In Vitro Report
AN OFFICIAL PUBLICATION OF THE SOCIETY FOR IN VITRO BIOLOGY VOL. 35, NO. 4 • OCT-DEC 2001

President's Column

Scientific Exchange and the Freedom it Demands

Dear Colleagues,

In the SIVB, we have been particularly proud to extol the international flavor of our journals and our Annual Meetings. We hosted speakers and featured authors from every corner of the world. We have recently firmed up an active working partnership with the Japanese Tissue Culture Association, and for last summer's St. Louis meetings, we celebrated our Latin American counterparts within several key sessions. Our global members have worked cooperatively with the SIVB leadership as we've grappled with strategies for increasing our visibility and impact, and showcasing our expertise in controversial arenas (stem cell research, GMO food crops) that have captured public attention. Then, the chain of horrific events in mid-September ripped away our sense of security, altered our priorities, and served as a rebuke to our entire way of life.

The carnage in New York City, and so close to the SIVB headquarters in Washington DC, sobered us all. It compelled us to temporarily suspend those goals and carefully-crafted new initiatives we'd pursued so urgently, just several short weeks ago. The nation and the wider circle of our friends throughout the world have been daunted by the uncertainty faced in the months ahead. We have struggled to come up with contingency plans to replace our normal modes of operation, because no one really knows what to expect when touched in places we thought we knew were secure. Our upcoming 2002 SIVB Congress (hosted in tandem with the International Association of Plant Tissue Culture and Biotechnology) will be centered in Disney's splendid Orlando theme park - one of the sites that was hurriedly evacuated on 11 September as a precaution against terrorism. In eight month's time, what will the reception of the international community be to the opportunities presented by our Congress, and so many others? Will the unrest and apprehension propagated by the terrorists succeed to intrude on the free exchange of scientific information? It is this particular freedom that scientists in our Society, and in other associations, have most valued and celebrated.

When our foundations are shaken, when calamity and disruption invade our lives, we long for normalcy, and for going back to the things that are familiar and cherished. It is my sincere hope that we will expediently and courageously go back to the norms that have made our lives worth living, because every moment we remain in suspended animation is yet another moment a terrorist has stolen from us. While travel may never again be viewed with the same casual inattention by this generation, we need to move on. Our resolve to continue forward and embrace scientific exchange cannot be shaken. We can and will focus ahead, and not permit anyone to take that freedom out of our hands.

Another small step we can take now to strengthen the future is to give our attention to the vote for new SIVB leadership. This fall marks the first time that leadership slots for all governance committee chairs will be elected by the entire membership. This shift further ensures that you have a voice in every aspect of SIVB's operations. Vote now and give your support to shaping this Society for the years ahead. And thank you for standing with us, and for the freedom of scientific exchange.

My best wishes for a safe and happy holiday season. We can't help but hold extra tight to our loved ones this season, and realize with fresh certainty how blessed we are to have them in our lives.

This Issue

Nominations are being accepted for Research Investigator Awards

The Society for In Vitro Biology has established awards to recognize excellence and achievement for in vitro research by non-student members of the Society. Recognition can be given annually to one Established Investigator and one New Investigator within each of the four sections of the SIVB (Cellular, Invertebrate, Plant and Vertebrate). Deadline for receipt of nominations by the Awards Chair is March 1, 2002. A nomination packet can be obtained from the Awards Chair, Melissa Hinga, by email: mhinga@ricetec.com or by mail: Ms. Melissa Hinga, RiceTec, Inc., PO Box 1305, Alvin, Texas 77512.

Maramorosch Receives Lifetime Award

The highest honor given by the Society for In Vitro Biology is the Lifetime Achievement Award. It is presented to scientists who are considered pioneers or highly influential researchers to the science and art of cell culture. They are men and women who have devoted their careers to exemplary research and|or teaching. The recipients of the Lifetime Achievement Award are selected by vote of the Board of Directors from a list of nominations received and recommendation by the Awards Chair. Melissa Hinga was the chairperson for 2001.

The Lifetime Achievement Award was presented to three scientists at the 2001 SIVB Annual Meeting in St. Louis, Missouri. The Awardees were ***Dr. June Bradlaw, Dr. Ian Freshney and Dr. Karl Maramorosch****. Dr. Bradlaw was highlighted in the last issue, Dr. Maramorosch in this issue, and Dr. Freshney will be highlighted in the next issue.*

Dr. Karl Maramorosch

Dr. Karl Maramorosch is past Chair of the Eastern Branch, Tissue Culture Association, and past Secretary and President of the History Branch of the SIVB. He joined the society in 1975, and earlier participated in the Decennial meetings in Hershey, Pa in 1965, and in 1955 in Woodstock, VT. Twice he conducted an invertebrate cell culture course at Lake Placid, NY. He first thought of using insect cell culture 8 years after meeting Dr. Ross. G. Harrison, the founder of vertebrate tissue culture at a symposium in Burlington, VT in 1948. At that time Karl worked at Rockefeller University, where he was influenced, and advised by Dr. William Trager. Karl started his first trial to culture leafhopper vector tissues to demonstrate plant phytoplasma multiplication in invertebrate cells devoid of plant material. He followed Trager's classical experiments in which the multiplication of equine encephalitis virus in hanging drops tissue culture of mosquito vectors was achieved in 1938. Dr. Maramorosch cultured cut pieces of six-spotted leafhopper nymphs *(Macrosteles fascifrons)* exposed previously by feeding on plants infected with aster yellows phytoplasma. After maintaining the cut pieces in his culture medium for 10 days, Karl was able to recover the phytoplasma from the cultured tissues. Using this method he was able to demonstrate the multiplication of the phytoplasma in cultured vector tissues for the first time (Virology 2:369-376, 1956).

At that time, attempts to culture insect cells were encountering great difficulties. It was commonly believed that insect cells were unable to proliferate in vitro. However, Karl thought that insect cell culture could provide a very useful tool and that it should be promoted further. In 1956 he invited T.D.C. Grace from CSIRO, Australia to his laboratory for 2 years in order to promote insect tissue culture studies. By 1962, after returning to CSIRO in Canberra, Grace succeeded in establishing continuous insect cell lines.

