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11. Genetics

University of Central Oklahoma

Abstracts from the 2013 Oklahoma Research Day

Held at the University of Central Oklahoma

05. Mathematics and Science

11. Genetics

05.11.01 The Role of Streptococcus Pyogenes Chromosomal Islands (SpyCI) in Survival: Response to Environmental Conditions and LmrP Expression

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Group A streptococcus is a pathogen that causes a wide range of human disease. A prophage-like chromosomal island regulates an operon of DNA repair genes in GAS, including the multi-drug resistance protein LmrP, by dynamic excision and re-integration. LmrP confers resistance to lipophilic antimicrobials in other species. We show the contribution of LmrP to survival in GAS following antimicrobial treatment. Cell growth and survival was determined following treatment with lipophilic antimicrobial ethidium bromide in strains SF370, 4A, OKM28, and OKM29. Growth was done in normal or buffered media and at either 30,37 or 39 degree of centigrade. Cells were treated with reserpine, sodium orthovanadate, or both. SF370 showed higher sensitivity to EtBr killing when compared to 4A . Surprisingly, inactivation of LmrP in these strains did not result in equivalent sensitivity. Treatment with RS increased both strains' sensitivity to EtBr, demonstrating the role of proton pumps in antimicrobial survival. Altered environmental conditions showed that strains were more resistant to EtBr in buffered media compared to unbuffered media. Strain SF370 had increased sensitivity to EtBr in 39 degree. Surprisingly, OKM29 was more resistant to EtBr than the parental SF370 strain in the presence of MDR pump inhibitor NaOV. A SpyCIM1 encoded protective mechanism may exist that directly protects cells from DNA damage. Temperature also may regulate the integrative state of SpyCIM1.

05.11.02 De Novo Next-Generation Sequencing, Assembling and Annotation of Arachis Hypogaea L. Spanish Botanical Type Whole Plant Transcriptome

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Peanut is a major agronomic crop within the legume family and an important source of plant oil, proteins, vitamins, and minerals for human consumption, as well as animal feed, bioenergy, and health products. Peanut genomic research effort lags that of other legumes of economic importance, mainly due to the shortage of essential genomic infrastructure, tools, resources, and the complexity of the peanut genome. This is a pioneering study that explored the peanut Spanish Group whole plant transcriptome and culminated in developing unigenes database. The study applied modern technologies, such as, normalization and next-generation sequencing. It overall sequenced 8,308,655,800 nucleotides and generated 26,048 unigenes amongst which 12,302 were annotated and 8,817 were characterized. The remainder, 13,746 (52.77%) unigenes, had unknown functions. These results will be applied as the reference transcriptome sequences for expanded transcriptome sequencing of the remaining three peanut botanical types (Valencia, Runner, and Virginia), which is currently in progress, RNA-seq, exome identification, and genomic markers development. It will also provide important tools and resources for other legumes and plant species genomic research.

05.11.03 Online Database Research on Marfan Syndrome

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Online Mendelian Inheritance in Man (OMIM), Genbank, Basic Local Alignment Tool (BLAST), Spidey, and Molecular Modeling Database (MMDB) are all databases used in research of genetic disorders such as Marfan Syndrome, or MFS. MFS is a complex multisystem connective tissue disorder with a highly variable phenotype. This somewhat rare disorder is attributed to a defect in Fibrillin-1 (FBN1). FBN1 is an essential component of connective tissue, and binds to calcium. OMIM provided the phenotype (#154700) for MFS. This search displays the chromosomal locus of FBN1 to be 15q21.1. OMIM also provides the mRNA RefSeq Identifier of NM_000138.4 and a protein designation of NP_000129.3. Through GenBank we were able to find that the sequence length of the gene is 11695 base pairs. Through BLAST we were able to identify similar genomic sequence to mRNA found in Pygmy Chimpanzees, Western Lowland Gorillas, and Northern White-Cheeked Gibbons. Through Spidey we aligned XM_004056150.1 a gorilla mRNA, and NM_000138.4 a Homo sapiens mRNA. The results displays a highly conserved sequence with 99.4% overall identity. MMDB indicated the gene is 1828 amino acids long and the Protein Database (PDB) number is 2W86. MMDB and the protein software known as Cn3D displays the structure of the FBN1 Homo sapiens protein.

