In Vitro Cellular & Developmental Biology - Animal (2019) 55 (Suppl 1):S2–S5
https://doi.org/10.1007/s11626-019-00353-4

Plenary Symposia

PS-1

Beyond Gene Editing: Current Status and Future Applications of Synthetic Biology in Plants. C. NEAL STEWART, JR. Department of Plant Sciences and the Center for Agricultural Synthetic Biology, University of Tennessee, Knoxville, TN. Email: nealstewart@utk.edu

Synthetic biology is still a nascent area in plant biology. Synthetic biology can be defined simply as applying engineering principles to biology. It relies on designing, building, and testing components, just like engineers do, when making a device. In our case the device is a modified plant consisting of new 'parts.'The parts are mostly made of DNA. They are genes, gene expression regulators, parts of genes, or, collections of genes. So far, the biggest application of synthetic biology in plants is gene editing using CRISPR systems–essentially 'rewriting' genes– but my goal is to extend the full power of synthetic biology into 'writing' DNA at a large scale into genomes. Theoretically the 'writing' can result in the entire genome of plants, which is far beyond what is currently feasible. The best I've hoped for is to rewrite the chloroplast genome of 100 genes: a synthetic chloroplast genome or "synplastome." I will discuss the work of my group toward designing, building and installing the first synplastome in plants as well as mini-synplastomes.

PS-2

Developing Plant Synthetic Biology Tools for Complex Metabolic Engineering. PATRICK M. SHIH[1,2,3], Amy Calgaro-Kozina[4], Khanh Vuu[2,3], Jay D. Keasling[2,5,6,7], Dominique Loqué[2,3], and Elizabeth S. Sattely[4]. [1]Department of Plant Biology, UC Davis, Davis, CA; [2]Joint BioEnergy Institute, Emeryville, CA; [3]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, One Cyclotron Road, Berkeley, CA; [4]Department of Chemical Engineering, Stanford University, Stanford, CA; [5]Department of Chemical and Biomolecular Engineering. UC Berkeley, Berkeley, CA; [6]Department of Bioengineering. UC Berkeley, Berkeley, CA; and [7]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, One Cyclotron Rd., Berkeley, CA. Email: pmshih@ucdavis.edu

Plants produce a wealth of biologically active compounds, and thus a major impetus for studying plant natural product biosynthesis has been the goal of elucidating and harnessing these metabolic pathways as a means to produce molecules of interest. The field of synthetic biology has opened the door not only to the engineering of new metabolisms into heterologous hosts, but also the designing of novel pathways that are not known to exist in nature. Here, we expand the biological repertoire of plant metabolism by taking a "plug-and-play" approach to rationally engineer synthetic pathways for the production of target non-natural compounds. As future endeavors in plant metabolic engineering are becoming increasingly complex, more sophisticated methodologies will be required to deliver on such ambitions. We have developed a number of plant synthetic biology tools to enable the coordinated expression of multiple transgenes simultaneously in order to ultimately facilitate the introduction of these synthetic pathways into relevant crop species.

PS-3

Profiling the Diversity and Development of Human Organs and Organoids at Single Cell Resolution. JASON SPENCE. Department of Internal Medicine, Gastroenterology; Department of Cell and Developmental Biology; Department of Biomedical Engineering; and Center for Organogenesis, University of Michigan Medical School, Ann Arbor, MI. Email: spencejr@med.umich.edu

Human pluripotent stem cell (hPSC)-derived organoids have provided researchers with routine, on demand access to live human tissues. As a proxy for naturally derived human tissues, it is critical to understand how well these models recapitulate the native tissue, and where possible, improve the resemblance of organoid models to the native tissue. For example, previous work has shown that hPSC-derived intestinal organoids (HIOs) are immature, and upon xenotransplantation

into a host mouse, the HIOs mature at the morphological level where they develop a complex crypt-villus architecture, at the cellular level where they differentiate a complex repertoire of intestinal cell types, and at the molecular level where they express genes associated with the mature intestine. However, it is still unclear how well HIOs recapitulate the diversity of cell types in the human intestine. In pursuit of better understanding how well organoids recapitulate native human tissue, we used single-cell RNA-seq to derive a high-resolution cell atlas of human fetal organs, and corresponding organoids. Using this data, we have carried out a comparative analysis of all developing and mature cell types both from native and hPSC-derived human intestine and we identify and validate novel cell populations in both settings. Further, we identify populations that are lacking from HIOs, and make inferences about epithelial and mesenchymal lineage decisions during human development.

