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I-1

Primary Cell Cultures from the Midgut of Lepidoptera Larvae. SILVIA CACCIA[1,2,3], Els JM Van Damme[2], and Guy Smagghe[1]. [1]Dept. Crop Protection and [2]Dept. Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Ghent, BELGIUM and [3]Present address: Dept. Biology, University of Milan, Milan, ITALY. Email: silviacacciax@hotmail.com

Lepidopteran larvae are extensively used as models in insect biology studies. In particular, there exists high interest in the lepidopteran midgut, the second largest organ after the skin, for its central roles in insect growth and physiology and as a major target for a number of insecticides, both chemically or biologically based. The lepidopteran midgut consists of a simple monolayer of epithelium separated from a framework of muscle and trachea by a thin basal lamina. It is constituted by two main mature cell types, columnar and goblet cells, and small round stem cells that mainly differentiate into mature phenotypes during the molt. Despite that the whole midgut epithelium has been largely and profitably used in *in vitro* experiments, the simplified context of primary cultures represent the most proper tool to finely elucidate the processes at cellular level. The availability of primary cultures from lepidopteran midgut, successfully established since the 1990s, gave rise during the last two decades to a revolution in insect midgut biology. Protocols to establish the cultures are mainly based on enzymatic dissociation of the midgut into mature cell types or from multiplication and differentiation of midgut stem cells. The latter protocol is based on the collection, by mechanical action, of midgut stem cells, which are not linked to the other cells with junctions. Once collected, thanks to the presence of the insect molting hormone 20-hydroxyecdysone and growth factors from lepidopteran fat body, stem cells enter mitosis and differentiate into new columnar and goblet cells that appear morphologically similar to mature cells *in vivo*. In respect to enzymatic dissociation, this protocol provides a semi-stable culture during several months. The first studies on primary cultures from lepidopteran midgut focused on the characterization of the mechanisms and the factors involved in the regulation of stem cell mitosis, the differentiation to mature forms and cell death. Primary cultures have also been exploited in a number of other studies concerning midgut biology such as the interaction between *Bacillus thuringiensis* toxins and midgut cells, protein absorption mechanisms, effect of secondary plant metabolites and new delivery strategies for pest control that target insect midgut. The interaction of plant lectins with lepidopteran midgut has also been studied *in vitro* using dissociated midgut cells, in particular lectin binding specificity to columnar cell microvilli and uptake mechanisms involved. The overall studies performed by means of primary cell cultures from lepidopteran midgut corroborate the effectiveness of this tool for midgut biology studies and new strategies development for pest control.

I-2

Cultures of Kenyon Cells from Bee Brains to Study Behaviour Side-effects by Pesticides on Pollinators. RODRIGO A. VELARDE[1], Veerle Mommaerts[2,3], Daniel Wilson[1], Guy Smagghe[3], and Susan E. Fahrbach[1]. [1]Department of Biology, Wake Forest University, Winston-Salem, North Carolina; [2]Laboratory of Cellular Genetics, Department of Biology, Faculty of Sciences, Free University of Brussels, Brussels, BELGIUM; and [3]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, BELGIUM. Email: velardra@wfu.edu; velarde.rodrigo.a@gmail.com

Over the world honey bees and bumblebees are important pollinators in agriculture/horticulture and are likely to be exposed to pesticides while performing their foraging duty. Today risks assessments are essential for all plant protection products and in this context several tests have been developed to assess the side-effects of pesticides on bee survival, growth/development and reproduction. Imidacloprid, an agonist of the insect nicotinergic acetylcholine receptor (nAChR), interferes at low doses with processes as learning and memory in bees. Its main target is the nAChR which is expressed in the Kenyon cells of the mushroom bodies. Here we report on a new bioassay using primary cultures of Kenyon cells (intrinsic neurons of the mushroom bodies) to evaluate side-effects of imidacloprid that have potential to affect pollinator behaviour. Kenyon cells derived from mushroom bodies of adult bees were cultured and directly exposed to imidacloprid in the culture medium. To assess the effect of this pesticide on neurons, we scored different cell parameters including process outgrowth, length of the

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major neurite, and area encompassed by the branches of the major neurite. The obtained data are discussed in relation to the side-effects of imidacloprid. We believe this new method allows to obtain new insights to better understand the mechanism behind behavioral effects caused by pesticide exposure.