05.11.04 The Bioinformatics Analysis of the Cystic Fibrosis Transmembrane Conductance Regulator

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The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene encodes a chloride channel protein normally found in cell membranes. A delta F508 mutation of the CFTR gene results in a deficiency of this protein, upsetting the sodium and chloride ion balance between the cell and tissue. This imbalance creates a thick, sticky mucus layer of the passageway linings. For this project, information about the autosomal recessive CFTR gene was collected utilizing the following databases: Online Mendelian Inheritance in Man (OMIM), Genbank, Basic Local Alignment Search Tool (BLAST), Spidey, and Molecular Modeling Database (MMDB). The OMIM database indicated that CFTR is located on chromosome 7, loci 7q31.2. Genbank revealed the CFTR gene mRNA sequence is made up of 6132 base pairs and translates 1,480 amino acids. The mRNA studied in this project has a coding sequence that starts at the 133rd base pair, ends at the 4575th base pair, and contains 27 exons. A BLAST search revealed similar genomic sequences to the Sumatran orangutan (NM_001168545.1). Utilizing the Spidey database, an interspecies mRNA comparison of this organism with the human mRNA of the CFTR gene (NM_000492.3) confirmed a 98.6 % identity with 2 exons. Protein databases and MMDB provided structural representation of the gene's image.

05.11.05 Bioformatics Research of Classic Maple Syrup Disease

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Classic Maple Syrup Disease type II is classified by its sweet urine odor in infants and is an inherited disorder where the body is unable to metabolize certain amino acids. The name of the gene is dihydrolipoamide branched-chain transacylase or DBT (OMIM *248610).This information was obtained from OMIM (Online Mendelian Inheritance in Man). OMIM was accessed to search for the genetic disorder. OMIM is commonly used by medical professionals and research scientists to study human genes and genetic diseases. The cytogenetic location is on chromosome 1 p21.2. Next, research was performed using GenBank to obtain a genetic sequence. GenBank lists the accession number as NM_001918. This is a cDNA sequence that has 10831 base pairs. The organism source is Homo sapien. BLAST is a tool used by molecular biologists, which stands for Basic Local Alignment Search Tool. BLAST compares different nucleic acid sequences. From BLAST the genomic DNA sequence for DBT was identified as accession number NG_011852. Using the Genbank's cDNA accession number NM_001918 along with BLAST accession number NG_011852, Spidey was utilized to compare the mRNA-to-genomic alignment. Spidey showed 11 exons with an overall percent identity of 100.

05.11.06 Anaylsis of the X-Linked KX Blood Group Gene (McLeod Syndrome)

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Mcleod syndrome is a rare and historically significant genetic disease in humans caused by a mutation in the X-linked Kx blood group gene, given by the symbol XK. Using the gene catalog, Online Mendelian Inheritance in Man (OMIM), we found the XK gene is responsible for encoding a putative membrane transporter expressed in all parts of the body, but is primarily found in nervous tissue, heart, and red blood cells. The chromosomal location of the gene is Xp21.1. Using Genbank, another online database, we ascertained that the accession number is NM_021083, the coding sequence is 5091 base pairs, and is derived from an mRNA molecule. Using a BLAST search we were unable to obtain a genomic sequence for this gene, but the data showed a strong correlation to genomic sequences in other species. Using Spidey we performed a cross-species analysis between the XK gene on a human and a gorilla which indicated a 99.6% match in identity; only 10 of the base pairs out of the 1456 base pairs do not align. The OMIM report shows that the XK gene encodes for an antigen of the Kell blood group system often resulting in acanthocytosis. Through Genbank we learned the locus of this protein (NP_066569) and the amino acid chain length (444); however, we were unable to obtain the structure of the protein using the molecular model database.

