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Plenary Symposia

PS-1

Cytoplasmic and Nuclear Trafficking of Plasmids During Gene Delivery. DAVID A. DEAN. Departments of Pediatrics and Biomedical Engineering, University of Rochester, Rochester, NY 14642. Email: david_dean@urmc.rochester.edu

The success of viral and non-viral gene delivery relies on the ability of DNA-based vectors to traverse the cytoplasm toward the nucleus and cross the nuclear envelope into the nucleus, the site of transcription in the cell. While the dense latticework of the cytoskeleton impedes free diffusion of large macromolecules, including DNA, transfections do work, and consequently there must be mechanisms by which DNA moves toward the nucleus. We and others have shown that plasmids utilize the microtubule network and its associated motor proteins to traffic toward the nucleus. We have identified a set of proteins that interact with the DNA to form protein-DNA complexes that mediate this trafficking and that into the non-dividing nucleus. While studying the effects of mechanical strain on the transfection process, we discovered that cyclic stretch causes large scale reorganization of the microtubule cytoskeleton and induction of a pool of stabilized microtubules that are post-translationally acetylated and which lead to increased gene delivery to the nucleus. Using a variety of approaches, we have found that by increasing the levels of acetylated microtubules we can increase gene transfer, not at the level of transcriptional activation, but rather at the level of cytoplasmic trafficking toward the nucleus. Taken together, these results suggest that modulation of the microtubule network and the formation of protein-DNA complexes can increase the efficiency of gene transfer.

PS-2

Gene Delivery. R. HELLER[1,2], B. Marrero[2], D. Jackson[1], and S. Beebe[1]. [1]Frank Reidy Research Center for Bioelectrics, Old Dominion University, 830 Southampton Ave, Norfolk, VA 23510 and [2]Department of Molecular Medicine, University of South Florida, College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612. Email: rheller@odu.edu

Gene medicine has held great promise for effective treatment of a variety of disorders including cancer, metabolic disorders, cardiovascular diseases and genetic disorders as well as a potential prophylactic approach for infectious diseases and cancer. One of the critical aspects of gene transfer is effective delivery of the material to the appropriate target. A key factor in the development of therapeutic protocols is to achieve the appropriate expression levels and kinetics to achieve the desired effect. This requires delivery of the gene to the appropriate target cells. Both biological (viral) and non-viral approaches have been evaluated. There are positive and negative attributes to both approaches. A major drawback of non-viral is inefficient expression of the transferred gene. This is typically related to the inability of the plasmid DNA to enter the cellular target. Electrically mediated delivery (electroporation) of plasmid DNA is an efficient non-viral delivery approach that allows better control over expression profile. Careful selection of tissue target and delivery parameters including electrode and electrical conditions allows an investigator to obtain the type of transgene expression necessary for a particular therapeutic application. The use of electric fields in the micro- or millisecond range can facilitate transport of the plasmid across the cell membrane. It may also be possible to use ultra-short pulses in the nanosecond range to facilitate uptake in the nucleus. Our group is studying the combination of these types of pulses to enhance delivery and expression. This approach was evaluated in HaCat cells as well as tumor spheroids made of co-culture of B16.F10 and HaCat cells.

PS-3

Motivating Gene Targeting: How an Understanding of the Basic Mechanisms and Regulation of Recombination May Yet Lead to Efficient Gene Replacement in Higher Plants. ANNE BRITT[1], Tomoyuki Furukawa[1], Fyodor Urnov[2], and Bryan Zeitler[2]. [1]Dept. Plant Biology, U. C. Davis, Davis, CA and [2]Sangamo Biosciences, Richmond, CA. Email: abbritt@ucdavis.edu

Yeast cheerfully perform gene targeting; higher plants refuse to do the same. Here we will discuss some possible explanations for the remarkably low rate of homology-dependent T-DNA transformation (perhaps 1/3000 homology-independent events) in Arabidopsis, and what steps can be taken to create a more rational protocol for gene targeting in this organism. We

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also will discuss a variety of approaches already taken—with varying degrees of success—to promote gene replacement in higher plants. These include: the addition of flanking counter selectable markers, the manipulation of host DNA repair pathways, and the promotion of target site recombination through the use of custom designed zinc-finger endonucleases.