I-3

Dissection of the Function of the RNAi Response in the Silkmoth and the Silkmoth-derived Bm5 Cell Line. Anna Kolliopoulou[1], Jisheng Liu[2], Hanneke Huvenne[2], Kostas Iatrou[1], Guy Smagghe[2], and LUC SWEVERS[1]. [1]Insect Molecular Genetics and Biotechnology, Institute of Biosciences and Applications, NCSR "Demokritos", Aghia Paraskevi, Athens, GREECE and [2]Lab. Agrozoology, Dept. Crop Protection, Fac. Bioscience Engineering, Ghent University, Ghent, BELGIUM. Email: swevers@bio.demokritos.gr

Since its discovery, RNA interference (RNAi) has become a powerful tool in functional genomics, and to date it is widely used in insect genetic research. Gene silencing through RNAi has revolutionized the study of gene function, particularly in non-model and non-genome sequenced insect species, which is the case for most agricultural pest insects. More recently, a new hot point is to find a feasible way to use RNAi as a new method for crop protection to combat pest insects. Efficiency of RNAi can differ extensively among insect groups, with coleopterans being characterized as highly susceptible and lepidopterans as refractory and greatly variable. Our studies focus on the analysis of the process of RNAi in the silkmoth, *Bombyx mori*, a model insect that represents the order of Lepidoptera. Four parameters are being evaluated for their role in the efficiency of RNAi: (1) expression levels of core factors of the RNAi machinery (Dicers, Argonautes, dsRNA-binding proteins) in different tissues and developmental stages; (2) rate of uptake of dsRNA by cells from the extracellular medium; (3) presence of enzymes that degrade the dsRNA triggers or siRNA effectors of RNAi; (4) inhibition of the RNAi response originating from the cryptic presence of RNA viruses in tissues. Besides experiments that involve silkmoth larvae, the Bm5 cell line is also used to evaluate the involvement of cellular and viral factors in RNAi through knock-out and over-expression studies. Our studies attempt to clarify the natural function of the RNAi pathway in the silkmoth with the ultimate goal of developing more effective approaches to RNAi-mediated inhibition of gene expression in lepidopteran insects. Such methods will have important applications for RNAi-mediated agricultural pest control.

I-4

Development, Characterization and Applications of Invertebrate and Fish Cell Lines - Tricks of the Trade. L. E. J. LEE[1], R. Hanner[2], and N. C. Bols[3]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [2]Department of Integrative Biology, University of Guelph, Guelph, ON; and [3]Department of Biology, University of Waterloo, Waterloo, ON, CANADA. Email: llee@wlu.ca

The development and use of non-mammalian cell lines, especially for economically valuable species like honeybees, crustaceans and molluscs, as well as for many fish species have lagged far behind despite their vast diversity and ecological/economical importance. Although a few fish species are represented among the piscinid cell lines reported to date, many more are needed to assist in understanding growth, developing tools, screening compounds, evaluating nutrients, manipulating genes, controlling pathogens, testing therapeutic agents, etc. The same is desirable for many invertebrates of economic importance, like bees for advancing apiculture or for aquatic invertebrates to enhance aquaculture and mariculture, yet to date and despite many attempts, no cell lines have been derived for bees nor shrimps, crabs or lobsters, whose economic value to Canada and the world are in the billions of dollars. My laboratory is expanding beyond the development of piscinid cell lines, to bee cell cultures and crustacean cells to establish continuous cell lines. To date, we have been successful in maintaining larval lobster cells in culture for up to a year with limited growth and passaging for up to 4 generations. We have also began attempts to generate embryonic and larval bee cell cultures. Best culture conditions, media supplements, physical parameters for maintaining long-term cell cultures from lobsters and bees will be reported along with the use of fish cell lines as models to elucidate best conditions for developing cell lines and to study pathogen interactions. A review of the literature on the attempts and progress to date towards the establishment of some of these species cell lines will be presented.

I-5

Fish Cell Lines: Some Strengths, Weaknesses and Mysteries. NIELS C. BOLS[1], L. E. J. Lee [2], N. T. K. Vo[1], and F. Zeng [1]. [1]Department of Biology, University of Waterloo, Waterloo, ON, CANADA and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA. Email: ncbols@uwaterloo.ca

Since the first fish cell line, RTG-2, was reported 50 years ago from gonads of rainbow trout, the number of cell lines has increased enormously and uses for them have exploded in different directions. Initially they were developed for the purpose of virology, and even now, this rationale often

drives cell line development. However, several trends in the last 20 years have led to new reasons for culturing fish cells. These include the emergence of zebrafish as a model organism, the emphasis on ecotoxicology or environmental toxicology, and the expanding importance and range of fish being used in aquaculture. In all cases the strength of cell lines is in allowing studies to be conducted inexpensively on material from the species of actual interest. One of the main weaknesses is the paucity of cell lines that expressed differentiated functions or can be induced to differentiate. Among the mysteries of fish cell lines are the ease with which the cells from many species appear to immortalize, whereas for a few species immortalization appears difficult. The future of fish cell lines should be bright as they s erve as a convenient bridge between the subcellular world of 'omics' and the life aquatic of fish.