In 1961, Dr. Maramorosch accepted the invitation to become Program Director of Virology and Insect Physiology at the Boyce Thompson Institute in Yonkers, NY. There he continued his studies and he invited many postdoctoral scientists from various countries. His associates were from the United States, Canada, Japan, Netherlands, Poland, Rumania, Korea, China and India, followed by Yugoslavia, France and Germany. Among the Japanese associates were Drs. Hirumi, Mitsuhashi, Tokumitsu, Yamada, who were working on insect cells culture, and from the United States, Karl was joined, among others, by Drs. A.H. McIntosh, T.J. Kurtti, U.G. Munderloh and R.R. Granados.

Between 1956 and 2000 Maramorosch and his associates published more than 40 papers dealing with invertebrate cell culture. With Prof. Mitsuhashi a method was established for the primary cultivation of cells from all developmental stages of leafhoppers (Contrib. Boyce Thompson Institute 22:435-460, 1964). In the course of this study, Mitsuhashi and Maramorosch developed a culture medium now known as the M&M medium. This formulation was attained after much trial and error. It is the simplest and the cheapest medium which can be used to culture various invertebrate cells. It contained originally 20% fetal bovine serum, but later it became evident that it could be used as a serumfree medium. (Science 144:1465-1467).

Jointly with Dr. Takashi Tokumitsu, attempts were made to culture aphid cells in vitro. Cytoplasmic protrusions that appeared in insect cells during mitosis in vitro were described in the J. Cell. Biol. 34:677683, 1967.

Jointly with Dr. K. Yamada, Karl published 4 papers dealing with baculoviruses in *Heliothis zea* cell lines.They carried out a serial passage of the singly embedded virus in a homologous cell line (J. Invert. Pathol. 39:185-1944). Dr. Arthur H. McIntosh joined forces with Karl in 1972 and they jointly published several papers. They first dealt with the retention of insect virus infectivity in mammalian cell cultures (J.N.Y. Entomol. Soc. 81-175-182, 1973).

Jointly with Prof. C. Vago, Karl organized the First-International Conference on Invertebrate Tissue Culture in Montpellier, France in 1962. It was the start of regularly held international conferences, promoting invertebrate cell culture, and held every four years. Proceedings of many of these conferences were edited by Karl and published, becoming useful texts for investigators of invertebrate tissue culture. Additional conferences, organized under the sponsorship of

the U.S. National Science Foundation and the Japan Society for Promotion o f Science were organized in 1965, 1974, and 1985.

The Society for Invertebrate Pathology selected Karl as their Founder's Lecturer in 1990 and he delivered the Founder's Lecture in Adelaide, Australia, honoring his former associate Dr. T.D.C. Grace. In turn, in 1998, Dr. Mitsuhashi became SIP Founder's Lecturer, in Sopporo, Japan, honoring Karl (J. Invert. Pathology 74:99-102, 1999).

Dr. Karl Maramorosch & Leonard Hayflick

In 1980 Karl was awarded the coveted $100,000 Wolf Prize in Agriculture, highest international award in agriculture, and a year later the Alfred Jurzykowski Foundation Award in Biology. In 1983 he received the AIBS Award of Distinction and the same year Rutgers University elected him a Named Professor (Robert L. Starkey Professor of Microbiology).

Professor Maramorosch is a former Vice-President of the New York Academy of Sciences and their Recording secretary and Chair of the Microbiology Section. He is a member of the oldest European Academy, Leopoldina, and an Honorary Member of the Indian Virological Society and Foreign Fellow of the Indian National Academy. In 1998 he was elected Honorary Member of the Entomological Society of America, where he became a Fellow in 1986. He is also a fellow of ESA, American Phytopathological Society and AAAS.

In this study Mitsuhashi and Maramorosch found that fetal bovine serum, already known as a strong promoter in mammalian cell culture, was indispensable in growing insect cells. Leafhopper cells could not multiply without fetal bovine serum and other sera, such as calf serum, bovine serum, horse serum, and sheep serum could not be substituted for fetal bovine serum. Only newborn calf serum could partially do this. After the paper in which this was described in 1964, most invertebrate cell culture workers began to use fetal bovine serum instead of insect hemolymph.

In 1964, jointly with Dr. Hiroyuki Hirumi, Karl described the in vitro cultivation of embryonic leafhopper tissues (J. Expd. Cell Res. 36:625-631), and tissue culture of the Monarch butterfly (Contrib. Boyce Thompson Inst. 22:259-268). A joint paper in Science, the same year, described the use of the blastokinetic stage of the leafhopper.

"The Lifetime Achievement Award of the Society for In Vitro Biology bestows a great honor on me. On this occasion I wish to extend my personal and professional gratitude. Beyond personal and professional recognition, this SIVB award also brings honor and recognition to all my former associates who have worked with me during the past 45 years. The first was T. D. C. Grace, who started his insect cell culture in my laboratory at Rockefeller University in 1956, when I received my first USPH/NIH grant for this work. During the following years, 1961 - 1974, at the Boyce Thompson Institute, Jun Mitsuhashi, Hiroyuki Hirumi, Takashi Tokumitsu, Robert and Jos Granados have worked with me, and after 1974, at Rutgers University, Arthur H. McIntosh, Timothy Kurtti, Ulrike Munderloh, and 38 postdoctoral associates from the United States, Japan, Australia, Germany, Romania, Yugoslavia, Turkey, Canada, India, and Slovakia continued cell culture work supported by grants from USPH, NSF, USDA, Rockefeller Foundation, WHO, and Merck Company.

If not for the excellent work of my associates, I would not be here today to receive this award. To them, many of whom are in this audience, I wish to express my sincere thanks and best wishes for their continuous successful research and happy life."

