

The Phenotype of Transgenic Mice Over-Expressing Calpastatin In Skeletal Muscle



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Muscle wasting in diseases such as muscular dystrophy includes the actions of proteases called calpains. The proteolytic activities of calpains are inhibited by the endogenous protein calpastatin. The present study tests whether over-expression of calpastatin affects muscle structure as a foundation for future studies to test whether calpastatin over-expression can slow muscular dystrophy. Two strains of calpastatin over-expressing mice have been examined, 381 and 69.1. Tissue sections were stained with hematoxylin and their average muscle fiber cross section area was determined. Anti-calpastatin and anti-slow myosin heavy chain (MHC) or anti-fast MHC stainings were then performed using a MOM Kit. This information may provide a better understanding of how calpastatin affects healthy muscle fibers *in vivo* and will provide the background necessary for studying the effects of calpastatin over-expression in dystrophic muscle.

Detailed Characterization of Superman, the Clark Kent Stable Strain, and Its Relationship to Kryptonite in *Arabidopsis Thaliana*



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DNA methylation of cytosine is essential to most organisms because it plays a crucial role in the regulation of gene expression, serving as a signal that determines whether a particular gene is expressed. We are using *Arabidopsis* as a model to study the superman phenotype. These plants are abnormally methylated at the *superman* locus and are characterized by their unfused carpels and an abnormal number of stamens. Methylation of the *superman* locus is unstable, and the gene spontaneously reverts to normal. In order to stop this reversion, an inverted repeat of the *superman* gene was randomly inserted into the genome of a superman plant. To understand the nature of our superman model and the

mutants that have been found using this model, we have characterized the *superman* inverted repeat. Now that the nature of the inverted repeat is understood, crossing two plants that suppress the superman phenotype at separate loci, Chromomethylase

Locomotor Recovery Studies Following Spinal Cord Injuries



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Inflammation has been implicated in secondary damage to healthy tissue after spinal cord injury (SCI). Neutrophils are the first inflammatory cells recruited to the primary site of injury. They secrete matrix metalloproteinases (MMPs), which are thought to be detrimental to spinal cord recovery. Previous studies have shown the general MMP inhibitor, GM-6001, given every 12 hours for 3 days was efficacious in improving locomotor recovery in post-SCI mice. In this study, we determined if similar recovery occurred with a daily dose for 5 days. We performed a double blind study for 42 days post-SCI. Results showed no significant difference in locomotor recovery in the drug- and vehicle-treated animals. Vehicle-treated animals displayed significantly faster and improved recovery compared to previous studies suggesting that animals from the second study received a milder level of injury. This suggests that GM-6001 is only neuroprotective in moderately injured mice. Ultimately, these findings may establish an important basis in optimizing treatment in the spinal cord injured patient.

Resistance to Chemotherapy-Induced Apoptosis: APAF-1 Regulation In Ovarian Cancer Eglulation In Ovarian Cancer



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A unique sequence of events comprises apoptotic cell death: cellular shrinkage, nuclear condensation, externalization of plasma membrane phosphatidyl serine, and DNA fragmentation. For this to occur, molecules called apoptotic substrates must be cleaved by a family of aspartate-specific cysteine proteinases, called caspases. Caspase activation via the mitochondria plays a significant function in the induction of apoptosis in response to chemotherapeutic agents and UV irradiation. Ovarian epithelial cancer, a very lethal gynecological malignancy, resists chemotherapy-induced apoptosis, and some ovarian cancer cell lines resist cytochrome c-dependent caspase activation while showing diminished APAF-1 activity. This suggests that APAF-1 regulation may play an important role in the establishment of apoptotic resistance in ovarian cancer. To further characterize the regulation of APAF-1 in ovarian cancer cell lines that demonstrate apoptosis resistance, Hsp70 expression, caspase-9 activation, and APAF-1 oligomerization were analyzed. A more complete understanding of the regulation of APAF-1 activity in normal ovarian epithelium cells and in ovarian cancer cell lines will facilitate the development of therapies for cancers resistant to chemotherapy-induced apoptosis.

Computation of a Piece of Unstable Manifold



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In 1963 Lorenz investigated a system of three first-order differential equations that contained solutions that tended towards a "strange attractor." Henon showed that the same properties could be observed in a simple mapping defined by a two-dimensional function.