PS-4

Zinc Finger Nuclease-Mediated Gene Targeting. JOSEPH F. PETOLINO. Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: jfpetolino@dow.com

Targeted modification of plant genomic sequences enable basic studies of gene function and could enhance the development of new crop varieties. Recently, designed zinc finger nucleases have been shown to mediate the formation of site-directed double strand breaks thereby facilitating naturally-occurring cellular repair mechanisms. Non-homologous end joining appears to be the most common means of double strand break repair in plants - often resulting in deletions, i.e., targeted mutations, at the cleavage site. In addition, providing cells with exogenous DNA comprising regions of sequence homology to the cleavage site has resulted in homology-directed repair of the double strand break, including targeted transgene integration and gene editing. Given that zinc finger nucleases can be designed to recognize a wide range of target DNA sequences, novel approaches for targeted genome modification are now available.

PS-5

Introduction to Research in Systems Biology. J. A. JORDAN. Department of Natural Sciences, Clayton State University, Morrow, GA. 30260. Email: jacquelinejordan@clayton.edu

For many years, researchers focused on understanding the biological response of individual genes and proteins. An important investigative tool in science is reducing complex systems like the cell into simpler components that are easier to study. Many critical scientific questions have been answered using this common reductionism strategy. A key limitation of reductionism is the study of novel properties that emerge following complex interactions between cellular components, tissues, and organs. The goal of systems biology is construct models of the whole body system. The sequencing of the human genome generated massive amounts of data. New tools became necessary to process this information and study the functions and complex interactions of genes and proteins. The systems biology approach allows scientists to predict how a change in one or more genes/proteins may affect the entire system. This has lead to the "omics" revolution and the use of key tools like high-throughput technology and bioinformatics to model the whole body system.

PS-6

Beyond Gene Clustering of Microarray Data. W. JIM ZHENG[1], Linyong Mao[1], Omar Moussa[2,3], John S. Yordy[2,3], Suiquan Wang[3], Andrew S. Kraft[3], and Dennis K. Watson.[2,3]. [1]Department of Biostatistics, Bioinformatics and Epidemiology; [2]Departments of Pathology and Laboratory Medicine and Biochemistry and Molecular Biology; and [3]Hollings Cancer Center, Medical University of South Carolina, Charleston, SC. Email: zhengw@musc.edu

Microarray data analysis has advanced from identifying gene clusters that share similar expression patterns to understanding the transcription regulatory pathways that manifest such changes. Mechanistic insight can be provided by combining comparative genomics and de novo motif discovery to significantly reduce genomic noise, allowing identification of sequence motifs potentially responsible for transcriptome changes. Modular and flexible web based application built on this combined approach can provide biologists with an easy to use tool to allow in depth analysis of their microarray data. We have developed a web application, MDframe, for the identification of human regulatory motifs by combining transcriptional profiling, comparative genomics and de novo motif discovery. MDframe has a modular design and integrates comparative genomics with the de novo motif discovery algorithm based on the exhaustive pattern enumeration technique. MDframe is a flexible tool that has been extensively validated by well-characterized data set. While applied to analyze genes regulated by Ets transcription factors, not only Ets binding motifs, but also the motifs for Ets interacting proteins can be identified by MDframe. Moreover, MDframe has been applied to analyze transcription regulation of gene clusters identified from microarray experiments. The discovered Ets binding motifs have been demonstrated to be occupied by Ets proteins in vivo using Chromatin Immunoprecipitation (CHIP) assay. The web interface of MDframe can be freely accessed at: http://genomebioinfo.musc.edu/MDframe.html.

