

Invertebrate Conference Symposia

I-1

Prostaglandin Actions in an Established Insect Cell Line. DAVID STANLEY and Cynthia Goodman. USDA, ARS, BCIRL, 1503 S. Providence Rd., Columbia, MO 65203–3535. Email: David.Stanley@ars.usda.gov

This presentation will report on using an established insect cell line (BCIRL-HzAM1 cells) and a primary hemocyte culture (*Manduca sexta* larval hemocytes) to investigate three hypotheses. In the first thrust, we posed the hypothesis that prostaglandins (PGs) and other eicosanoids influence the permissiveness of HzAM1 cells to viral replication. We treated cells with selected pharmaceutical inhibitors of eicosanoid biosynthesis, then challenged the cells with a recombinant virus engineered to express a Red Fluorescent Protein behind the heat shock promoter, AcHSP70-RFP. This cell line is non-permissive to viral replication, however, cells treated with inhibitors of eicosanoid biosynthesis became semi-permissive as seen by expression of the viral RFP and by TCID₅₀ values. We infer from these experiments that PGs and other eicosanoids act in virus/host cell interactions, although much remains to be learned about the eicosanoid actions. The second hypothesis is that one mode of PG action in HzAM1 cells is through influence on gene expression. We will show that PGE₁, PGA₁, PGA₂ and PGE₂ all exert important actions on gene expression as seen on 2D gels. Our data strongly support the hypothesis that PGs influence gene expression in this insect cell line. We used primary cultures prepared with *M. sexta* hemocytes to test our hypothesis that eicosanoids mediate insect hemocyte migration toward the bacterial-specific peptide fMLP. We recorded substantial and statistically significant reductions in hemocyte migration in primary hemocyte cultures prepared from *M. sexta* larvae that had been pre-treated with selected inhibitors of eicosanoid biosynthesis. We infer from these data that eicosanoids mediate insect hemocyte migration toward sites of bacterial infection. Overall, all three experimental thrusts show tremendous potential of using insect cell lines to investigate the biological actions of PGs and other eicosanoids in insect cells.

I-2

Signaling Interactions between Olfactory Receptor Axons and Glial Cells in the Axon Sorting Zone of the Developing Moth Olfactory Pathway. LYNNE A. OLAND, Nicholas J. Gibson, James T. Pearson, and Leslie P. Tolbert. University of Arizona, Arizona Research Laboratories Division of Neurobiology, P.O. Box 210077, Tucson, AZ 85721. Email: lao@neurobio.arizona.edu

The olfactory pathway of the moth *Manduca sexta* offers a system in which to examine developmentally critical neuron-glia interactions involved in axon sorting and targeting. During their growth to the olfactory lobe, receptor axons (ORNs) in the moth grow through a glia-rich region of the nerve where the axons separate from their neighbors, dramatically change directions, and fasciculate according to their target glomerulus in a glia-dependent manner. When the number of glial cells is severely reduced, this sorting process is markedly disrupted. Because these changes occur in a discrete region of the nerve that is easy to dissect differentially and the ORN cell-bodies reside peripherally, we can use *in vitro* approaches with well-defined cellular populations to study the underlying signaling interactions. Our *in vivo* data in the SZ show that fibroblast growth factor receptors (FGFRs) are activated on the glia, epidermal growth factor receptors (EGFRs) are activated on ORN axons, and the cell adhesion molecule neuroglian (an L1 homolog) becomes tightly anchored in axonal and glial membranes. In co-cultures of ORN axons and SZ glial cells, the growth cones of ORN axons become more complex shortly after contact with SZ glia. Together these data support a model of signaling interactions in which interaction of ingrowing ORN axons with SZ glia elicits a glia-derived signal that subsequently alters axonal behavior by regulating interactions between IgCAMs that then affect activation of the FGFR and EGFRs. We are using both time-lapse imaging and immunocytochemistry to examine the effects of blocking growth factor activation on axonal growth cone behavior, axon outgrowth, glial morphology and movement, and neuroglian stabilization. Funded by NIH DC004598.