We consider the Henon family mappings with the function $T = T_{a,b} = (x, y) \mapsto (y - ax^2, b - a - y)$. Fixing b and giving an iterate $N > 1$, the algorithm computes a set of parameters giving an indication for which there is a "bifurcation" on the topology of the strange attractor. We have also con-

firmed Henon's finding that depending on the initial point $\{x_0, y_0\}$, the sequence of points obtained by iteration of the mapping either diverges to infinity or leads to a strange attractor, which appears to be the product of a one-dimensional manifold by a Cantor set. Due to advances in technology, we are able to increase the precision of calculation and describe the mapping in more detail.

Attenuation of P-Wave Amplitude in Concrete With Simulated Deterioration



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Three cylinders made from mortar with varying degrees of simulated damage were tested using ultrasonic Non-Destructive Evaluation (NDE) techniques.

The recently developed Frequency-dependent Amplitude Attenuation Characteristics Technique (FACT) was used to measure the P-wave amplitude attenuation in the concrete. FACT uses the P-wave amplitude reduction rather than velocity as an indicator of internal damage in concrete structures. Simulated damage was achieved by mixing various quantities of styrofoam into the cement mix to replicate microvoids. The three samples contained 0%, 4%, and 8% volume of styrofoam respectively. The sample with 0% represents a sample with no damage, and the other two samples represent various degrees of damage. The behavior of the waves in the simulated damage and undamaged cylinders was then compared to characterize the degree of damage present. Using FACT, we hypothesized that there would be up to a 40% difference in amplitude attenuation between the samples with simulated damage and those with no damage. The actual percent difference varied from approximately 40% to 95%.

Identification of Mutants Causing Defects in Hindgut Morphogenesis of *Drosophila*



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To identify genes that affect epithelial morphogenesis during development of the *Drosophila melanogaster* hindgut, we are carrying out a gain-of-function ge-

netic screen. This screen uses the yeast transcription factor (GAL4) and its DNA binding site, the upstream activation sequence (UAS), to cause ectopic expression in *Drosophila*. In our screen, we cause a P-element carrying the UAS sequence at one end to "hop" to random positions in the genome. The mutant males in which such a "hop" has occurred are crossed with females carrying the *bynGAL4* (*brachyenteron* GAL4) chromosome. This chromosome causes expression of the yeast GAL4 transcription factor specifically in the hindgut. The GAL4 in the hindgut cells binds to the UAS in the P-element. This will result in over-expression and possibly lethality. The morphology of the affected hindgut can be observed through the microscope because the *bynGAL4* chromosome also causes expression of Green Fluorescence Protein (GFP) in the hindgut. From this screen, we have generated many P{y+ UAS} insertion lines that cause a gain-of-function defect in the hindgut.

Functional Role of Sur2, a Subunit of the Mammalian Srb/Mediator Complex, in Adenovirus Replication.



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Adenovirus (Ad) has been an excellent model system for studying transcription in mammalian cells. The large adenovirus E1A protein is involved in expressing its early viral genes. However, E1A itself is not a DNA binding protein and requires host cellular transcription factors to start transcription. Past *in vitro* studies have shown that the conserved region 3 of E1A binds to the Sur2 subunit of the mammalian Srb/Mediator complex, which then together bind to Ad early promoters. The question addressed here is whether the E1A protein requires Sur2 for its functional role *in vivo*. This question was answered using RNA interference, a post-transcriptional silencing method, in order to silence *sur2* expression in *Drosophila* S2 cells. Simultaneously, Gal4-fused E1A was expressed in the same S2 cells and used to test if E1A activity decreased with the loss of Sur2. E1A activity was measured using a luciferase reporter gene driven by UAS (Upstream Activating Sequence), to which Gal4 binds. As expected, a decreased level of luciferase activity was found in cells lacking Sur2.

Emergence of Trematode Parasites from the Marine Snail *Cerithidea californica*



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Light microscopy, video/visual analysis, and dissections of *Cerithidea californica* indicate that there is a specific route of emergence taken by the trematode cercaria *Himasthla rhigedana*, *Euhaplorchis californiensis*, and *Renicola buchanani* within their mollusc host. Video microscopy has shown that the cercaria emerge from the right side of the mantle cavity. Upon dissection, groups of *H. rhigedana* and *R. buchanani* were observed to be burrowing through the tissue of what appears to be an excretory duct that leads into the right side of the mantle cavity. These findings may help understand parasite dispersal and population control within an estuarine environment.