PS-4

Enhanced Gene Delivery Utilizing Non-viral Approaches. RICHARD HELLER[1,2], Chelsea Edelblute[1], Cathryn Lundberg[1], Sezgi Arpag[1], Guilan Sh[1], and Loree Heller[1,2]. [1]Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA and [2]School of Medical Diagnostics and Translational Science, Old Dominion University, Norfolk, VA. Email: rheller@odu.edu

Gene therapy has held great promise for effective treatment of a variety of disorders. Although Gene therapy has made great strides, effective delivery is still a critical element that remains a challenge. One area that has seen tremendous growth is non-viral gene delivery systems including the use of physical delivery approaches. We have been exploring the use of electroporation (gene electrotransfer; (GET)) for quite some time and have established several protocols to several tissue types and for a variety of potential therapeutic applications including immunotherapy for cancer, delivery of DNA vaccines, wound healing and the treatment of ischemic tissue. One area of focus has been delivery to skin utilizing various approaches for delivery including GET, plasma as well as the combination of the two. These methods are simple and direct for delivering genes for therapy and can be accomplished in a minimally invasive way. We have demonstrated that GET was an effective tool for delivering plasmid DNA to the skin. While use of GET has been effective, we have established that the addition of moderate heat in combination with GET can significantly enhance delivery. In addition, we recently began evaluating the use of cold plasma as a delivery tool for plasmid DNA. Plasma assisted gene transfer is an area that is gaining more attention, since it is a non-invasive method and it minimizes potential discomfort of treated area. We investigated plasma assisted delivery and the combination with GET both in vitro in a full thickness skin construct as well as in vivo in a guinea pig model. Delivery was assessed utilizing two plasmids each encoding a different reporter gene (luciferase or green fluorescent protein). Sites treated with plasma, GET or the combination, had higher levels of expression than just injecting the plasmid alone. Expression lasted for approximately 2 weeks. These studies are continuing to determine if delivery could be further improved and to evaluate the approach in therapeutic models.

PS-5

High Aspect Ratio Nanomaterials Enable Delivery of Functional Genetic Material Without Transgenic DNA Integration in Mature Plants. Gozde S. Demirer[1], Huan Zhang[1], Juliana L. Matos[2,3], Natalie Goh[1], Francis Cunningham[1], Younghun Sung[1], Roger Chang[1], Abhishek J. Aditham[1], Linda Chio[1], Myeong-Je Cho[3], Brian Staskawicz[2,3], and MARKITA P. LANDRY[1,4,5]. [1]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720; [2]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720; [3]Innovative Genomics Institute (IGI), Berkeley, CA 94720; [4]California Institute for Quantitative Biosciences, QB3, University of California, Berkeley, CA 94720; and [5]Chan-Zuckerberg Biohub, San Francisco, CA 94158. Email: landry@berkeley.edu

Genetic engineering of plants is at the core of sustainability efforts, natural product synthesis, and agricultural crop engineering. The plant cell wall is a barrier that limits the ease and throughput with which exogenous biomolecules can be delivered to plants. Current delivery methods either suffer from host range limitations, low transformation efficiencies, tissue regenerability, tissue damage, or unavoidable DNA integration into the host genome. Here, we demonstrate efficient diffusion-based biomolecule delivery into tissues and organs of intact plants of several species with a suite of pristine and chemically-functionalized high aspect ratio nanomaterials. Efficient DNA delivery and strong protein expression without transgene integration is accomplished in mature *Nicotiana benthamiana*, *Eruca sativa* (arugula), *Triticum aestivum* (wheat) and *Gossypium hirsutum* (cotton) leaves and arugula protoplasts. We also demonstrate a second nanoparticle-based strategy in which small interfering RNA (siRNA) is delivered to mature *Nicotiana benthamiana* leaves and effectively silence a gene with 95% efficiency. We find that nanomaterials both facilitate biomolecule transport into plant cells, while also protecting polynucleotides such as RNA from nuclease degradation. DNA origami nanostructures further enable siRNA delivery to plants at programmable nanostructure loci. Our work provides a tool for species-independent, targeted, and passive delivery of genetic material, without transgene integration, into plant cells for diverse plant

biotechnology applications. Reference: Demirer et al. *Nature Nanotechology* In Press (2019).

