Welcome to the 7th Annual P.U.R.E. Symposium!

Monday April 9, 2012

LSF 102, 3:15 to 5:00 pm

PROGRAM FOR UNDERGRADUATE RESEARCH EXPERIENCE

Session 1:

3:25-3:37pm:

Initial Characterization of Expression of a Putative Member of the p53 Superfamily in *Daphnia pulex.*

Students: Maigen M. Bethea, Krissy L. Smith; **Faculty Mentor**: Dr. Erin M. Eaton

Recently, the entire genome of the aquatic organism *Daphnia pulex* has been sequenced (1). The sequence has revealed the presence of a putative member, based on homology, of the p53 superfamily of proteins; in humans this superfamily includes p53, p63 and p73. The human protein displaying the closest homology to the *D. pulex* protein is p63. The two polypeptides show 40% amino acid identity and 56% amino acid similarity between the two DNA binding domains. Significantly, both the dimerization sites and the zinc binding sites are conserved between the two proteins. The p63 protein has been demonstrated to be important in induction of nucleotide excision repair following UV irradiation in human cell lines (2); the manner of induction of p63 in these cell lines is yet unclear. Using reverse transcriptase-PCR and mRNA isolated from control and UV-irradiated *D. pulex*, we provide evidence of the presence of this gene product at the transcript level. This transcript appears to not be significantly upregulated by UV irradiation, as it is seen in both non-irradiated and UV irradiated samples.

Cloning of Daphina Pulex p53-like gene.

Student: Wendell Jones; **Faculty Mentors**: Dr. Erin M. Eaton and Krissy Smith

The p53 protein, also known as the "DNA watchdog", functions as a tumor suppressor protein in humans and is expressed in response to DNA damage. When DNA has been exposed to harmful reagents that may cause genomic damage (radiation or carcinogens), this protein functions to protect the genome from spontaneous mutations and is therefore critically important in protecting cells from developing cancer. Recent studies have indentified that these types of proteins are also found in other multicellular organisms and more importantly, tend to function by the same mechanisms. The sequenced genome of *Daphnia pulex* reveals a possible homolog to the human p53 gene. Although the genome has been sequenced, as to our knowledge there have been no attempts to isolate and clone the gene. The purpose of this project has been to isolate the putative p53 gene using RT-PCR and perform a bacterial transformation using *E. coli*.

3:49 – 4:01pm:

Effects of the antimalarial drug chloroquine on response to ceramide in prostate cancer cells.

Student: Frank Pezzimenti; **Faculty Mentor** : Dr. Lori Turner

When prostate cancer cells undergo chemotherapy or radiation, the signaling molecule ceramide is produced to initiate a cell death pathway. The cells can become resistant to ceramide-initiated cell death pathways by upregulating expression of the enzyme acid ceramidase to convert ceramide into sphyngosine-1-phosphate, which signals for proliferation rather than cell death. Since acid ceramidase is localized in the lysosomes, the anti-malarial drug chloroquine was used to destabilize the lysosomes. We concluded that a pre-treatment of chloroquine was effective in decreasing cell viability by increasing sensitivity of prostate cancer cells to ceramide cell-death signaling.

4:01 – 4:13pm:

An Investigation of Permeability Transition in Isolated Mouse Liver Mitochondria in Response to Elevated Cadmium.

Student: Anthony N. Gavalas; **Faculty Mentor**: Dr. Latha Malaiyandi

Mitochondria are well known targets during many pathologies, including neurodenegeneration, cancer and diabetes. More specifically, the intracellular accumulation of free ions, such as calcium or zinc, can lead to mitochondrial dysfunction, such as loss of membrane potential or elevated production of reactive oxygen species. Cadmium, although not biologically relevant, is an environmental toxin that accumulates in industrial areas. We have previously shown that cadmium is a more potent mitochondrial toxin compared to calcium or zinc and appears to behave through a mechanism similar to calcium. In this study the induction of permeability transition by the three ions wasinvestigated. Damaged mitochondria may undergo this event, which is the opening of a large, non-specific pore that causes mitochondrial swelling and may lead to apoptosis or necrosis. Here mitochondria were isolated from mouse liver and swelling was monitored in a sucrose buffer by measuring absorbance using a platereader. Upon addition of a range of concentrations of calcium, zinc or cadmium, concentration-dependent swelling rates were determined in comparison to alamethicin, a positive control for mitochondrial swelling. Permeability transition in the presence of a known inhibitor, cyclosporin A or the calcium uniporter blocker, ruthenium red was also investigated. These studies help further elucidate the specific mechanism for cadmium-induced mitochondrial toxicity in relation to biologically-relevant ions.