Dr. Karl Maramorosch

Nomination packets for the 2002 Lifetime Achievement Award are required to be submitted to the Awards Chair no later than January 5, 2002. The nomination packet must include the nominee's Curriculum Vitae, a letter of nomination and two additional letters of recommendation. Any other supporting material is welcome. The nominator must secure a donation of $1500 to defray the cost of giving the award. A letter from the donor acknowledging their contribution must be included in the nomination packet. The nominator is also required to collect materials for a poster for display at the SIVB annual meeting on the achievements of the nominee. Please send nomination packets to the Awards Chair, Melissa Hinga, by email: mhinga@ricetec.com or by mail: Ms. Melissa Hinga, RiceTec, Inc., PO Box 1305, Alvin, Texas 77512.

Journal Highlights

V. Illcheva

Interspecific Hybrids of Tobacco

A protoplast fusion experiment was carried out aiming to obtain somatic hybrid plants of transgenic Nicotiana tabacum (bar) (+) N. rotundifolia (npt II). The bialaphos resistance marker (bar) was introduced into N. tabacum via Agrobacterium tumefaciens using vector pGV1500 carrying the bar gene phosphinothricin acetyltransferase. N. rotundifolia (npt II) was recovered after direct gene transformation of protoplasts by the pGP6 plasmid carrying the npt II gene for neomycin phosphotransferase. Both plasmids possessed 35S CaMV promotors. Hybrid selection was based on dual bialaphos – kanamycin resistance. AFLP analysis of regenerated plants showed the presence of species specific bands for both parents, which confirmed their hybrid nature. N. tabacum (bar) (+) N. rotundifolia (npt II) hybrids exhibited a great diversity in morphology. Fertile hybrids which possessed N. tabacum or N. rotundifolia morphology were recovered. Flow cytometric analysis revealed that the N. tabacum and N. rotundifolia - like hybrids had nuclear DNA contents near that of N. tabacum (9.40 ±0.24pg) or N. rotundifolia (5.29 ±0.36pg), respectively and were highly asymmetric. Other hybrids combined traits from the two species at various levels - N. tabacum habit or branched, similar to N. rotundifolia. Their leaves varied in shape. The flowers of the hybrid plants were of N. tabacum or N. rotundifolia type, or had N. rotundifolia dimentions, pink with N. tabacum corolla or white with curly fused petals. All were self sterile or male sterile. The male sterility was characterized by feminized anthers, anthers without pollen and the entire lack of anthers. The nuclear DNA content varied from 8.90 ± 0.30pg to 19.57 ±0.33pg. The data from the morphological and cytological analysis provided evidence that the parental chromosome elimination in the hybrid clones was spontaneous and not species - specific and that diploidization of the tobacco genome might have occurred in some clones during in vitro culture. This reflects the genomic incompatibility between the two species. ***V. Illcheva**, L. H. San, N. Zagorska, and B. Dimitrov, Production of Mal Sterile Insterspecific Somatic Hybrids Between Transgenic N. tabacum (BAR) and N. rotundifolia (NPTII) and Their Identification by AFLP Analysis, In Vitro Cellular and Developmental Biology – Plant 37: 496 – 502, 2001.*

Toyoki Kozai

Growth of In Vitro Banana

In vitro banana (Musa spp.) shoots were cultured under photomixotrophic (30 g l^{-1} sucrose and 0.2 h^{-1} number of air exchanges of culture vessels) and photoautotrophic (0 g l^{-1} sucrose and 3.9 h^{-1} number of air exchanges) conditions for 28 d in 370 cm3 Magenta boxes (GA7-type) containing 70 ml of half-strength Murashige and Skoog (MS) medium with 22.2 μM N6- benzylamino purine (BA). The effects of varying CO_2 concentration (475 or 1340 μmol mol^{-1}) and light intensity (PPF of 100 or 200 μmol m^{-2} s^{-1}) were investigated. Fresh and dry weights of banana shoots grown photomixotrophically were significantly greater on day 28 than those grown photoautotrophically. Photoautotrophic shoots had a larger number of unfolded leaves and greater leaf area than photomixotrophic plants by days 14 and 28, regardless of CO_2 concentration. The shoot fresh and dry weights on day 14 in photoautotrophic conditions were significantly greater at PPF of 200 μmol m^{-2} s^{-1} than at 100 μmol m^{-2} s^{-1}. The increase in net photosynthetic rate of photoautotrophic banana shoots was significant compared with photomixotrophic shoots. The multiplication ratio of *in vitro* banana shoots grown photoautrophically in a 28-d culture period was the greatest at 100 μmol m^{-2} s^{-1} PPF and 475 μmol mol^{-1} CO_2. *Quynh Thi Nguyen* and ***Toyoki Kozai***, *Growth of* In Vitro *Banana* (Musa spp.) *Shoots Under Photomixotrophic and Photoautotrophic Conditions, In Vitro Cellular and Developmental Biology – Plant, 37: 824 - 829, 2001.*

M. C. Christey

RI Mediated Plant Transformation

Agrobacterium rhizogenes–mediated transformation has been used to obtain transgenic plants in 89 different taxa, representing 79 species from 55 genera and 27 families. A diverse range of dicotyledonous plant families are represented, including one Gymnosperm family. In addition to the Ri plasmid, over half these plants have been transformed with foreign genes, including agronomically useful traits. Plants regenerated from hairy roots often show altered plant morphology such as dwarfing, increased rooting, altered flowering, wrinkled leaves and/or increased branching due to rol gene expression. These altered phenotypic features can have potential applications for plant improvement especially in the horticultural industry which such morphological alterations may be desirable. Use of A. *rhizogenes* and *rol* gene transformation has tremendous potential for genetic manipulation of plants and has been of particular benefit for improvement of ornamental and woody plants. ***M. C. Christey**, Invited Review: Use of Ri-mediated Transformation for Production of Transgenic Plants, In Vitro Cellular and Developmental Biology – Plant 37: 687 - 700, 2001.*

