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I-1

Applications of Insect Cell Lines in Industry. JESSICA MONSERRATE[1], C. Peters[1], C. Oppert[1], S. Geibel[2], and D. Joo[1]. [1]Bayer CropScience, 3500 Paramount Parkway, Morrisville NC, 27560 and [2]Bayer CropScience, Monheim, GERMANY. Email: jessica.monserrate@bayer.com

For over five decades cell lines have taken center stage in an untold number of scientific advancements both in biomedical research and in biotechnology. Some of the key qualities that make cell lines desirable to countless academic labs are the same attributes industry seeks such as, modeling complex biological processes, the relative ease in scaling up experimental designs, and the exploitation of protein synthetic pathways for heterologous expression. In fact, in industry a very highly sought after scenario emerges when these three qualities converge to create a functional high-throughput screen in which a cell line can be used to screen libraries of active agents. However, in the field of insect control the number of well characterized cell lines that closely model important agricultural pests is very low. Thus, the question of biological significance often arises when cells lines obtained from model species are used. Expanding our research to include more relevant insect cell lines can potentially expedite our ability to deliver new classes of insecticidal agents with new modes of action in a manner that sufficiently meets the needs of farmers globally.

I-2

RNAi in Insects: Use of Insect Cell Cultures to Better Understand and Increase Efficacy. GUY SMAGGHE. Dept. Crop Protection, Ghent University, Ghent, BELGIUM. Email: guy.smagghe@ugent.be

Over the past decade, RNA interference (RNAi), the sequence-specific suppression of gene expression triggered by specific dsRNA molecules, has proven to be a very promising strategy in crop protection. The main advantages of RNAi are its selectivity, as well as the lack of persistency in and damage on the environment as a whole. There have been promising results against pest insects such as the western corn rootworm *Diabrotica virgifera*, and also successes have been reported against other beetle pests as Colorado potato beetle, but also pest insects. In this paper, a number of challenges will be discussed to implement RNAi as a widely-used pest control strategy and the specific use of insect cell cultures will be presented. Primary cultures and continuous insect cell lines engineered with a reporter system will be discussed to investigate possible causes for this variability in RNAi sensitivity as degradation of the dsRNA in the insect body and an insufficient uptake into the target cells.

I-3

Manipulating Wolbachia Infection and Maintenance in Mosquito Cell Lines. A. M. FALLON. University of Minnesota, Department of Entomology, 1980 Folwell Ave., St. Paul, MN 55108. Email: fallo002@umn.edu

Wolbachia pipientis is an obligate intracellular alphaproteobacterium that is widespread in arthropods, including insects that vector diseases of humans. In infected mosquitoes, Wolbachia causes a reproductive distortion known as cytoplasmic incompatibility (CI). CI provides an attractive mechanism for introducing transgenic mosquitoes into the field to reduce disease transmission, and our goal is to characterize the biological and molecular mechanisms that underlie CI. Using a proteomics approach, we have identified candidate proteins from Wolbachia-modified sperm that have molecular properties consistent with a role in CI. Because Wolbachia can be produced only within insect host cells, its implementation for control purposes requires an understanding of metabolic interactions that can be maximized to develop effective transformation protocols and improve its recovery after genetic manipulation. Using mosquito cell lines, we have developed flow cytometric methods for evaluating Wolbachia infection, have identified conditions that maximize production of Wolbachia, and have characterized a Wolbachia proteome including 800 proteins. A complementary proteomic analysis of differentially expressed host cell proteins provides a framework for identifying

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pharmacological agents that may influence production and retention of Wolbachia in cell lines.

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Prostaglandin Actions in Insect Immunity: Results from Primary Hemocyte Cultures. DAVID STANLEY and Cynthia L. Goodman. USDA, ARS, BCIRL, 1503 S. Providence Rd., Columbia, MO 65203. Email: David.Stanley@ars.usda.gov

We broaden appreciation of insect cells lines by focusing attention on primary hemocyte cultures to investigate prostaglandin (PG) actions in insect immunity. Insect hemocytes are able to detect and migrate toward chemical signals released from sites of wounding and infection. We posed the hypothesis that hemocytes prepared from tobacco hornworms could migrate toward the signature bacterial tripeptide, formyl-methionyl-leucyl-phenylalanine (fMLP). We used an apparatus in which fLMP was added to the bottom chambers, separated from the upper chambers charged with primary hemocytes by a polycarbonate membrane. After 1 h incubations, the membranes were inverted, stained and hemocytes on the lower surface of the membranes counted. Hemocytes migrated through the membranes toward the fMLP-charged lower chambers and pharmaceutical inhibitors of prostaglandin (PG) biosynthesis reversibly blocked the migration. In a similar vein, we used primary hemocyte cultures prepared from beet armyworms to test the hypothesis that plasmatocyte spreading peptide (PSP) acts through PGs. PSP and PGE2 independently stimulated hemocyte spreading in the primary cultures which, again, was reversibly blocked by selected pharmaceutical inhibitors of PG biosynthesis. We used primary hemocyte cultures to demonstrate the role of the small G-protein, Rac1, in hemocyte spreading. In more basic work, the enzymatic source of PGs in insects was unclear. In particular, detailed searches in genomic databases have not found gene sequences similar to the enzymes responsible for PG synthesis in mammals. We used cell spreading in primary hemocyte cultures to identify two peroxidases in the beet armyworm genome responsible for PG production. We discuss this work in the broader context of primary cell cultures.