Session 2:

4:13 – 4:25pm:

Interplay between Acid Ceramidase and Cortisol in Adrenal Cortex Cells.

Student: Lenton Holley; **Faculty Mentors**: Dr. Teresa Herzog and Dr. Lori Turner

We are trying to find out if acid ceramidase affects cortisol production in adrenal cells. Acid ceramidase converts ceramide to sphingosine, which is converted to sphingosine-1-phosphate. Ceramide signals cell death whereas sphingosine-1-phosphate signals cell proliferation. Acid ceramidase is of interest in cancer research because it has been shown to be overexpressed in some cancer cell types. Cortisol is a steroid stress hormone produced by adrenal cells in response to adrenocorticotropic hormone (ACTH) which is released by the pituitary gland. Published studies have shown that ACTH can also alter acid ceramidase expression. Therefore, we have hypothesized that acid ceramidase expression may affect cortisol levels and therefore may play a role in stress response. To test this hypothesis, we are producing an adrenal cell line that overexpresses acid ceramidase and a control cell line expressing lacZ.

4:25 – 4:37pm:

Isolation and Cloning of the lacZ Gene into the DNA of a Plasmid Vector.

Student: Tiffany Phillips; **Faculty Mentor**: Dr. Vernon Bauer

Beta-galactosidase is a hydrolase enzyme resulting from the expression of the lacZ gene of the lac operon. It is important for blue-white selection when cloning a DNA insert into a plasmid. Our goal was to isolate the lacZ gene itself and insert it into a plasmid vector in order to provide the ability to observe successful cloning of the DNA by the appearance of blue colonies on media containing X-gal but no IPTG. Thus, the lacZ gene was isolated from pBLU, with pSP72 being the vector of choice. A culture of each of the two plasmids was prepared and purified. Both plasmids were digested using restriction enzymes NdeI and PstI. The desired fragments were then extracted from a gel, ligated, transformed, and plated on media containing X-gal and IPTG. After incubation, a blue colony was formed, indicating cleavage of X-gal by the enzyme β -galactosidase and, thus, the successful insertion of lacZ into the DNA of vector pSP72. Further cultures, prepared using the acquired blue colonies, resulted in the formation of blue colonies when streaked on a plate containing only X-gal and no IPTG. Using the same cultures, the plasmid was prepared and loaded on a gel. A band of proper length was acquired, providing further indication that lacZ had been successfully cloned into vector pSP72. The results indicate a successful attempt to design a “blue” construct without need of IPTG to express β -galactosidase.

4:37 – 4:49pm:

The Importance of Biological Studies in Everyday Work and Research in Veterinary Medicine

Student: **Ashley Volskay**; Veterinary Mentors: Dr.Shrum, Dr. Powell, and Dr.Storm

The main purpose of my internship was to shadow a Veterinarian in order to gain some perspective as to what my career path would be like if I was going to go to school for Veterinary science. When I got there I didn't realize how much affect my other biology classes would have in the field of Veterinary science. I also soon began to realize that every day brought an opportunity for research no matter how big or small. Some of the areas of biology that were frequently used were microbiology, anatomy, and genetics. For example, a dog came in and had dislocated its hip. Dr. Shrum hadn't seen many cases like this one due to the various variables surrounding the patient's medical history so he consulted Veterinarian literature as well as other Veterinarians in the area to figure out how he would go about the procedure that would be efficient and most beneficial towards the patient's recovery and future. Other forms of research that was done every day included urinalysis, skin testing, and pathology just to name a few.

The Department of Biology at FMU strongly encourages student participation in research activities. We offer many opportunities for undergraduates to assist in faculty research or develop their own independent research projects. Students can earn academic credit through Special Studies (BIOL 497) and Honors Independent Study.

If you are interested in learning more about P.U.R.E. or available research opportunities, please visit our website at: http://departments.fmarion.edu/biology/PURE.htm. You can also contact Dr. Barbeau (tbarbeau@fmarion.edu) or Dr. Pryor (gpryor@fmarion.edu), the coordinators of P.U.R.E. We can answer any questions you might have and get you started on a research project!