Vivanco and Bais

Root Specific Metabolism

The roots of higher plants comprise a metabolically active and largely unexplored biological frontier. Some of their prime features are the ability to synthesize a remarkably diverse group of secondary metabolites, and to adjust their metabolic activities in response to different abiotic and biotic stresses. This adjustment includes the ability to exude a wide array of micro- and macromolecules into the rhizosphere and to phytoremediate toxic metals, with the potential to affect and alter the relationships between plants and beneficial and deleterious soil-borne pathogens. In the past, research on root biology has been hampered by the underground nature of roots and the lack of suitable experimental systems to study the root-root and root-microbe communications. However, recent progress in growing roots in isolation with other elements of the rhizosphere has greatly facilitated the study of root-specific metabolism and contributed to our understanding of this remarkably active plant organ. ***Harsh Pal Bais**, Victor M. Loyola - Vargas, Hector E. Flores, and **Jorge M. Vivanco**, Root Specific Metabolism: The Biology and Biochemistry of Underground Organs, In Vitro Cellular and Developmental Biology – Plant 37: 730 - 741, 2001.*

G. Jayachandran

New EcR Isoform

The insect steroid hormone 20-hydroxyecdysone (20E) initiates a cascade of regulatory events in a temporal and tissue-specific manner by first binding to a complex of an ecdysone receptor protein (EcR) and ultraspiracle protein (USP). Using an antisense RNA approach, we show that disruption of EcR expression in transfected C7-10 cells from the mosquito Aedes albopictus affects survival and growth. From stably transfected cells, we recovered a new isoform of Aedes albopictus AalEcRa, which is named AalEcRb. The deduced amino acid sequence of AalEcRb was almost identical to that of AalEcRa, with the exception of a seven amino acid sequence near the C-terminus. Using PCR followed by restriction enzyme analysis, we found that AalEcRa is the predominant species expressed by wild type C7-10 cells, while cells transfected with antisense EcR expressed bothisoforms at approximately equal levels. ***G. Jayachandran** and A. M. Fallon, Antisense Expression of the 20-Hydroxyecdysone Receptor (EcR) in Transfected Mosquito Cells Uncovers a New EcR Isoform That Varies at the C-Terminal End, In Vitro Cellualr and Developmental Biology – Animal 37: 522 – 529, 2001.*

Classifieds

Assistant/ Associate Scientist, Winchester, KY, GenApps, a company dedicated to serving the needs of agriculture has a position immediately available for an Associate Scientist located in our Winchester, Kentucky facility. The qualified applicant should possess a M.S. degree or a B.S. degree with 3-5 years relevant working experience in biology/molecular biology, agricultural or related field. Three years laboratory experience emphasizing molecular biology required. Strong knowledge of and outstanding skills in a wide range of recombinant DNA techniques, gene expression systems, enzyme activity assays and plant tissue culture are desired.

Qualified applicant must be able to provide technical support under minimum supervision by planning and implementing individual duties and participating in interdisciplinary projects in support of overall company objectives. Must possess accurate oral and written communication skills, math knowledge associated with technical problems, computer capability, and the ability to perform assigned duties in varied environments including laboratory, greenhouse, and field. Responsibilities will include, but are not limited to, conducting assigned projects to meet department goals and providing assistance to Scientist to initiate new projects.

We offer competitive wages, excellent company benefits and the opportunity to become part of a dynamic organization. If you meet the above qualifications and wish to explore this opportunity further, please send your resume with salary history/ requirements and references to: Human Resources Coordinator, GenApps, Inc., 4262 Colby Road, Winchester, KY 40391

Research Scientist, **Sunseeds Co., Keiser, Oregon,** a Research Scientist position is immediately available at Sunseeds Co. Emphasis of the position will be on molecular marker systems, DNA fingerprinting, RAPDs and AFLPs. Experience with many types of molecular techniques, as well as with automated DNA sequencers and analyzers preferred. The position is open to Ph.D's or equivalent. Willingness to help on the many different types of projects in the lab, including tissue culture, when appropriate. Ability to work independently, but also cooperate with an international team. Competitiv salary and attractive benefits package co mensurate with experience. Sunseeds is a member of Aventis Crop Science world wide vegetable seed division.

Send letter of application, a detailed CV and three letters of reference to Sunseeds Co., PO Box 20672, Keizer, OR 97303.

Scholarships and Stipends, MS Graduate Program, Salem International University, Salem, WV, Scholarship and stipends are available in the Department of Bioscience, Salem International University, Salem WV for the MS Graduate Program in Molecular Biology and Biotechnology. A position is available to work on the genetic transformation of wetland monocots with novel genes with activity against specific metals and to develop a plant model for the study of metal remediation.

Interested individuals should submit official transcripts, cv and three letters of reference to Dr. S Rogers, Department of Bioscience, Salem International University, Salem, WV 26426-0500 Telephone 304-782-5585 FAX 304-782-5579 Make e-mail inquiries to Rogers@Salemiu.edu EOE/AA.

One PhD candidate & one Post Doctoral Research Associate Seeking one PhD candidate & one Post Doctoral Research Associate for a project which will examine the health beneficial phytochemicals in tomatoes, berries, and the phytochemicals produced in the counterpart in vitro suspension cultures of tomato and berry germplasm. The project involves in vitro production of carotenoids and flavonoids (anthocyanins and proanthocyanidins), phytochemical extraction and separation of component bioactive compounds, and bioactivity assays (antioxidant, cancer-chemopreventive).

Also seeking qualified MSc or PhD candidate for a project examining proanthocyanidin production in soybean cell cultures. Looking for candidates in the spring and summer semesters.

Please send inquiries and vitae/references to M.A.L. Smith via email initially, at imagemal@uiuc.edu.

2001 SIVB Student Awards

The following student awards were presented at the 2001 Congress on In Vitro Biology, St. Louis, Missouri. Information related to the available specific student awards can be found on the SIVB Web site (www.sivb.org) or by contacting the SIVB Office at (301) 324–5054, sivb@sivb.org or Dr. Gertrude Buerhing, Chair, Student Affairs & Awards, (510) 642-3870, buehring@ulink4.berkeley.edu.

H. Kobayashi

Hope E. Hopps Award

Extraction and detection of kavapyrones from in vitro cultures of kava (*Piper methysticum* Foster).