05.11.07 Bioformatic Analysis of Charcot-Marie-Tooth Disease Dominant Intermediate B; CMTDIB

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Online Mendelian Inheritance in Man (OMIM), Genbank, Basic Local Alignment Tool (BLAST), Spidey, and Molecular Modeling Data Base (MMDB) are databases utilized in research of genetic disorders such as Charcot-Marie-Tooth disease (CMT). CMT is one of the most common inherited neurological disorders. CMT comprises a group of disorders that affect peripheral nerves. There are several forms of CMT; this research will focus on information pertaining to Charcot-Marie-Tooth Neuropathy, Dominant Intermediate B DI-CMTB CMTDI1. CMTDIB is caused by a mutation in the dynamin-2 (DNM2) gene. By using OMIM database, which focuses on the relationship between phenotype and genotype of a disorder, we were able to obtain the chromosomal locus of DNM2. The locus is 19p13.2 with an accession number NM_001005360. Through Genbank we found the mRNA for the gene is 3684 base pairs with the coding region starting at 191 and ends at 2803 of this sequence. Using Spidey, we are able to find that the sequence for DNM2 is 88.4% comparable to the mRNA sequence for Mus muculus gene accession number AK_171049. The gene has six highly conserved DNA coding sequences between mouse and human. By utilizing MMDB and the program Cn3D, we were able to visualize a structural representation and digital image of the protein.

05.11.08 Melatonin Sensitivity Mutants in Caenorhabditis elegans

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The Caenorhabditis elegans genetic system would be a valuable tool in determining the impact of melatonin on neuronal plasticity and long term potentiation. However, components of the melatonin signaling pathway in C. elegans remain ambiguous. The purpose of this study is to identify the C. elegans melatonin receptor(s). Worm G-protein coupled receptors (GPCRs) with homology to human melatonin receptors (hMRs) were identified through BLAST searches of the GenBank database. Multiple sequence alignment of homologous protein regions was also performed using ClustalW2 software. The field was narrowed down to less than 25 genes by limiting bioinformatic analyses of C. elegans GPCRs to regions of functional importance. A locomotion assay was developed to determine crawling rates of appropriate strains before and after exposure to melatonin. Wild type worms demonstrated significantly slowed locomotion after exposure to melatonin, but several GPCR mutant strains carrying mutations in potential homologues to hMRs were insensitive to melatonin. A GFP transgene marking all neurons allowed measurement of tissue cultured axons in response to melatonin. C. elegans neurons in tissue culture have longer axons when exposed to 1 mM melatonin. Worm mutants do not exist for some potential hMR homologues, so RNAi will be used to analyze the effects of melatonin on their behavior. Of special interest will be the srh-135 and srh-287 GPCR genes, which have the same NRY motif that is unique to hMRs.

05.11.09 Two Proteins May Protect Red Algal Photosynthesis From High Light Damage

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One helix protein (OHP) is necessary for recovery of photosynthetic activity after exposure of an organism to high light levels. Although the functional mechanism of OHP is not known, it is associated with the photosynthetic apparatus in green plants. Red algae are distant relatives to green plants, whose photosynthetic apparatus is structurally different from the well-studied green plant photosystems. We investigated if red algae contain one or more proteins with similar photoprotective function as green plants OHP. We have identified two homologous proteins relative to green plant OHP in Porphyra umbilicalis (nori), an ocean-dwelling, multicellular red alga. We are researching the function and regulation of OHP in Porphyra. Green plant OHP mRNA and protein expression responds to high light intensity, so we will investigate the regulation of the protein by comparing expression of Porphyra OHP mRNA before and after high light exposure by using qPCR. Also, we will use an OHP mutant and a complemented mutant with Porphyra OHP to compare the difference in response to high light levels by measuring the rate of growth and photosynthesis in each type. We expect to see that the function and regulation of Porphyra OHP is similar to green plant OHP and thus that red algae have similar protection mechanisms against high light as green plants.

05.11.10 Genetic interaction studies with Drosophila Ard1 suggest a role in regulating alternative cell death pathways

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Cancer is a widespread problem, and an estimated 1.6 million people are diagnosed with cancer every year. The enzyme Ard1 is expressed in most tissues, but Ard1 has been found to be overexpressed in a wide range of cancers. Ard1 is the catalytic subunit of the NatA complex, an Nα-terminal acetyltransferase, which can alter the function of many proteins through acetylation. Our present study investigates how Ard1 plays a role in cell survival in Drosophila. Genetic crosses were performed to demonstrate that RNAi knockdown of Ard1 during eye development causes a small eye phenotype. Expression of DIAP1, a caspase inhibitor, mitigated the Ard1 loss-of-function phenotype. However, expression of caspase inhibitor p35 exacerbated the Ard1 RNAi mutant phenotype. Since DIAP1 is known to also regulate autophagic cell death, these genetic interactions suggest that Ard1 may play a role in regulating alternative cell death pathways. Our research into the function of Ard1 and the role it plays in cell survival may help to advance our understanding of cancer genetics.