Investigating Barriers To Cancer Screening among First-Degree Relatives of Colorectal Cancer Patients



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Colorectal Cancer (CRC) is the second most common form of cancer in the United States, and ~40% of those diagnosed will die from the disease. Early detection and screening decreases mortality rates and increases the chance of a cure, but rates of screening have been estimated at only 20% in the general population. Although first-degree relatives of CRC patients are at increased risk, their rates of screening are similar to those of the general population. The current study examined differences in barriers to screening among first-degree relatives of colorectal cancer patients. Information was gathered from 29 first-degree relatives through telephone interviews. Unscreened relatives had less knowledge regarding CRC, less knowledge of appropriate screening methods, poorer doctor-patient communication, and more concrete barriers to screening. However, relative groups did not differ in fear of CRC. The higher number of barriers reported by unscreened versus screened relatives may have impeded these individuals from getting screened and could lead to a poorer health outcome. Results will be used to design education programs in the future.

Observation of high-energy gamma-rays derived from Active Galactic Nuclei (AGN) present in nebulae and active galaxies



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We have observed 10-300 GeV gamma rays from the blazar Markarian 501 (Mark 501) and calculated its gamma-ray flux. Previous experiments were unable to see Mark 501 clearly because of attenuation factors associated with observing Mark 501 at too high or too low of energy.

We examined Cherenkov Radiation produced in the Earth's atmosphere due to incoming high-energy gamma-ray radiation. Canting over the source and subtracting the cosmic-ray background derived from an off-source run determined gamma-ray excesses. Bad data was removed through a series of run cuts designed to eliminate spurious figures. The significance of the gamma-ray signal was calculated, with a significance of 5.0 sigma or greater yielding strong evidence that Mark 501 is active. We also calculated the gamma-ray flux result from the significance and gamma-ray excess values using an integral flux method that incorporates the effective area of the gamma-ray signal on the ground. A significance of 0.018 sigma was calculated, corresponding to a gamma-ray flux limit of 2.73×10^{-10} at an energy threshold of ~ 125 GeV.

Perturbation of Homeobox-Containing Transcription Factors Six3 and Six6 During Chicken Retinal Development Using Retroviruses



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Molecular mechanisms responsible for the differentiation of multipotent neural progenitor cells in the retina remain to be determined. The homeobox-containing genes Six3 and Six6 are vertebrate

homologues of the *Drosophila* eye determining gene, *optix*, and may be critical in retinal development. The chick is an excellent model to study eye morphogenesis and retinal development because of the accessibility of the early embryo and the availability of retrovirus-mediated gene transfer technology. We have designed a retrovirus-based strategy to perturb Six3 and Six6 function during chick retinal neurogenesis. RT-PCR has been used to generate full-length

or partial cDNAs of chick Six3 and Six6. Both sense and antisense viral vectors have been constructed by molecular cloning. Viral stocks have been generated by transfecting producer cell lines and collecting supernatants. The expression and biological functions of the viruses will be characterized by Western blots, RT-PCR and/or *in situ* hybridization assays. Disruption of Six3 and Six6 expression during chicken retinal neurogenesis using retroviruses may cause developmental perturbations and thus elucidate roles of the homeobox-containing transcription factors during eye development.

Molecular Dynamics Simulations of Glass-Forming Liquids



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We study two-dimensional binary mixtures of repulsive Lennard-Jones discs over a range of temperatures and densities using constant temperature molecular dynamics simulations. For densities above a critical packing fraction density, the structural relaxation time and average potential energy per particle should display a change in behavior when temperature is lowered below the glass transition temperature T_g . In contrast, for densities below critical packing fraction density, one would expect the relaxation time and the average potential energy to display the same behavior over the entire range of temperatures and there should not be glass transitions. Above the critical packing fraction density, the relaxation time should become immeasurably long at nonzero T_g , and the average potential energy should remain constant below T_g . Below the critical packing fraction density, the relaxation time should become immeasurably long only at $T=0$, and the average potential energy should go to zero at $T=0$. We measured the glass transition temperatures as a function of density to determine whether or not the glass transition temperature vanishes at random close packing of hard discs.