The roots and rhizomes of kava (Piper methysticum Foster), a South Pacific medicinal herb, are used as a phytomedicinal treatment for anxiety, tension, agitation, and insomnia. Slow maturity, sterility, and diseases threaten the supply of this medicinal herb. The objectives of this study are to develop kava micropropagation and kavapyrone production in vitro to support conventional kava production and future bioreactor-based production of kava phytomedicinals. Young, expanding leaves from greenhouse kava plants ('Awa' and 'Makea') were introduced to modified 1/2 Murashige and Skoog media containing Plant Preservative Mixture (PPM, 2.0 ml L^{-1}), and, in mg L^{-1}, 2,4-dichlorophenoxyacetic acid (2,4-D, 2.0), or a-naphthaleneacetic acid (NAA, 0.1) and N6-benzylaminopurine (BA, 0.5). Despite severe and persistent contamination, callus initiation subsequently occurred on media with 2,4-D after four weeks, and formation of protuberances resembling embryos were observed within two months. Root regeneration occurred after transfer of calli within one month to 1/2 MS media with NAA at 2.0 mg L^{-1}. High Performance Liquid Chromatography and Thin Layer Chromatography were utilized to examine the kavapyrone content of methanolic extracts of callus and regenerated roots from callus, along with roots of greenhouse plants. The amount of kavapyrones detected from the callus sample by HPLC was significantly less than that of kava roots from the greenhouse, while the amount of kawain from regenerated roots was comparable to that of roots in vivo on the TLC plate. *H. Kobayashi, Dept. of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801. E-mail: hkobayas@uiuc.edu. In Vitro Cellular and Developmental Biology 37:31-A, 2001.*

Cellular Toxicology Award

Three-Dimensional Transgenic Model for Genotoxic Assessment Using Macroporous Cultispheres.

Human exposure to space environment imposes genetic hazards that must be identified and alleviated. Currently, a model test system is needed that is representative of cellular interactions in tissue, and capable of quantifying genetic damage induced by low levels of space radiation and chemicals. We describe a three-dimensional, multi-cellular tissue-equivalent model, produced by culturing genetically engineered mammalian cells in a NASA-designed rotating wall bioreactor. Rat 2lambda fibroblasts, genetically engineered to contain high-density (>60 copies/cell) target genes for mutagenesis, were co-cultured with human epithelial cells on macroporous Cultispher-S beads. Light microscopy and histology were used to confirm cell attachment, distribution, and viability over the 8-day culture period. Key cell culture parameters (glucose, pH, and lactate) were monitored daily. Cells attached and completely covered the bead surface including the inner channels by day 4. Treatment of 4-day samples with dispase II dissolved the cultisphere and produced stable, bead-less spheroids. The spheroids were multi-cellular, had a well-organized extracellular matrix, and retained cell viability. The results suggest that stable multicellular spheroid models of uniform size can be produced in NASA bioreactors with genetically engineered cells for Earth-based studies as well as quantifying the potential health hazards attributed to the space environment. (Supported by NASA NRA-98-HEDS-02.)

D.N. FRAGA,University of Notre Dame, South Bend, IN, 46637. E-mail: Fraga.3@nd.edu. In Vitro Cellular and Developmental Biology 37:45-A, 2001.

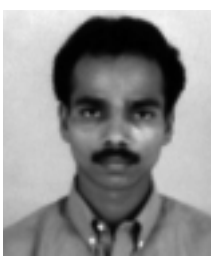
Bushra Sadia

Wilton R. Earle Award/Student Travel Award

Somatic Hybrids of Solanum tuberosum cv. Desiree and S. chacoense Bitt: A Baseline for Disease Resistance in Potato

Solanum chacoense, a wild tuber-bearing species ($2n=2x=24$), is resistant to potato cyst nematode, colorado beetle, common scab and bacterial wilt. It contains glycoalkaloids (leptines) associated with insect/disease resistance. In attempts to introduce these characteristics into tetraploid ($2n=4x=48$) cultivated potato (*S. tuberosum* cv. Desiree), mesophyll protoplasts of *S. chacoense* were electrofused with cell suspension protoplasts of potato, giving 12% heterokaryon formation. Cultured protoplasts of *S. chacoense*, did not produce colonies. However, in the same medium (MS with 1.25 mgl^{-1} NAA, 0.25 mgl^{-1} 2,4-D, 1.0 mgl^{-1} zeatin or 0.1 mgl^{-1} 2,4-D, 0.3 mgl^{-1} BAP), protoplasts of Desiree were totipotent. Selection of putative somatic hybrid tissues was based on heterosis, with such tissues exhibiting both purple and green pigmentation. Two hundred calli were obtained from 4 experiments; 75 vigorously growing putative hybrid calli were selected and transferred to regeneration medium (MS with 0.02 mgl-l NAA, 0.02 mgl^{-1} GA_3, 2.0 mgl^{-1} zeatin). After 16 weeks, tissues regenerated 24 plants with anthocyanin pigmented stems, a characteristic of *S. chacoense*. RAPD analyses indicated the hybridity of 9 plants after transfer to the glasshouse. Three of twenty four 10-mer primers tested showed the presence of parental DNA bands in these plants. The latter were intermediate in their vegetative, floral and tuber characters compared to both *S. chacoense* and *S. tuberosum*. After 4 months in the glasshouse, plants produced larger, red tubers similar to those of Desiree, compared to small white tubers of *S. chacoense*. As expected, regenerated plants were amphidiploids, with a chromosome complement of $2n=6x=72$. Somatic hybrid plants are being evaluated for their disease resistance.

B. SADIA, Plant Science Division, School of Biosciences, University of Nottingham, Nottingham NG7 2RD, UK. E-mail: mike.davey@nottingham.ac.uk. In Vitro Cellular and Developmental Biology 37:34A, 2001.



