tam; Daniel Yeh

Project Number

S0425

Project Title

A Screen for Mutants in Drosophila melanogaster Affecting Triglyceride Levels

Abstract

Objectives/Goals

In our project, we overexpress the progeny of our Ryan-Daniel (aptly named after ourselves) fly line cross using a UAS-GAL4 system and test them for high or low triglyceride levels which deviate from the calculated average value. We will isolate the progeny with notable deviation because if the lines of the overexpressed gene show an increase in triglyceride level, it means that the genes regulate the triglyceride levels. We want to isolate new genes that change the triglyceride levels of the fly, and we predict that we will find lines causing both an increase and decrease of triglyceride levels in the fly.

Methods/Materials

For our methods, we grinded eight male Drosophila Melanogaster fruit flies (the results of our cross) each for thirty different microtubes. Each of the Drosophila constitutes a different fly line, or P-element. We continually add triglyceride reagants and buffer and undergo several incubation steps in order to get our desired solution. We then put each of our thirty different solutions into the microplate, which we load into a microplate reader to get our triglyceride results.

Materials include the triglyceride reagant, PBS buffer, our Drosophila cross, and the microplate reader.

Results

We found that line 5 of our cross had the highest and most consistent deviation and thus had the most reproducible phenotype. Through this project, we want to understand fat regulation and the effects of high triglyceride levels.

Conclusions/Discussion

The success of Drosophila Melanogaster as a model organism has largely been due to its ability to carry out large-scale genetic screens in identifying the genes involved in a biological process. Although most genes are based on a loss-of-function phenotype, our gene is based on a gain-of-function phenotype, or overexpressed genes.

Summary Statement

We will isolate the progeny of our RD-cross and test them for notable deviations in triglyceride levels, using triglyceride assays; this may show that the overexpressed gene regulates the triglyceride levels.

Help Received

Our mentor, Brian Zid, helped guide us through the project, helping us with the background information and the procedures.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Vinay Tripuraneni

Project Number

S0426

Project Title

Characterization of the Arabidopsis thaliana plsp1-1 and plsp1-2 Lines: Toc75 Suggests a Novel Protein Targeting Pathway

Abstract

Objectives/Goals

Toc75 forms the only known protein translocation channel in the chloroplast outer envelope membrane, and is thus crucial to plastid biogenesis. Toc75 is an unusual transmembrane protein in that it has 2 transit peptides; most membrane targeted proteins have none. A type 1 signal peptidase, Plsp1, is proposed to cleave the second transit peptide.

Methods/Materials

When PLSP1 is disrupted/knocked out with a tDNA insertion in the fourth intron, plants of the line, plsp1-1, exhibit a pale phenotype with underdeveloped chloroplasts. However, when the same gene is disrupted with a tDNA insertion in the first exon, plants of the line, plsp1-2, exhibit a phenotype similar to the wild type plants.

Results

The tDNA insertion portion of plsp1-2 was sequenced, and found to be immediately after the first methionine of the Plsp1 sequence. Because the beginning of Plsp1 has more than one methionine, interrupting the first methionine may not cause a knockout of PLSP1. This was examined by reverse-transcriptase mediated PCR, which revealed that the plsp1-2 plants homozygous for the tDNA insertion are transcribing PLSP1. Immunoblotting and chloroplast import in Pisum savitum of Toc75 suggests that Plsp1 is the protease responsible for cleaving the second transit peptide of Toc75.

Conclusions/Discussion

This evidence reveals a novel protein targeting mechanism where Toc75 demonstrates a completely new model for protein targeting pathways in all organisms. This new pathway has ramifications beyond chloroplast protein import; it may provide a model for new and more sensitive tests for genetic illnesses, give further insight into membrane chemistry and drug delivery, and may also be a step towards a cure for peroxisomal and mitochondrial disorders.

Summary Statement

Proteins make up nearly everything in an organism. A new way to get proteins where they are supposed to go (protein targeting) has been discovered!

Help Received

Used lab equipment at California State University, Fresno under supervision of Dr. Calderon-Urrea. Also used lab equipment at University of California, Davis under supervision of Dr. Inoue

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Stephanie J. Yaung

Project Number

S0427

Project Title

Selective Expression of Ligands Slit2, SlitL2, and their Putative Receptors Robo1 and Robo4 in Hematopoietic Stem Cells

Abstract

Objectives/Goals

The goal of the project is to investigate novel factors in hematopoietic, or blood-forming, stem cell (HSC) migration by testing for Slit or Roundabout (Robo) expression in HSC and performing chemotaxis assays to assess the effects on HSC migration.