I-6

Cell Cultures from Cartilaginous Fishes. DAVID W. BARNES. School of Science and Technology, Georgia Gwinnett College, 1000 University Center Lane, Lawrenceville GA 30043. Email: dbarnes1@ggc.edu

Elasmobranchs (sharks, rays and skates) have been used for many decades in comparative biology as models of primitive vertebrates, yet continuously proliferating cell lines only recently have been derived. Establishment of these lines was based on the concept that best results are obtained by attempting to mimic in culture the natural *in vivo* environment of the cell. This approach departs from conventional mammalian culture medium formulation, in which cells routinely are exposed to 5-20 % heterologous serum. The methodology has been successful in establishing cell lines from a variety of teleosts that are important experimental models, including zebrafish, *Xiphororous* (swordtail/platyfish) and pufferfish (*fugu*). Similarly, cell lines were derived from two species of cartilaginous fishes by formulating basal nutrient media for elasmobranchs, and supplementing with peptide growth factors and a minimal concentration of serum. The issue of misidentification or contamination with other lines can be problematical with these cultures, since karyotype information on cartilaginous fishes is rare, and karyotyping of the lines is difficult for technical reasons. A quantitative flow cytometric approach was used to confirm the species-relatedness of fish cell lines. Often fish lines derived in the manner described remain euploid or near-euploid. In addition, high-throughput nucleic acid sequencing was employed to construct partial transcriptomes for cartilaginous and other fish cell lines. This information provides species confirmation, identifies sequences previously unknown in any organism, and introduces a basis for gene diversity investigations and developmental-evolutionary studies at a genomic level. For instance, mRNAs were detected in a shark cell line for which the 3'-untranslated regions show elements that are remarkably well conserved evolutionarily, potentially identifying novel regulatory gene sequences. These cell culture systems provide new, physiologically valid tools to study functional genomics and many aspects of comparative biology.

I-7

Development of an In Vivo-In Vitro Bridging Model for the Investigation of Stress-induced Bystander Signaling in Aquatic Organisms. CARMEL MOTHERSILL, Richard Smith, and Colin Seymour. McMaster University, Hamilton, Ontario, CANADA L8S 4 K1. Email: mothers@mcmaster.ca

Validation of data obtained in vitro in organisms is often an issue and leads to a tendency to dismiss research to develop tissue culture approaches for organisms other than humans. However in threatened environments it is becoming very important to develop reliable non-invasive techniques for monitoring population stress responses due to physical or chemical pollutants. Our group has developed a technique for measuring stress response in fish. Fish which have been exposed to low concentrations of heavy metals (Cu, Cd, or Al) and/or low dose irradiation were allowed to swim with unexposed fish for 2 hrs. Fin clips and small gill filament samples were taken from all fish at the end of the period. Sham exposed fish and their partners were also examined. Gill samples were used for proteomic analysis and fin samples were used for tissue culture to look for stress signal production using a calcium flux reporter assay and for expression of biomarkers such as p53, bcl2, cmyc and MAPK in the cells cultured from the explanted fin. The fish were unharmed by the sampling of these tissues. The results show that the exposed animals exhibited a stress response resulting in elevated expression of proteins associated with early stage carcinogenesis. However they communicated signals to the unexposed fish resulting in an up-regulation of a suite of proteins associated with protective and adaptive responses in the cultures. The harvested medium from cultures of these fish demonstrated strong calcium fluxes in an in vitro reporter culture upon stimulation which again suggests a population level adaptive response to stressors which affected individuals in the population. Since the technique does not require sacrifice of the fish, it may have applications in ecotoxicology monitoring in sensitive environments.

I-8

Long-term Storage and Impedance-based Water Toxicity Testing Capabilities of Fluidic Biochips Seeded with

RTgill-W1 Cells. LINDA M. BRENNAN[1], Mark W. Widder[1], Lucy E. J. Lee[2], and William H. van der Schalie[1]. [1]US Army Center for Environmental Health Research, Fort Detrick, MD and [2]Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, CANADA N2L 3 C5. Email: linda.brennan@us.army.mil, mark.widder@us.army.mil, william.van derSchalie@us.army.mil, and llee@wlu.ca

Rainbow trout gill epithelial cells (RTgill-W1) are used in a cell-based biosensor being developed for detection of toxic industrial chemicals (TICs) in drinking water supplies. RTgill-W1 cells seeded on enclosed fluidic biochips and monitored using electric cell-substrate impedance sensing (ECIS) technology could detect 18 out of 18 TICs tested within one hour; The RTgill-W1 cells remain viable on the biochips at ambient carbon dioxide levels at 6°C for 78 weeks without media changes. Stored RTgill-W1 biochips were challenged with 9.4 $B&L(BM sodium pentachlorophenate (PCP), a benchmark toxicant, and impedance responses were significant ($p<0.001$) for all storage times tested. This poikilothermic cell line has toxicant sensitivity comparable to a mammalian cell line (bovine lung microvessel endothelial cells (BLMVECs)) that was tested on fluidic biochips with the same chemicals. In order to remain viable, the BLMVEC biochips required media replenishments 3 times per week while being maintained at 37°C. The ability of RTgill-W1 biochips to maintain monolayer integrity without media replenishments for 78 weeks, combined with their chemical sensitivity, make them excellent candidates for use in low cost, maintenance-free field-portable biosensors. Additional information will be provided