G. Franklin

Student Travel Award

High Efficiency Transformation of Egg Plant *(Solanum melongena L.)* by Agobacterium tumefaciens

An efficient method of producing transgenic eggplant *(Solanum melongena L.) via Agrobacterium-* mediated genetic transformation was developed. Explants were transformed by cocultivation with

Agrobacterial strain LBA4404. The strain harbours a binary vector pBAL2 carrying the reporter gene GUS intron (GUS-INT) and the marker gene neomycin phosphotransferase (NPTII). Transformation efficiency depends on an efficient regeneration system, in addition to other parameters. Hence regeneration potential of different explants was studied in detail and the most optimum concentration was used in the present study. Among the different combinations of TDZ and NAA tested, 0.1 mg/l of TDZ and 0.2 mg/l NAA in the medium influenced efficient regeneration of shoots *via* indirect organogenesis. Callus induction and shoot regeneration occurred subsequently in the same media. The frequency of transgenic calli formation was better with cotyledonary explants compared to leaf explants. Shoot buds elongated in the same media in 3-4 weeks time. The putatively transformed shoots were harvested and placed directly for rooting on soil (soilrite) watered with sterile water containing 100 mg/l kanamycin. Molecular analysis of the field established plants was carried to confirm the transgenic nature from the genomic DNA isolated. The presence of GUS and NPTII genes in the transgenic plants was verified by histochemical GUS assay and PCR analysis respectively. Integration of T-DNA into the genome of putative transgenics was further confirmed by Southern blot analysis. GUS hitstochemical assay was also positive in the T1 plants. A total of 124 transgenic plants were raised in pots and mature fruits were collected. Progeny analysis of these plants showed a pattern of classical Mendelian inheritance for both NPT11 and â-glucuronidase (GUS) gene expression. *G. FRANKLIN and G. Lakshmi Sita. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560 012, INDIA. Email: gfrank@mcbl.iisc.ernet.in. In Vitro Cellular and Developmental Biology 37: 22-A, 2001.*

Shirley Lin

Phillip White Award Winner

Ms. Lin would like to thank the society for rewarding her with the Phillip White Award. She was able to obtain the training opportunity by attending the 5th Canadian Plant Tissue Culture & Genetic Engineering Workshop in Saskatoon, May 12 - 15, 2001 and will be training in the area of producing doubled haploid plants through tissue culture and identifying the haploid plants using molecular technique in Dr. Julian Thomas' lab in Cereal Research Center, Agricultural Agri-Food Canada, Winnipeg, Manitoba, Canada. Dr. Thomas has extensive research experience in plant tissue culture and molecular genetics. Currently, she is working as a flax doubled haploid research technician in Morden Research Station, Agricultural Agri-Food Canada, Manitoba, Canada. Her research is focused on increasing the efficiency of anther / microspore culture and the percentage of doubled haploid plants. The Award was used to purchase round-trip airplane tickets from Winnipeg to Saskatoon, hotel, and registration fees of the Plant Tissue Culture Workshop as well as the training from Dr. Julian Thomas. Additional costs for the training will be covered through her supervisor's program, as the award will not be enough to cover the costs for all of her training.

2001 Congress on In Vitro Biology

ACKNOWLEDGMENTS

The Planning Committee acknowledges the contributions received from the following companies and organizations for their support of scientific and educational programs.

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Points To Ponder

The Stem Cell Controversy

The use of stem cells is so controversial that it took the historical events of September 11th to displace it from the front-page news. The topic remains sufficiently controversial that it is sure to once again erupt into a national debate. This Edition of Points to Ponder examines some of the pros and cons related to stem cells.

–Wayne Parrot

Research and Development Issues Facing Human Embryonic Stem Cell Therapies

Alison Venable and Steven Stice*
University of Georgia
Athens, GA
*Email sstice@arches.uga.edu

Human embryonic stem cells can be described as unspecialized, self-renewing cells that have the potential to form any of the 206 different cell types of the body. These cells are isolated from the inner cell mass, a ball of cells in a seven-day old embryo. The first human embryonic stem cells were isolated and propagated in 1998 [1], and thus sparked public interest due to the promise of this technology coupled with the legal and ethical issues that these cells raise. It is believed that human embryonic stem cells can one day ameliorate the debilitating effects of Parkinson's disease, multiple sclerosis, spinal cord injury, Alzheimer's, and other diseases in which functional cells could replace impaired cells and so there is an urgency to pursue areas of human embryonic stem cell research.

To get the cells to clinics many critical issues remain. Our experience and reports from Dr. Jamie Thomson [1, 2] indicate that both human and primate embryonic stem cells are highly prone to spontaneous differentiation. Thus, they must be managed carefully, nurtured manually on an extremely small scale. In addition, unstable human embryonic stem cells undergoing spontaneous differentiation are not ideal candidates to initiate uniform differentiation towards specific cell types such as neural lineages. Our goal is to develop a method of culturing a stable cell line (equivalent to primitive ectoderm) that can be directed homogenously down neural pathways rather than randomly differentiating down lineages that happen to include neural cells. It is our expectation that stepwise, directed differentiation to neural stem cells to dopaminergic neurons will yield a uniform population of cells suitable for testing in animal models of Parkinson's disease and then patients.

As we are poised at the beginning of this promising technology, it is important to not only conduct the basic research but also start addressing safety issues that are associated with stem cell research before beginning any replacement therapy. This article will briefly address some of the safety issues facing stem cells therapies as they move toward clinical trials.

It is important to have a multifaceted safety net to safeguard those that may receive cell therapy treatments. This means that all isolations of stem cell lines, culturing and maintenance of stem cells be conducted with high standards. The first human embryonic stem cell lines were isolated under the conditions that were proven effective for isolating mouse embryonic stem cells. The mouse embryonic stem cells required a mouse fibroblast feeder layer to provide nutrients and growth factors such as leukemia inhibitory factor to prevent spontaneous differentiation of the stem cells. Successful attempts in isolating any of the NIH-qualified human embryonic stem cell lines most likely have been achieved using a mouse fibroblast feeder layer. This could pose a potential risk to anyone receiving cell therapy as these cells have been in intimate contact with cells of another species and could potentially transfer viruses to the patient if the cells are used in clinical trials. Currently, research is underway in private sectors to maintain and potentially isolate human embryonic stem cells without using a mouse fibroblast feeder layer. It is likely that by the time cell therapies reach the clinics new cell lines proven free of animal viruses will be available.