I-3

Intercellular Signaling Regulates Heart Development in *Drosophila*. STUART J. NEWFELD and Aaron N. Johnson. School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501. Email: newfeld@asu.edu

Intercellular signaling by secreted protein of the TGFbeta family play important roles in the development of animals from sponges to humans. During early stages of heart development in *Drosophila*, the TGFbeta family member Decapentaplegic (Dpp) signals from the dorsal ectoderm to the underlying mesoderm to maintain Tinman expression. This signal specifies the primary cardiac field and homologous genes (BMP2/4 and Nkx2.5) perform this function in mammals. We recently showed that there is a second Dpp signal from the dorsal ectoderm to the underlying mesoderm that occurs late in heart development. This second signal maintains the boundary between pericardial cells and the adjacent muscle cells thereby defining the ultimate size of the heart. In the absence of the second Dpp signal, pericardial cells overgrow. This results in first instar larvae with enlarged hearts and significantly reduced cardiac output. Our study parallels a report indicating the existence of a second round of BMP signaling in mammalian heart development.

I-4

Applications and Potential Uses of RTgill-W1, a Cell Line Derived from Gills of Rainbow Trout. LUCY E. J. LEE¹, Kristin Schirmer², and Niels C. Bols³. ¹Wilfrid Laurier University, Department of Biology, Waterloo, Ontario, CANADA; ²EAWAG, Department of Environmental Toxicology, Dübendorf, SWITZERLAND; and ³University of Waterloo, Department of Biology, Waterloo, Ontario, CANADA. Email: llee@wlu.ca

Gills are unique structures involved in respiration and osmoregulation in piscinids and in many aquatic invertebrates. The availability of the trout derived gill cell line, RTgill-W1, is beginning to make impacts in fish health and toxicology. These cells are available from the American Type Culture Collection as ATCC CRL 2523. The cells have an epithelioid morphology and form tight monolayer sheets that can be used for testing epithelial resistance. The cells can be grown in regular tissue culture surfaces or in transwell membranes in direct contact with water on their apical surfaces. The ability of RTgill-W1 to withstand hypo- and hyper-osmotic conditions and their optimal growth capacity at room temperature, make these cells ideal sentinel models for in vitro aquatic toxicology as well

as model systems to study fish gill function and gill diseases. RTgill-W1 support growth of paramyxoviruses and orthomyxoviruses like salmon anemia virus. RTgill-W1 also support growth of *Neoparamoeba pemaquidensis*, the causative agent of Amoebic gill disease. The cells have been used to understand mechanisms of toxicity, ranking the potencies of toxicants, and evaluating the toxicity of environmental samples. These cells are also valuable for high throughput toxicogenomic and toxicoproteomic studies which are easier to achieve with cell lines than with whole organisms. RTgill-W1 cell line could become a valuable complement to whole animal studies and in some cases as gill replacements in aquatic toxicology.

I-5

Unique Cell Characteristics for the Development of a Portable Cell-based Toxicity Sensor for Drinking Water Protection. MARK WIDDER¹, Linda Brennan¹, Tommy Shedd¹, David Trader¹, William van der Schalie¹, Noe Salazar², Ethan Lerner³, and Aurel Iuga³. ¹U.S. Army Center for Environmental Health Research, Fort Detrick, MD; ²Agave Biosystems, Austin, TX; and ³Harvard University, Cambridge, MA. Email: mark.widder@us.army.mil

The U.S. Army Center for Environmental Health Research is developing an Environmental Sentinel Biomonitor system platform that includes sensors capable of rapidly identifying toxicity associated with a broad range of industrial and agricultural chemicals in drinking water. One sensor that incorporates cellular impedance technology has been well characterized using a primary mammalian cell line (bovine lung microvessel endothelial cells). Cells are maintained on a closed-system fluidic biochip (Agave Biosystems, Austin, TX) with a fluidic microelectrode array for non-invasive toxicant exposure and monitoring of the cells. The technology facilitates long term storage and maintenance and greatly improves portability of the sensor in support of field testing requirements. Additional research has been initiated to evaluate the use of non-mammalian cell lines to improve long term viability and maintenance and to increase toxicant sensitivity. Evaluations of the fish cell line RTgill-W1 (*Oncorhynchus mykiss*) and a frog melanophore cell line (*Xenopus laevis*) will be discussed. **Disclaimer** – The views, opinions, and/or findings contained in this abstract are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation. Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.