Methods/Materials

Experiments involved the secreted proteins Slit1, Slit2, and Slit3, the membrane-bound protein Slit-like2 (SlitL2), and the receptors Robo1 and Robo4. Gene expression analysis consisted of isolating RNA from mouse bone marrow, reverse transcribing it into DNA, and testing that with Slit and Robo primers in quantitative real-time PCR.

After amplifying Slit DNA and transfecting the vector into cell cultures, the supernatant from the cells, which produce Slit from the DNA, was collected and used in chemotaxis assays. In transwell plates for migration assays, live HSC were placed in the upper chamber and allowed to migrate through the membrane and into the lower chamber containing the protein of interest. Cells were collected from the bottom chambers and analyzed on a FACS machine to determine the percentage of migrated HSC.

Results

Quantitative real-time PCR runs detected selective expression of Slit2, SlitL2, Robo1, and Robo4 in HSC. Most notably, Robo1 and Robo4 were differentially expressed at several-fold greater levels in HSC than in other hematopoietic populations, indicating that Slits may play a larger role in HSC migration. Chemotaxis assay data suggested that while Slits alone had no noticeable effect on hematopoietic cell migration, Slit2 did inhibit HSC migration towards stromal cell-derived factor-1ALPHA (SDF-1ALPHA) by 50%.

Conclusions/Discussion

For continual blood cell replacement in adults, a small number of HSC migrate between the bone marrow and circulating blood. Although a vital process, little is known about these movements and only a few factors have been found. Recent research has shown that the Slit protein family and their receptors Robo have similar effects as repellants or inhibitors of migration in various cell types. This study discovered that there was differential expression of Robos in HSC, highly suggestive of Slit and Robo's important role in HSC migration. Indeed, preliminary migration assay data identified Slit2-induced inhibition of HSC migration towards SDF-1ALPHA, a clinically important finding for bone marrow transplantations because SDF-1ALPHA is a chemoattractant in HSC mobilization and homing.

Summary Statement

Selective HSC expression of Slit2, SlitL2, Robo1, and Robo4, in addition to Slit2 inhibition of HSC migration towards SDF-1ALPHA, indicated that Slit/Robo interplay had a role in HSC migration, a valuable finding with clinical applications.

Help Received

Many thanks to mentor Dr. Camilla Forsberg (Dept. of Developmental Biology, Stanford Univ. School of Medicine) for her invaluable guidance, and the entire Dr. Irving Weissman lab for their kind support and technical advice. Also special thanks to the CCIS internship program for making this project possible.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Hann-Shuin Yew

Project Number

S0428

Project Title

Investigation of Homopolymeric Runs in C. elegans Genome with Novel Model for Control Sequences

Abstract

Objectives/Goals

To create, test and optimize a new algorithm, the Guided Localized Model (GLoM), for simulating DNA that is more precise than currently available methods. It was hypothesized that GLoM would provide a closer approximation to the homopolymeric run frequencies in the C. elegans genome than current controls. Secondly, using a larger window size would create a more precise DNA simulation, with reduced overemphasis of repeated regions.

Methods/Materials

GLoM was developed in Java, a platform-independent programming language from Sun Microsystems. Processing and generating 97MB of data on a 1.3GHz processor with 512MB RAM and a 1000nt window size required 68 minutes.
The C. elegans chromosome sequence was downloaded from WormBase. Markov models were created from the Regulatory Sequence Analysis Tools (RSAT) of the Université Libre de Bruxelles, and skewed-random sequences - random controls with built-in nucleotide biases - were generated with Java. Five runs of each sequence were analyzed to minimize statistical fluctuations.
Homopolymeric run frequencies were then calculated and plotted with Logger Pro to provide a graphical comparison of the different DNA sequences.

Results

Of all the controls, GLoM provides the best fit to the original genome. However, it tends to overemphasize the nucleotide and codon biases in the original DNA.
Unexpectedly, changing the window size does not appreciably improve the precision of the algorithm, especially for the A/T runs. However, in the case of the C/G runs, expanding the window size creates a slightly closer fit to the original genome. Shrinking the window size also produces overly long (>40nt) runs.

Conclusions/Discussion

GLoM, the Guided Localized Model, is a fast, elegant method of generating a control sequence of any length. Compared with a standard Markov model, it gives a superior reflection of the homopolymeric run frequencies in C. elegans. Through this improved fit, GLoM demonstrates that the non-random character of the homopolymeric runs is probably due to localized and holistic mechanisms. In short, GLoM has a wide range of potential applications in the fields of computational and molecular biology.

Summary Statement

In this project, an algorithm that simulates DNA sequences more comparable to real DNA than those from currently used methods was created and tested, extending our understanding of the C. elegans nematode genome in the process.