Today bovine serum is often used in human embryonic stem cell culture media. Since the development of cattle in some countries other than the U.S. with a disease called Bovine Spongiform Encephalopathy (BSE), it is imperative that culture medium be made using serum derived from cattle that are certified-free of BSE and other disadvantageous agents. Alternatively, serum substitutes are being tested and refined for use in stem cell culture media. However, a serum substitute that completely eliminates all animal derived components is unlikely in the short term.

Another factor to consider while isolating stem cell lines is the quality and genetic history of resulting human embryonic stem cell lines. Obviously cardiac tissue transplants should be derived from human embryonic stem cell lines devoid of genetic defects that might impair cardiac function. So, genetic characterization of any isolated stem cells used for clinical treatment is important. Genomic, proteomic and others technologies will be important tools used to characterize stem cell lines.

Prior to use, the stem cells or the population of cells to be used in the actual cell therapy will be fully characterized. Uniformity of the cell population will be closely monitored including, morphology, expression of the cell surface antigens, karyotype analysis, and biological efficiency testing. It is possible for cells to spontaneously differentiate in culture to a specialized cell type, such as muscle or neurons, and the first detection would be checking the appearance of cells. Also, important would be testing the cells for surface antigens that are indicative of the desired cell type. Similarly, karyotype analysis should be performed on the stem cells frequently to determine any chromosomal abnormality that might result after substantial time in culture. Finally, the biological activity or functionality of the cells to be administered should be detected before cell therapy. For example, neurons that are to be generated for cell therapy should be checked initially for the secretion of the neurotransmitter desired.

The isolation of stem cells and the culture to desirable transplantable cell types must meet certain regulations and follow standardized practices in order to provide a strong foundation for embryonic stem cell-based therapies. This step will undoubtedly have certain issues to be addressed as well. For example, a transplanted cell type may to migrate to a non-target site, and depending on the interactions these cells have with neighboring cells, could possibly differentiate to a cell type that is inappropriate for that location. Likewise, the proliferative potential of the human embryonic stem cells is another characteristic that raises concern. It is known that human embryonic stem cells can develop into complex and unorganized growths, or teratomas, when transplanted into immunologically incompetent strains of mice[1, 2]. So, there is a risk of unregulated growth, meaning these cells could quite possibly result in tumor formation in a patient if subpopulations of the transplanted cells maintain the embryonic stem cells' pluripotent properties. Therefore, a homogenous population of step–wise differentiated stem cells will likely reduce the potential risks of teratomas.

So how do we test the safety and efficacy issues prior to clinical trials? As in the past, scientists will use animal models before testing of stem cell-derived lines in clinical trials. Since most human maladies do not exist in animals, methods involving chemical, surgical, and immunological procedures are implemented to damage neurons, induce diabetes, or stimulate heart attacks, stroke, hypertension, or compromise organ function. However, some animal models work quite well. For example, a rat model is used to study Parkinson's disease. The model is generated by stereotaxical administration of 6-hydroxydopamine in the striatum of the mouse brain. When taken up by the dopaminergic neurons, the 6-hydroxydopamine induces lesions. So, using this model it is possible for researchers to use differentiated human embryonic stem cells that are characterized as dopaminergic neurons and inject these into the lesioned region of the brain and the animals can be tested for reversal of physiological changes induced by the lesions. Then, the researchers can check the various uncertainties associated with human embryonic stem cell-based therapies.

While it is important to understand the risks associated with human embryonic stem cells to be used in therapies, it is certainly a field of research that will receive much attention in the decades to come. Scientists are just learning of the factors that control differentiation and maintain pluripotency, and there is still a wealth of knowledge waiting to be unveiled about the potential of these cells. It is with great anticipation that many researchers, patients, and others affected by these diseases, wait for the first clinical trials of injecting differentiated human embryonic stem cells into patients. However, precautionary measures need to be taken to ensure that stem cell therapy can be fully realized. Following standardized procedures in all areas of stem cell research should be paramount among researchers and physicians. Furthermore, society and especially patients affected by theses diseases need to be aware of the potential of this revolutionary science, but proper preclinical and clinical testing must be performed to ensure efficacy and safety of any stem cell therapy including embryonic stem cell therapies.

1. Thomson, J.A., J. Itskovitz_Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, and J.M. Jones, 1998 *Embryonic stem cell lines derived from human blastocysts*. Science. 282(5391): 1145-7.
2. Thomson, J.A. and V.S. Marshall, 1998 *Primate embryonic stem cells*. Current Topics in Developmental Biology. 38: 133-65.

2001 FUND FOR THE FUTURE CONTRIBUTORS

We would like to acknowledge with thanks the contributions made by our members for the future endeavors of the Society for In Vitro Biology.

The Scotts Company & Subsidiaries
UST, Inc.
Katalin G. Abraham
Helen M. Allison
Suad Al-Mazrooei
David W. Altman
Sara Amancio
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Herman Yeger
Edward C. Yeung
Shinso Yokota
James M. Ziegenmeyer

Future Meetings

SIVB MEETINGS

2002 – June 23-28, International Association for Plant Tissue Culture and Biotechnology (IAPTC&B), Orlando, FL. Contact the IAPTC&B Congress Secretariat, Society for In Vitro Biology, 9315 Largo Drive W, Suite 255, Largo, MD 20774, (301) 324-5054, fax (301) 324-5057, email: sivb@sivb.org
2002 – June 26-29, Congress on In Vitro Biology, Orlando, FL
2003 – May 31-June 5, Congress on In Vitro Biology, Portland, OR