PS-6

Current and Future Gene Editing Delivery Methods for Plant Genome Modification. DOANE CHILCOAT. Applied Science and Technology, Corteva Agriscience™ Agriculture Division of DowDuPont. Email: doane.chilcoat@dow.com

Recent improvements in reference genomes, plant transformation technology, and CRISPR/Cas applications are enabling a new method of plant improvement: genome engineering. Corteva Agriscience™, Agriculture Division of DowDuPont is using these technologies to improve a number of high value crops, including yield potential, changing grain composition, and enhancing biotic resistance. We are developing methods for genome editing in major crops, by optimizing the delivery of genome editing reagents, including Cas9, sgRNA, morphogenic genes, selectable markers and repair template.

PS-7

Improving CRISPR-Cas9 Activity and Specificity by Chemical Modification of the sgRNA Backbone. D. E. RYAN[1], D. Taussig[1], I. Steinfeld[1], S. M. Phadnis[1], B. D. Lunstad[2], M. Singh[1], X. Vuong[1], K. D. Okochi[2], R. McCaffrey[2], M. Olesiak[3], S. Roy[2], C. W. Yung[1], B. Curry[1], J. R. Sampson[1], D. J. Dellinger[2], and L. Bruhn[1]. [1]Agilent Research Labs, Santa Clara, CA 95051; [2]Agilent Research Labs, Boulder, CO 80303; and [3]University of Colorado, Boulder, CO 80303. Email: Daniel_Ryan@agilent.com

CRISPR systems have provided transformative tools for altering genomes in living cells, yet there remains keen interest in increasing their specificity to minimize off-target alterations. Multiple approaches have been explored to further increase the specificity of CRISPR-Cas9 systems including truncation of the guide RNA and directed evolution or rational design of the Cas protein for higher specificity. We developed a novel approach for enhancing specificity by employing site-specific chemical modifications in the 20-nucleotide DNA recognition sequence ("guide sequence") in single-guide RNAs (sgRNAs). Our targeted deep sequencing results from human cells transfected with complexes (RNPs) of Cas9 protein and chemically synthesized single guide RNAs show that a chemical modification (2′-*O*-methyl-3′-phosphonoacetate, or "MP") incorporated at select sites in the ribose-phosphate backbone of sgRNAs can markedly reduce off-target cleavage activities, while maintaining high on-target performance as demonstrated for four clinically relevant genes. For instance, addition of a single MP modification to a guide RNA reported by several groups to be highly efficient for editing the sickle cell disease (SCD) allele dramatically reduced off-target activity at a site in chromosome 9 (which typically undergoes high rates of off-target cleavage) by an order of magnitude in three different cell types, without sacrificing the on-target activity. Through extensive studies in which we systematically walked a single MP across the 20-nt guide sequences of sgRNAs targeting four different genes, *HBB*, *IL2RG*, *VEGFA*, and *CLTA*, we identified specific positions where MP and similar modifications of the backbone are particularly effective at improving specificity across differently-targeted guide sequences while maintaining high on-target activity. These positions map onto known contacts between Cas9 protein sidechains involved in monitoring the extent of complementarity in the gRNA–target DNA duplex as Cas9 detects whether helix formation and specificity are sufficient to allow cleavage. Our results demonstrate that selective incorporation of modifications such as MP in the 20-nt guide sequence can enable users to tailor the specificity of sgRNAs for select targets and cellular environments, thus providing a versatile new tool for augmenting the performance of CRISPR systems for research, industrial and therapeutic applications.

PS-8

Activity and Specificity of CRISPR-Cas9 and Cas12a Systems in Plant Genome Editing. YIPING QI. Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742 and Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD 20850. Email: yiping@umd.edu

Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 and Cas12a are two leading genome editing nuclease systems for plant genome editing. In recent years, we have developed efficient and easy-to-use Cas9 and Cas12a toolkits, which have been used for plant genome editing by many researchers. Here, we will report successful examples of using these reagents to edit the genomes of Arabidopsis, rice, maize, tomato and carrot. To understand the scope of off-target mutations in Cas9 or Cas12a-edited crops, we recently used whole-genome sequencing (WGS) and CIRCLE-seq to assess off-target effects of these nucleases in rice and maize. Our comprehensive and rigorous analysis suggests both Cas9 and Cas12a nucleases are very specific in generating targeted DNA modifications in plants.