Help Received

Many thanks to Dr. Andrew Fire of the Departments of Pathology and Genetics at the Stanford University Medical School for his invaluable mentoring.

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Joanne Y. Zhang

Project Number

S0429

Project Title

Effects of MMP-Carrying Inflammatory Leukocytes on Tumor Cell Intravasation

Abstract

Objectives/Goals

To determine the role of inflammatory leukocytes, specifically chicken heterophils carrying MMP-9 and monocytes/macrophages carrying MMP-13, on tumor cell intravasation (entrance of blood vessels) and metastasis.

Methods/Materials

Western blotting determined if chMMP-9 and chMMP-13 were the proteins aiding tumor cell lines 45a and 47a in remodeling and intravasation. Proteins from tumors were separated by gel electrophoresis. Proteins transferred onto PVDF membranes and incubated separately with chMMP-9 and chMMP-13 antibodies. Membranes developed in a dark room after the addition of a chemiluminescent substrate. Proteins from tumor environment run through zymogram gel by electrophoresis. Gelatin ingrained in zymogram gel reveals which proteins are gelatinases (implies they are also collagenases). Gelatin stained with blue dye and gelatinase MMPs shown as white bands in blue gel.

DNA samples collected from the lower chorioallantoic (CAM) membrane and liver. Samples underwent real-time alu PCR analysis for alu sequences unique to primates (45a and 47a came from a human tumor). PCR machine records the number of cycles needed to detect alu sequences. Cycle number is converted into cell numbers using a mathematical function. High number of cells equates superb ability to intravasate and metastasize.

Same numbers of tumor cells from each cell line were seeded on the upper CAM. Six days after seeding, tumors were excised and stained with antibodies specific for chMMP-9 or chMMP-13 to visualize heterophils or monocytes/macrophages, respectively. Images of the tissue were taken and stained inflammatory leukocytes were quantified using Adobe Photoshop.

Results

47a has more chicken MMP-9, MMP-13, and higher ability in intravasation and metastasis than 45a. More inflammatory leukocytes are found near 47a tumors than 45a. This means that the heterophils and monocytes/macrophages carrying MMP-9 and MMP-13 help 47a intravasate (and thus metastasize) at higher frequencies than 45a.

Conclusions/Discussion

The hypothesis of this study was that MMP-9-carrying heterophils and MMP-13-carrying monocytes/macrophages directly contribute to tumor cell intravasation. The results obtained during the experiments verify this hypothesis.

Future studies may include the effects of anti-inflammatory drugs on tumor intravasation and metastasis.

Summary Statement

This research focuses on the effects of inflammatory leukocytes--specifically chicken neutrophils and monocytes/macrophages--on tumor cell intravasation (entrance of vasculature) and metastasis.

Help Received

Dr. Elena Deryugina for guidance, suggestions, trainings; used Dr. James P. Quigley's lab equipment at The Scripps Research Institute

CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

Phillip Y. Zhang

Project Number

S0430

Project Title

Immunohistochemical Identification of Early Disease Markers in Amyotrophic Lateral Sclerosis

Abstract

Objectives/Goals

Amyotrophic Lateral Sclerosis (ALS or "Lou Gehrig's Disease") is a neurodegenerative disease characterized by the death of motor neurons, causing complete paralysis and death by respiratory failure within three to five years in human patients. The exact cause of motor neuron degeneration remains unknown. My project attempted to develop a means for the early detection of ALS.

Methods/Materials

Thus far, scientists have identified a number of genes that differ in mRNA expression between healthy and ALS mice, even before they show symptoms. As proteins are the base for cellular functions and changes at the mRNA level do not necessarily cause changes in the expression of corresponding proteins, I selected three specific proteins coded by previously identified genes for further examination. Through the application of immunohistochemistry, I compared the level of these proteins in spinal cord tissue taken from healthy and diseased mice.

Results

My experimental results showed that expression levels of all three proteins correlate with the known mRNA expression increase, but their temporal patterns were different. Two of the proteins tested, GFAP and Urocortin, showed significant changes only in the late stages of the disease, and may therefore not be useful for early detection of the disease. However, the remaining protein, TGFalpha, showed significant difference between healthy and diseased mice at a relatively early age.

Conclusions/Discussion

As TGFalpha protein is secreted from cells, it has the potential to be used for the early detection of ALS using relatively non-invasive methods. Additional research is warranted, as detection of early disease markers will be critical for the diagnosis and treatment of ALS.

Summary Statement

My project attempted to find a means for the early detection of amyotrophic lateral sclerosis (ALS or "Lou Gehrig's Disease").

Help Received

Used equipment and facilities of the Burnham Institute for Medical Research under the supervision of Dr. Rengang Wang.