PS-7

Integrating High-Throughput and Genomics Technologies in Least Expected Places. M. KODANI. Centers for Disease Control and Prevention, Atlanta, GA 30333. Email: ibt1@cdc.gov

Recent developments in high-throughput nucleic acid extraction, real-time PCR, and microarray technology allow

for faster and more efficient ways of performing large-scale experiments. Advances in nucleic acid extraction and automation allow for rapid extraction of nucleic acids from many tissues, cells, formalin-fixed paraffin-embedded blocks, viruses, and bacteria. Depending on the nature of the experiment, expression and gene detection studies can be done on a large scale using microarray technologies, or on a small scale using real-time reverse transcription technologies. Experiments can be broad, generating expression differences within the entire genome, or they can be focused on an area of interest, such as cancer or apoptosis, or cell-cell signaling. Customized array experiments, tailored to specific needs of different laboratories, have become more common. We are developing a tool for detection of multiple pathogens in respiratory illness outbreak samples. Our TaqMan low density array (TLDA) cards were designed to screen a panel of 21 different microorganisms, including both viruses and bacteria. The respiratory panel TLDA cards are currently being evaluated for specificity and analytical sensitivity. The TLDA cards allow investigators to fully customize experiments in which multiple assays are performed on a large number of samples.

PS-8

Metabolomics and Integrated Functional Genomics Reveal Novel Information Related to Medicago Secondary Metabolism. LLOYD W. SUMNER[1], Mohamed A. Farag[1], Corey D. Broeckling[1,2], David V. Huhman[1], Ewa Ubanczyk-Wochniak[1,3], Wensheng Li[1,3], Mohamed Bedair[1], Zhentian Lei[1], Bonnie S. Watson[1], Marina Naoumkina[1], Bettina Deavours[1,2], and Richard A. Dixon[1]. [1]The Samuel Roberts Noble Foundation, Ardmore OK; [2]Colorado State University, Ft. Collins, CO; and [3]Monsanto Company, St. Louis, MO. Email: lwsumner@noble.org

Legumes are a fundamental food source for most global cultures due to their substantial protein content achieved through symbiotic nitrogen fixing rhizobia. Legumes also produce an array of natural products that have a substantial impact upon mutualism as well as plant disease/defense. Particularly important are the flavonoids, isoflavonoids, and saponins which serve as key signaling molecules in plant-microbe interactions, as primary defense compounds, and increasingly as important neutraceuticals and pharmaceuticals. *Medicago truncatula,* a close relative of the premium and global forage legume alfalfa (*Medicago sativa*), is an ideal model organism for the study of legume biology due to the availability of a near complete genome sequence and a 61,200 probe set Affymetrix GeneChip for DNA microarray analyses. Unfortunately, the application of such molecular tools to the study of plant secondary metabolism is currently limited by deficient and/or inaccurate annotation of secondary metabolic enzymes and by the incomplete knowledge of the full secondary metabolic composition of plants. However, the union of non-targeted metabolite profiling i.e. metabolomics with parallel analysis of the transcriptome and/or proteome, provides a powerful integrated platform for the discovery and hypothesis driven assessment of metabolic networks and the in vivo functions of biosynthetic genes. This presentation will provide multiple examples of how metabolomics and integrated functional genomics are yielding novel discovery and mechanistic insights related to Medicago secondary metabolism and its role in stress and plant-microbe interactions.

PS-9

Towards a Systems Analysis of Plant Molecular Networks. SIXUE CHEN[1,2], Mengmeng Zhu[1], Yan He[1], Qiuying Pang[1], Shaojun Dai[1], Ning Zhu, Linda Abraham[1], and Johanna Strul[1]. [1]Department of Botany and Zoology, University of Florida, Gainesville, FL 32611 and [2]Interdisciplinary Center for Biotechnological Research, University of Florida, Gainesville, FL 32610. Email: schen@ufl.edu

Plant molecular networks include signal transduction networks and metabolic networks. Our knowledge about how different molecules and pathways connect and relate to each is very limited. With the advances made in genomics and functional genomics that includes transcriptomics, proteomics and metabolomics, research in systems analysis of plant molecular networks becomes possible. In this seminar, our recent progress on developing and implementing proteomics and targeted metabolomics tools to elucidate guard cell ABA signaling networks and glucosinolate metabolic networks will be reported. The future directions of mapping proteins and metabolites onto pathways and integrating into molecular networks will be discussed.