PS-9

Systematic Evaluation of CRISPR-Cas9 Specificity and Its Relevance in Crop Improvement. SANDEEP KUMAR[1], Joshua Young[1†], Gina Zastrow-Hayes[1†], Stéphane Deschamps[1†], Sergei Svitashev[1], Mindaugas Zaremba[2], Ananta Acharya[1], Sushmitha Paulraj[1], Brooke Peterson-

Burch[1], Chris Schwartz[1], Vesna Djukanovic[1], Brian Lenderts[1], Lanie Feigenbutz[1], Lijuan Wang[1], Clara Alarcon[1], Virginijus Siksnys[2], Gregory May[1], and Doane Chilcoat[1]. [1]Corteva Agriscience™, Agriculture Division of DowDuPont™, Johnston, IA 50131 and [2]Institute of Biotechnology, Vilnius University, Vilnius, LT-10257, LITHUANIA. [†]These authors contributed equally to this work. Email: sandeep.kumar@corteva.com

The CRISPR-Cas9 genome editing system is a precise and powerful genome editing tool with broad applications for gene discovery, trait development and expedited breeding in crop species. In addition to the intended target (on-target) site, Cas9 can potentially cause double-strand breaks (DSBs) at unintended genomic locations (off-target) with significant sequence similarity to that of the intended target sequence. To interrogate Cas9 nuclease specificity, we paired computational prediction with genome-wide biochemical off-target detection, followed by validation in maize plants. Computationally unique targets demonstrated no evidence of off-target cutting in a cellular context, while on-target activity was observed up to ~90% of all alleles. Predictable off-target cutting was observed with a 'promiscuous' guide RNA, intentionally designed to validate our approach. Robust bioinformatics tools minimize the potential for off-target cleavage by identifying targets that are a) unique within the genome, or b) differ minimally from similar sequences by at least three nucleotide mismatches and/or bulge combinations, where at least one of the three mismatches is located within the PAM proximal "seed" region. We also report that inherent genetic variation in the genotype used in this study far exceeded potential genetic changes generated by CRISPR-Cas9 genome editing techniques. Taken together, our study indicates that with appropriately designed guide RNAs, CRISPR-Cas9 off-target editing is much less than the expected naturally occurring diversity in plants. As part of commercial product development, genome edited plants would similarly enter well-established rigorous plant breeding and selection processes, removing individuals with undesirable characteristics while retaining individuals with intended improvements. These breeding practices have a long history of effectively selecting new plant varieties that are safe and deliver value to producers and consumers. Therefore, concerns related to specificity of CRISPR-Cas9 technology in crop improvement have little relevance.

PS-11

Getting Outside the Lab: Endophytes as Modifiers of Plant Stress. B. K. WHITAKER[1], M. G. Bakker[2], and C. V. Hawkes[1]. [1]Plant and Microbial Biology, North Carolina State University, 112 Derieux Place, Raleigh, NC 27607 and [2]Mycotoxin Prevention & Applied Microbiology Research Unit, USDA-ARS, 1815 N. University St., Peoria, IL 61604. Email: bkwhitak@ncsu.edu

The impact of plant microbiota on host function can span a continuum from beneficial to pathogenic. Non-pathogenic members within the plant microbiome are increasingly seen as potentially useful symbionts for disease suppression or abiotic stress tolerance in agricultural systems. However, it remains difficult to understand the ecology of these potentially beneficial microbiota given the innate complexity of host-symbiont interactions that can occur simultaneously with variable abiotic conditions. Many research studies attempt to reduce this complexity by performing simplified competition assays on media plates or plant substrates in the lab. However, if functional roles are context dependent, lab-based assessments may not accurately represent functional outcomes under variable field conditions or inside living plants. This can lead to a failure of candidate biocontrol agents when transferred to field conditions. Given this conundrum, our research objectives were to test whether traits displayed by endophytic microbiota under laboratory conditions are predictive of disease and drought stress outcomes in living plants. In one system, we contested bacterial endophytes against *Fusarium graminearum*, a fungal pathogen that causes Fusarium head blight in wheat, in the lab and in living plants across both temperature and atmospheric CO_2gradients.In another system, we investigated whether fungal endophytes isolated from Switchgrass plants reduce drought stress in the field as predicted by measurements from the lab, and if inoculation success in the field varies by the identity of the fungal isolate. We conclude that endophytic impacts on plant performance cannot be easily inferred from many commonly-used assays, and that environmental gradients should be incorporated into future testing of microbial interactions in plant hosts.