I-5

Fish Cell Line: Development, Characterization and Conservation for In Vitro Research in India. MUKUNDA GOSWAMI. ICAR-Central Institute of Fisheries Education, Mumbai-400 061, INDIA. Email: mukugoswami@gmail.com

Fish cell lines have been widely used in vitro research because of their potential applications in pathology, toxicology, biomedical science & other biotechnological and developmental biology. The number of fish cell lines has been increasing tremendously covering a wide variety of species and tissues in recent years. Our research group developed more than 10 cell lines from different tissues from many commercially and aquaculture important fish species in India including a concept and subsequent actions on establishing a National Repository of Fish Cell Line in the country. The cell lines were authenticated and characterized using DNA barodes, karyotypes and protein expression signatures. The cell lines have been cryopreserved in liquid nitrogen (-196°C) for conservation as well as research purpose. Some of the cell lines have been utilized as rapid and cost-effective in vitro model for toxicological assessment of aquatic pollutants as well as gene expression studies. Development, characterization, conservation and applications of fish cell line as an in vitro model will be highlighted in the presentation.

I-6

Are Animal Cell Lines an Immortal Resource If Their Developers Are Mortal? NIELS BOLS[1] and Lucy E. J. Lee[2]. [1]University of Waterloo, Dept. of Biology, 200 University Ave. W., Waterloo, ON N2L 3G1, CANADA and [2]University of the Fraser Valley, 33844 King Road, Abbotsford Campus, Room B315, Abbotsford, BC V2S 7M8, CANADA. Email: ncbols@sciborg.uwaterloo.ca

Animal cell line development has been on going for over 70 years. The cell lines are either finite or continuous. Finite cell lines can be maintained for a limited number of population doublings, whereas continuous cell lines are immortal. For both types, large numbers of cells can be stored frozen, presumably forever. As a result, cell lines can be an invaluable scientific resource, supplying consistent populations of specific and general cell types for numerous uses in a wide range of scientific disciplines. Yet, to completely fulfill their promise as a dependable cell source, their reliable, long-term storage and retrieval is critical. Depositories, such as American Type Culture Collection (ATCC), accomplish this, cryopreserving and distributing cell lines to researchers for a fee. For fish, new cell lines from new species are being described every year. This is in part because the range of fish species being considered for aquaculture is expanding and cell lines from them can be used to investigate issues associated with their domestication, such as viral diseases. Whether depositories will want to accommodate all these new cell lines is unclear because a demand for them is difficult to predict. The cell lines are often developed for a

particular purpose in a project and when the project ends, they are put into liquid nitrogen storage. However, their long-term fate is uncertain. Every effort should be made to keep these cell lines available for future researchers as they could be invaluable in both anticipated and unanticipated ways.

I-7

Collagen Producing Fish Cell Lines and Their Use in Biomedical Research. L. E. J. LEE[1] and N. C. Bols[2]. [1]Faculty of Science, University of the Fraser Valley, Abbotsford, BC, and [2]Department of Biology, University of Waterloo, Waterloo, ON, CANADA. Email: Lucy.Lee@ufv.ca

Collagens are the main components of the extracellular matrix (ECM) of metazoans and the most abundant proteins in vertebrates including fish. It is a major structural protein, whose synthesis and deposition is regulated by hormones and diet, and is modulated by disease and physico-chemical factors. Despite its pivotal role in structural integrity, growth, and wound healing, comparatively little is known about fish collagen synthesis, regulation, and role in fish health. Furthermore, fish collagens have been shown to exhibit good biodegradability and weak antigenicity with excellent biomechanical properties suitable as key biopolymers in material sciences. In vitro models have been crucial for human and higher vertebrates to study collagen synthesis and secretion, yet very few models have been developed for fish. Like humans and other primates, teleosts require Ascorbic acid (AA) in their diet for collagen synthesis as they lack gulonolactone oxydase, a critical enzyme for AA synthesis. This study provides an overview on the use of novel fish cell lines to study collagen, as models to evaluate physico-chemical factors regulating synthesis, secretion and deposition of collagen, wound healing, disease progression, toxicity studies and nutraceutical effects.