PS-10

Interaction of Global Regulatory Approaches and Testing Methodology with Trade. R. SHILLITO. BioAnalytics Dept., Bayer CropScience LP, Research Triangle Park, NC 27709. Email: ray.shillito@bayercropscience.com

As agricultural crops developed using modern biotechnology (GM) become more widely adopted worldwide, their presence in global trade increases proportionally. Differences in the global acceptance of these crops, in the approaches taken by regulatory agencies worldwide, and in country labeling policies have implications for international trade. To meet with differing requirements seeds and agricultural products (including foods) may require testing for the

presence of biotech material or the customer may require confirmation that presence of biotechnology products is below a certain threshold to avoid labeling the end-product. In addition, testing for specific events may be required to exclude the presence of non-permitted materials. These factors can translate into specific testing needs at different points in the product value-chain which in turn leads to the application of a variety of different approaches to testing. These range from immunochemically based tests (lateral flow strips, ELISA), to qualitative and quantitative PCR. A requirement for such testing is that the methods must give comparable results in the originating and the receiving countries. This talk will cover the way in which such testing may be applied at each point in the chain, the practical limits on various types of testing, international efforts at harmonizing methods, and how testing methods relate to, and comply with the different regulatory and labeling regimes.

PS-11

Safety Assessment of Plant-Incorporated Protectants (PIPs) by the United States Environmental Protection Agency. J. L. KOUGH, Biopesticides and Pollution Protection Division, Office of Pesticide Programs, USEPA, Washington, DC. Email: Kough.John@EPA.gov

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act (FFDCA), the Environmental Protection Agency registers all pesticides sold in the U.S. The advent of plant transformation technology has led to the production of transgenic plants with new pesticidal properties. These biopesticides are termed Plant-Incorporated Protectants (PIPs). This category includes plants produced by introduction of traits that would not normally be available by traditional plant breeding methods with sexually compatible relatives. An example would be the integration of bacterial gene sequences into corn for insect resistance. Prior to registration, each PIP is examined to decide what data are needed to insure that a reasonable certainty of no harm to man and the environment will result from its use. In general, details of the gene construct and expression levels of the introduced pesticidal substance, as well as information on the digestibility and allergenicity of proteins produced and toxicity profiles for humans and non-target species are requested from the registrant.

PS-12

Animal Clones and their Progeny, FDA's GE Animal Guidance, and Other Recent Actions. J. JONES. Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD. Email: jeffery.jones@fda.hhs.gov

Animal biotechnology has long been an area of research interest. Recently, somatic cell nuclear transfer (SCNT or animal cloning) and the genetic engineering of animals have moved from theoretical discussions or limited laboratory utilization to broader use and potential commercialization. To provide structure and guidance to the field as well as to protect the public health, the Food and Drug Administration has recently released documents describing our scientific and regulatory approach to these technologies. Animal Cloning: A Risk Assessment and associated communications documents were released January 8, 2008 to provide the agency's assessment of the scientific concerns associated with consumption of food from animal clones and their progeny, and to provide some context to the public in understanding the technology. Guidance for Industry #187 - Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs was released January 15, 2009 to clarify the agency's legal and regulatory authorities to regulate rDNA constructs in GE animals, as well as to help industry understand their responsibilities and obligations under the Federal Food, Drug, and Cosmetic Act (FFDCA), and the National Environmental Policy Act (NEPA), as they apply to these animals. In this presentation, we will outline both approaches, and briefly describe the scientific underpinning of the regulatory approaches taken.

