Welcome to the 8th Annual P.U.R.E. Symposium!

Spring Session
Thursday Apr 18, 2013
LSF 102

<u>3:46 – 3:58pm:</u> "The expression of empathy in rats and its effects on the brain."
Student Researcher: Cody Beard ; Faculty Mentor: Dr. Shayna Wrighten

The research conducted on the expression of empathetic behavior in rats benefits society by giving deeper insight into what is occurring in the brain while such an emotion is being expressed in humans. The hypothesis for the experiment was that rats that are familiar with each other will experience empathetic behavior. The methods used for the experiment were that two familiar rats were placed in an arena. One of the rats was trapped and the other was allowed to roam freely. The testing was done over a 20 minute period at the same time every day. The test was repeated for 3 weeks to see if the free rat would free the trapped rat. The brain tissue was collected after a 3 week period to be analyzed. From the experimentation it was determined that the free rat would in fact free the trapped rat in the restrainer only with the trapped rat's aid. This suggests that the free rat wants to be with the trapped rat. Once the trapped rat was freed the amount of contacts with the trapped rat was recorded. The data collected suggest that the two familiar rats do not like being separated and when one sees the other in distress it will come to its aid and free it. The experiment suggests that the hypothesis that familiar rats will experience empathetic behavior is plausible. Rats do experience empathetic behavior similar but not the same as a human being.

<u>3:58 – 4:10pm:</u> "Cloning the cDNA for the p63-like protein in *Daphnia pulex.*"
Student Researcher: Will Brown; Faculty Mentor: Dr. Erin Eaton

Expressing a protein *in vitro* has a number of practical uses including validation of antibodies, protein domain folding analysis and DNA binding assays. However, in order for the protein to be expressed, the cDNA must be put into a proper expression vector. This project has focused on sub-cloning the cDNA for the *D. pulex* p63-like protein into a bacterial expression vector. Once cloned, the vector will be used to express the protein in E. coli for subsequent purification and analysis.

<u>4:10 – 4:22:</u> "Initial characterization of expression of a putative member of the p53 superfamily in *Daphnia pulex.*" Student Researcher: Stephanie Berry; Faculty Mentor: Dr. Erin Eaton

Recently, the entire genome of the aquatic organism *Daphnia pulex* has been sequenced. 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; the manner of induction of p63 in these cell lines is yet unclear. We hypothesized that irradiating *D. pulex* would produce an increase in transcription of the p63 gene. 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. Furthermore, quantitative real time reverse transcriptase-PCR confirms our observations using only reverse transcriptase-PCR.

Recently, we have obtained an antibody that will specifically recognize the *D. pulex* p63 protein. Studies using this antibody are underway to determine if UV-irradiation causes an increase in translation of the *D. pulex* p63 homologue.

4:22 – 4:34: "Establishing primary cell lines from *Daphnia magna* embryos."

Student Researcher: Chris Donaldson; Faculty Mentor: Dr. Erin Eaton

With the recent sequencing of the *Daphnia pulex* genome, interest in the molecular aspects of the members of the genus popular ecological model has grown. Our lab has been investigating several proteins and signaling pathways in whole animals; unfortunately such studies can be limited by the logistics of extracting molecules, such as protein, RNA and DNA from the whole animal. In other words, degradation often occurs during the processing and valuable amounts of material are lost. Another limitation is the ease of manipulation of the organisms during treatments, such as UV irradiation. One way around these limitations has historically presented itself in the use of cell cultures from your organism of interest. For example, cell cultures from humans, mice and *Drosophila* (just to name a few) are in common use in labs around the world. To date, however, there is no published presence of any adherent *Daphnia* cell lines.

This project focused on the development of a protocol enabling the extraction of embryos from *D. magna* and the subsequent creation of an adherent cell line. I have chosen *D. magna* as my model because, as the name suggests, they are larger and the embryos are more easily extracted than those from smaller species.

4:34 – 4:46: "Effect of KIF5B knockdown on response to ceramide in prostate cancer cells."

Student Researcher: Timothy Prince; Faculty Mentor: Dr. Lori Turner

Acid ceramidase (AC) over-expression has been observed in prostate cancer cell lines and primary tumors, and contributes to resistance to chemotherapy and radiation. The consequence of AC over-expression is the ability to convert ceramide, which is often produced as a pro-apoptotic response to stress, to sphingosine, which can then be converted to the pro-survival molecule sphingosine-1-phosphate. Published results have shown that prostate cancer cell lines over-expressing AC have increased expression of the lysosomal-stabilizing protein KIF5B. Since AC is localized to lysosomes, disruptions to lysosome stability may counter AC-mediated resistance to treatment. We have evaluated the effect of siRNA against KIF5B on cell viability and the response of these cells to ceramide treatment. Preliminary results indicate that KIF5B knockdown restores some sensitivity to ceramide treatment. These results suggest KIF5B may be a feasible therapeutic target in cancer cells that over-express AC.

4:46 – 4:58: "ACTH affects acid ceramidase expression in adrenal cortex cells."

Student Researcher: Heather Yancey; Research Mentors: Dr. Lori Turner and Dr. Teresa Herzog

Acid ceramidase (AC) is an enzyme that converts ceramide, a lipid molecule that can signal apoptosis, to sphingosine, which is converted to the pro-survival molecule sphingosine-1-phosphate. AC up-regulation has been linked to resistance to ceramide-induced cell death in prostate cancer cells. Cortisol is a steroid stress hormone produced by adrenal cells in response to adrenocorticotropic hormone (ACTH) released by the pituitary gland. Published studies have shown that ACTH can also alter acid ceramidase expression, suggesting a relationship between stress pathways and cancer. We are evaluating how exogenous ACTH treatment affects AC expression levels in H295R cells, and if this confers resistance to ceramide-induced cell death. Understanding the relationship between ACTH and AC may offer insight into the relationship between the biological factors associated with psychological stress and the development of cancer.

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!