OTHER MEETINGS

2002 – April 1-6, Cell Culture Engineering VIII, Snowmass, CO. For more information, contact (212) 591-7836, fax (212) 591-7441, or visit the conference website at http://www.engfnd.org/engfnd/2AC.html.
2002 - April 8 - 12, Society for Experimental Biology Annual Main Meeting, Swansea, Wales, UK. For more information contact Christine Trimmer at +44 (0) 207 439 8732, fax +44 (0) 207 287 4786, email c.trimmer@sebiology.org or visit the SEB website at http://www.sebiology.org

NE Chapter News

Currently the chapter is "quiescent". Our last main event was held on April 22, 1999, with a presentation by Dr. Robert Farrell of Penn State who spoke on current RNA methodologies. Since the response to that presentation was minimal, we have not organized other similar events. The chapter has, and will continue to cosponsor, events in conjunction with the Pace University chapter of Sigma Xi, which include our annual Bioethics Forum (November 9, 2001) and annual satellite (downlinked) presentation of the Howard Hughes Medical Institute "Holiday Lecture Series" in December 2001. We welcome input from any prior members of the NE Branch with regard to revitalizing the chapter.

Carl Candiloro, *Chapter President*

Invertebrate Section Plans Program for Orlando

The 2002 Congress on In Vitro Biology at the *Disney's Coronado Springs* Resort, Florida, will feature an Invertebrate Symposium "In Vitro Approaches to Production of Marine-derived Drugs" convened by **Shirley Pomponi**, **PhD**, Harbor Branch Oceanographic Institution. A Joint Invertebrate / Vertebrate / Toxicology Symposium on Stem Cells and Organogenesis is being convened by **Raziel S. Hakim, PhD**, Howard University, and **Nam-ho Huh, MD**, Okayama University. Other symposia planned: joint Vertebrate/Toxicology Symposium, "Regulatory Status and Funding for Stem Cell Research" convened by **Janis L. Demetrulias**, MS Technikos Research Associates, Vertebrate Symposium, "NASA Research" convened by **Neal R. Pellis, PhD**, NASA-Johnson Space Center, two Toxicology Symposia, "Tissue Engineering" convened by **Gordana Vunjak, PhD**, MIT, and **Jonathan Garlick, PhD**, SUNY, Stony Brook, and "Assessing the Predictive Capacity of In Vitro Tests" convened by **Leon Bruner, PhD**, Gillette Medical Evaluation Laboratories. The Distinguished Plenary Speaker will be **Dr. Gordon Sato,** Department of Fisheries, Asmara, Eritrea who will speak on "The Manzanar Project: Contributions of In Vitro Biology, Tissue Engineering, Proteomics, and Beyond". Shirley Pomponi will introduce **Dr. Sheldon Schuster**, Director, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville. His Plenary Talk is entitled "Research and Development in the Biotechnology Industry: Outlook for the Future".

Guido Caputo, *Secretary*, *Invertebrate Section*

"Cool Technologies" at the Next Congress

Want to learn more about confocal microscopy, bioinformatics, proteomics, or DNA and RNA microarrays? During the 2002 Congress on In Vitro Biology, we are planning special tutorials and demonstrations to be presented by companies or technical experts that may include these, as well as other, new and exciting technologies. More in depth then just visiting an exhibitor's booth, it is hoped that these workshops will provide attendees with a basic understanding of the latest technologies to aid them in their experimental endeavors. If you have any questions or suggestions for this fun event, please contact either Lia Campbell (lcampbell@organ-recovery.com) or Cindy Goodman (goodmanc@missouri.edu), 2002 Program Co-Chairs.

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Title of Publication: *In Vitro Report*
Publication No. 1077-3975
Date of Filing: October 31, 2001
Frequency of Issue: Quarterly
No. of Issues published annually: 4
Annual subscription price: $55.00
Complete mailing address of publication: 9315 Largo Drive West, Suite 255, Largo, Maryland 20774 (Prince George's County)
Complete mailing address of the headquarters or general business offices of the publishers: Society for In Vitro Biology, 9315 Largo Drive West, Suite 255, Largo, Maryland 20774.
Full names and addresses of publisher, editor, and managing editors:
PUBLISHER: Society for In Vitro Biology, 9315 Largo Drive West, Suite 255, Largo, Maryland 20774.
EDITOR: Martha S Wright, 7612 West 149th Street, Overland Park, KS 62223.
MANAGING EDITOR: Michele Schultz, 9315 Largo Drive West, Suite 255, Largo, Maryland 20774.
Owner: Society for In Vitro Biology, 9315 Largo Drive West, Suite 255, Largo, Maryland 20774—No stockholder owning or holding one percent or more of total amount of stock.
Known bondholders, mortgagees, and other security holders owning or holding one percent or more of total amount of stock: None
The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes have not changed during the preceding 12 months.

Publication Name: *In Vitro Report*
Issue date for circulation date: July-September 2001
Extent and Nature of Circulation:

	Avg. No. Copies Ea. Issue During Preceding 12 Mos.	Actual No. of Copies of Single Issue Nearest to Filing Date
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g. Total Distribution *(Sum of 15c and 15f)*	1106	1053
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i. Total *(Sum of 15g, 15h[1], and 15h[2])*	1500	1500
j. Percent Paid and/or Requested Circulation (15c/15g x100)	92%	91%

16. Publication is required. Statement of Ownership will be printed in the October – December issue of this publication.
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In Vitro Report

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IN VITRO REPORT (ISSN 1077-3975) is published quarterly, one volume per year, by the Society for In Vitro Biology (SIVB) 9315 Largo Drive, West, Suite 255, Largo, MD 20774. The subscription nonmember rate is $40 per volume ($55 outside the USA). payable in advance in U.S. funds drawn on a U.S. bank. SIVB members receive IN VITRO REPORT as part of annual dues. Claims of nonreciept should be made within 6 months of publication. Issues claimed after 6 months may be purchased as back issues and should be directed to IN VITRO REPORT. Periodicals postage paid at Upper Marlboro, MD, and additional mailing offices. POSTMASTER: SEND ADDRESS CHANGES TO IN VITRO REPORT, 9315 Largo Drive, West, Suite 255, Largo, MD 20774.