PS-13

GMAX Yeast Background Strain Made from Industrial Tolerant *Saccharomyces cerevisiae* Engineered to Convert Sucrose, Starch, and Cellulosic Sugars Universally to Ethanol Anaerobically with Concurrent Coproduct Expression. STEPHEN R. HUGHES. USDA/ARS/NCAUR/BBC, 1815 North University Street, Room 1106, Peoria, IL 61604. Email: Stephen.Hughes@ars.usda.gov

Tailored GMAX yeast background strain technology for universal ethanol production industrially: Production of the stable baseline glucose, mannose, arabinose, xylose-utilizing (GMAX) yeast will be evaluated by taking the genes identified in high-throughput screening for a plasmid-based yeast to utilize xylose and glucose anaerobically for ethanol production. The use of the xylose isomerase gene from *Piromyces* in combination with a modified xylulokinase gene from bacteria plus the anaerobic growth genes from *S. cerevisiae* allowed anaerobic growth on xylose and glucose simultaneously for cellulosic ethanol production and the gene set to be used as the basis for the background strain for other co-product gene expressions such as a lipase, antibacterial, and or an insecticidal gene set. The

resulting stable transformed plasmids into any industrial yeast strains of *Saccharomyces cervisiae* that are already tolerant to environments in the production biorefinery are being developed for universal ethanol production from any feedstock provided. Initially a cellulosic strain will be produced with XI, XKS, and one or more of the anerobic xylose utilization genes to be discussed for use on acid or base hydrolysates for high level production of cellulosic ethanol and use as a platform for coproduct production.

PS-14

Lowering the Cost of Biomass Conversion Through Expression of Cell Wall-degrading Enzymes in Transgenic Plants. SCOTT BETTS, Stacy Miles, Martin Allen, Sergio Arellano, Nateefa Wade, Bruce Link, Jason Nichols, Myoung Kim, Paul Oeller, and Simon Warner. Syngenta Biotechnology, Inc., 3054 Cornwallis Rd., Research Triangle Park, NC 27709. Email: scott.betts@syngenta.com

Although numerous processes have been described for the conversion of cellulosic biomass to bioethanol, none of them have proven to be commercially viable to date. Cell-wall degrading enzymes, or cellulases, are used in many of these processes to hydrolyze pretreated biomass feedstocks, such as corn stover and sugarcane bagasse, to soluble and fermentable sugars. The most widely used and best characterized cellulases are those secreted by *Trichoderma reesei*, a filamentous fungus. The major *T. reesei* cellulases are the exo-cellulases (CBHI, CBHII) and endoglucanase (EG). However the cost of producing sufficient quantities of fungal cellulases at the current minimum dose of tens of grams of enzymes per liter of ethanol produced remains prohibitively expensive and a major impediment to commercialization. The expression of these enzymes *in planta* has not been without significant challenges, as plants are composed of cellulose and many enzymes require post translational modification in order to achieve maximum functionality. Nevertheless, Syngenta has achieved high levels of expression of enzymes representing the exo-cellulase and endoglucanase classes. This plant based enzyme expression technology could offer significant economic advantages over fermented enzymes. Here we demonstrate the expression of heterologous CBHI, CBHII, and EG enzymes in transgenic corn. We also present evidence that the corn-expressed CBHI substitutes efficiently for the corresponding fungal-expressed enzyme in the hydrolysis of pilot-scale pretreated sugarcane bagasse.