HIV Induces Apoptosis in Cardiomyocytes



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HIV infection can lead to heart conditions such as cardiomyopathy and fibrosis. This led us to question how the virus was passing through coronary arteries and destroying the heart muscle. Previous studies have shown that HIV induces apoptosis in coronary artery endothelial cells, the cells that line the vessels of the heart. Our hypothesis was that HIV was also directly inducing apoptosis in cardiomyocytes, leading to a destruction of the heart muscle and cardiomyopathy. In our present study, we have determined through TUNEL (TdT-mediated dUTP Nick-End Labeling) staining that the virus is inducing apoptosis in tissue sections from hearts with HIV and cardiomyopathy. Experiments with HIV stimulated cardiomyocytes have also shown an induction of apoptosis. We believe that the destruction of the heart through apoptosis is a direct effect of the virus and not due to other factors such as macrophage supernatant. We have further confirmed apoptosis in these hearts by a more specific marker of apoptosis using PARP and anti-caspase 3 antibodies.

Morphology of Human Cortical Dysplastic Neurons Obtained From Children Undergoing Neurosurgery to Alleviate Intractable Seizures



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Cortical dysplasia (CD) is a condition that is often associated with epileptic seizures. Cortical dysplasia is characterized by anatomical malformations of the cerebral cortex. It consists of disturbances in lamination patterns, alterations in neuronal size and appearance, and changes in neuronal orientation. We have obtained samples of dysplastic tissues from children who are undergoing neurosurgery to alleviate their intractable seizures. We are studying the morphological properties of these cortical dysplastic tissues, comparing cells that have been described as abnormal-appearing with cells that are normal-appearing. In addition, we are also studying cells that come from non-dysplastic cortex. The specific cells that we are looking at in the cortex are pyramidal neurons.

These neurons exhibit a very distinct appearance and thus are usually easy to identify, as are any alterations or abnormalities that may be present. The hypothesis is that the abnormal-appearing cells will be most prevalent in cortical samples that have been previously classified as most abnormal by other criteria.

The Pits in the South Polar Cap



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For the past year, Malin and others have correlated the expansion of pits to the erosion of the south polar cap on Mars. The large number of Mars Global Surveyor images has caused difficulty in making a correlation between local observations and global trends. An analysis made by Malin et. al. of images containing pits in the same location during a variety of seasons support the hypothesis that the climate is changing, causing the erosion of the cap. Based on our analyses, it is not conclusive whether or not the south polar cap is in the process of erosion. We base our analysis on when the image was taken, location (longitude and latitude), season, solar time, and north and solar azimuths. The dynamics of the Martian surface with the atmosphere is not well understood at the moment. The cap could either be eroding or freezing, which would indicate climate change. If both are in effect, the cap is in equilibrium: the south polar cap is in a cycle of erosion and deposition (freezing).

Experimental Study of the Enhancement of the Efficiency of a Recuperator in a Micro Turbine Engine



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A recuperator extracts heat from exhaust gases from a micro turbine engine and preheats the compressed air entering a combustor. This cyclic process reduces the amount of fuel that must be burned in combustion and increases the efficiency of gas turbine engines. The micro turbines manufactured at Capstone Turbine Corporation are used for power generation. Optimization of the recuperator can provide improvements in performance for the micro turbine

engine, thus lowering fuel consumption, cost of maintenance for generation of power, and CO₂ emissions, which are a well-known environmental pollutant. Our objective is to construct a test fixture that will monitor the behavior of a specially designed recuperator in a high-speed airflow. We hope to verify that by modifying the manufacturing processes and geometrical features of the device, its heat transfer efficiency can be optimized, thereby further increasing the overall efficiency of the micro turbine engine.

Purification of L-Isoaspartylmethyl-Transferase From *Pyrobaculum Aerophilum*



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Proteins are susceptible to age-dependent inactivation and degradation. Formation of isomerized or racemized asparaginyl and aspartyl residues results in a distortion of the polypeptide backbone resulting in protein inactivation. L-isoaspartyl protein carboxyl methyltransferase (PCMT) initiates a series of reactions effective in repairing these *altered residues*. *Solving the structures of PCMT from several organisms* will provide an understanding of its mechanism. We have over-expressed and purified PCMT from the archaebacteria *Pyrobaculum Aerophilum*. We included a hexa-histidine affinity tag added to the C-terminus of the protein to simplify the purification process. We exploited the thermostability of the hyperthermophilic bacterium by heating the enzyme solution causing the precipitation of most other proteins. We further purified the protein using Ni²⁺ affinity column chromatography. The purification procedure yielded protein of sufficient purity for crystal growth and resolution of structure by X-ray crystallography. Since the successful completion of the purification, we have proceeded to growth trials using the hanging drop method and eagerly await crystal formation.