PS-15

Brachypodium distachyon: a New Model for Biomass Crops. J. VOGEL[1], K. Mayer[2], D. Rokhsar[3], J. Schmutz[4], T. Mockler[5], N. Huo[1], J. Bragg[1], J. Wu[1], Y. Gu[1], D. Garvin[6], and M. Bevan[7]. [1]USDA-ARS Western Regional Research Center, Albany, CA; [2]MIPS, Helmholz Zentrum Munich, GERMANY; [3]DOE Joint Genome Institute, Walnut Creek, CA; [4]Hudson Alpha Institute of Biotechnology, Huntsville, AL; [5]Oregon State University, Corvallis, OR; [6]USDA-ARS Plant Science Research Unit, Univ. of Minnesota, St. Paul, MN; and [7]John Innes Centre, Norwich, UK. Email: john.vogel@ars.usda.gov

Brachypodium distachyon (Brachypodium) is rapidly emerging as a model system to study questions unique to the grasses. This emergence is coincident with an increased need for basic research in grass biology to develop perennial grasses as a source of renewable fuel. The list of genomic resources available to Brachypodium researchers is increasing exponentially. We recently completed the sequencing and analysis of the complete genome. The final assembly and annotation is of extremely high quality and promises to be a powerful research tool. Other resources we have developed include: a highly efficient transformation system, a high density SNP-based genetic map, a physical map, BAC libraries and BAC end sequences, EST sequences, and mutagenesis protocols. In addition, the generation of a sequence-indexed insertional mutant population is underway with >3,000 mutants generated to date. When taken together, these resources enable researchers to utilize Brachypodium for a wide array of experimental approaches, including those that require complete genome sequence. An overview of the *Brachypodium* genome project, *Brachypodium* resources and the identification of mutants relevant to biomass crops will be presented.

PS-16

Switchgrass (*Panicum virgatum*) Transformation for Altered Cell Wall Biosynthesis in the DOE Bioenergy Science Center. C. NEAL STEWART, JR.[1], Laura G. Abercrombie[1], Holly Baxter[1], Jason Burris[1], Fang Chen[2], Richard A. Dixon[2], Raymond Equi, III[1], Mathew Halter[1], Hiroshi Hisano[3], Sakae Hisano[2], Zachary King[4], Peter Lafayette[4], David Mann[1], Mitra Mazarei[1], Rangaraj Nandakumer[3], Richard S. Nelson[2], Wayne A. Parrott[4], Charleson Poovaiah[1], Hema Ramanna[2], Mary Rudis[1], Zeng-yu Wang[3], Janice Zale[1], Jennifer S. Hawkins[5], Ryan Percifield[5], and Jeffrey L. Bennetzen[5]. [1]Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996; [2]Plant Biology Division, Noble Foundation, Ardmore, OK 73401; [3]Forage Improvement Division, Noble Foundation, Ardmore, OK 73401; [4]Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602; and [5]Department of Genetics, University of Georgia, Athens, GA 30602. Email: nealstewart@utk.edu

The Bioenergy Science Center (BESC) is one of three bioenergy centers created by the US DOE in 2007, and is focused on decreasing the recalcitrance of cellulosic feedstock for conversion into fuel. Biotechnology of switchgrass is important in screening potential cell wall biosynthesis genes, which in BESC is being performed by five laboratories in three institutions. Accomplishments include altering lignin biosynthesis, improved tissue culture and transformation systems, creation of a virus induced gene silencing (VIGS) system, a protoplast system, and a new vector set for monocot transformation. A transgene pipeline committee identifies transgenes to be evaluated (overexpression or knockdown) in switchgrass. To evaluate gene function while waiting for stable transformants in switchgrass, we are utilizing VIGS, which gives transient knockdown expression data. Our VIGS system uses a Brome mosaic virus vector, which infects foxtail millet (Setaria italica), a close relative of switchgrass. If a recalcitrance-altering phenotype is shown in VIGS, stable transformants are produced for that gene. One example is in the lignin biosynthesis pathway, in which we have produced stable transformants that are currently being evaluated. In order to coordinate gene expression and to facilitate more rapid screening of genes, we have developed a Gateway-compatible monocot transformation vector set for overexpression (high or low), RNAi, visible and selectable markers. BESC has facilitated coordination of expertise and duties to perform research in switchgrass biotechnology that would have been impossible in any individual laboratory.