Plenary Symposia

PS-1

In Vitro Generation of Human Pluripotent Stem Cell Derived 3-dimensional Organoids for Studying Human Development and Disease. JASON R. SPENCE. University of Michigan Medical School, Center for Organogenesis, Department of Internal Medicine, Department of Cell and Developmental Biology, Ann Arbor, MI 48109. Email: spencejr@umich.edu, Website: jasonspencelab.com

The generation of complex organ-like tissues from human embryonic and pluripotent stem cells (hPSCs) is an important goal, giving rise to novel in vitro models to study human development, homeostasis and disease. Several different studies have differentiated hPSCs into a multitude of “organoid” models, representing diverse organ and tissue types, using a process called directed differentiation. Directed differentiation uses different signaling cues in a step-wise manner to mimic embryonic events in a temporally controlled manner. Using this process, we have demonstrated that hPSCs can be directed to differentiate into self-organizing, 3-dimensional organoid structures from different endodermal lineages, including intestine and lung. For example, by manipulating developmental signaling pathways we have recently shown that hPSCs can give rise to human lung organoids (HLOs). HLOs possess epithelial and mesenchymal compartments of the lung that have structural features similar to the native human lung. Importantly, in the case of both intestinal and lung organoids, we are beginning to appreciate that tissues generated in the dish are more highly representative of immature, fetal tissue; however, by transplanting organoids into mice, they undergo maturation events and more closely resemble the adult organ. In this seminar, I will discuss our ongoing work using in vitro generated organoids resembling fetal tissue as well as in vivo transplanted, mature adultlike organoids to understand human development, tissue maturation and to study disease.

PS-2

New Regulators of Shoot Meristem Development in Maize and Arabidopsis, and Potential Benefits for Crop Yields. DAVE JACKSON, Byoung Il Je, Mike Pautler, Peter Bommer, Qingyu Wu, and Andrea Eveland. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724. Email: jacksond@cshl.edu

Shoot growth depends upon meristems, pools of stem cells that are maintained in a number of ways, including a negative feedback loop between the CLAVATA pathway and the WUSCHEL homeobox gene. CLAVATA signaling involves a secreted peptide, CLAVATA3 (CLV3), and its perception by cell surface leucine-rich repeat (LRR) receptors, including the CLV1 receptor kinase, and an LRR receptor-like protein, CLV2. We are interested in finding novel players in the CLV-WUS pathway, as well as new regulators that could affect meristem size in parallel pathways. Maize provides a rich source of new information, because there are many clavata-type mutants, and their isolation is becoming routine. We isolated the maize COMPACT PLANT2 (CT2) gene, and it encodes the predicted a subunit (Ga) of a heterotrimeric GTP binding protein. ct2 mutants have CLAVATA-like meristem proliferation phenotypes, and genetic, biochemical and functional assays indicate that CT2/Ga signaling transmits a stem cell restrictive signal from a maize CLAVATA LRR receptor, suggesting a new function for Ga signaling in plants. Heterotrimeric GTP-binding proteins are membrane-associated molecular switches that are commonly activated by ligand binding to an associated 7-pass trans-membrane (7TM) G-protein-coupled receptor (GPCR). Recent studies have questioned the idea that plant heterotrimeric G proteins interact with canonical GPCRs, and our findings suggest that single pass LRR receptors act as GPCRs in plants, challenging the dogma that GPCRs are exclusively 7TM proteins. We have also identified new regulators of maize shoot meristem size, including a new receptor, fea3, and a transcription factor fea4. The roles of these genes will be discussed, as well as their...
potential use in improvement of maize yields, for example through control of seed number. In addition, these genes could be useful targets to improve shoot regeneration in culture.

PS-3

Transcriptional Logic of Plant Stem Cell Maintenance. G. VENUGOPALA REDDY, Mariano Perales, Kevin Rodriguez, and Stephen Snipes. Department of Botany and Plant Sciences, Center for Plant Cell Biology (CEPCEB), University of California, Riverside, CA 92521. Email: Venug@ucr.edu

Classical cell ablation experiments have revealed the importance of positional information in plant development. The importance of protein and hormonal gradients in plant development is well documented, however the transcriptional mechanisms that underlie threshold dependent orchestration of gene expression have not been understood. Shoot apical meristems (SAMs) harbor a set of stem cells within the central zone (CZ) whose progeny differentiate in adjacent peripheral zone (PZ). We have analyzed expression profiles of at least 10 different cell population of SAM stem cell niche (Yadav et al., 2009 and 2014). We have used this resource to reconstruct transcriptional programs mediated by stem cell inducing TF-WUSCHEL (WUS). We have shown that WUS protein migrates from the RM/niche into adjacent cells to form a protein gradient (Yadav et al., 2011). Upon migration, WUS activates its own negative regulator-CLV3 in the CZ and also represses a large number of differentiation promoting transcription factors, both through direct transcriptional regulation (Yadav et al., 2013). Thus WUS-mediated direct transcriptional regulation links the three functional domains—the CZ, the PZ and the RM to provide a robust homeostatic control. Though WUS migration can explain stem cell maintenance, it remains unknown how the WUS gradient results in the transcriptional activation of some genes (for example the CLV3 in the CZ), and the repression of other genes (differentiation promoting transcription factors) in placing a negative regulator at a distance from its own domain of expression, while keeping the differentiation program repressed in stem cells. Our unpublished work reveals that WUS functions in a dose dependent manner in regulating target gene transcription. WUS utilizes same cis-elements on the CLV3 promoter to repress transcription at higher concentration and activate transcription at a lower concentration. This dose dependent transcriptional output is influenced by differential binding patterns of WUS to cis-elements. Biochemical evidence along with in vivo studies showing the effects of manipulation of WUS binding cis-elements and manipulation of WUS protein gradient on target gene expression will be presented. The threshold dependent transcriptional discrimination model will be discussed in the context of morphogen concept.

PS-4

Neonatal Epigenetic Predictors of Childhood Asthma. DONATA VERCELLI. Arizona Respiratory Center, Department of Cellular and Molecular Medicine, Arizona Center for the Biology of Complex Diseases and The Bio5 Institute, University of AZ. Email: donata@email.arizona.edu

The timing and mechanisms of childhood asthma inception remain imprecisely defined. Epigenetic mechanisms are worth investigating because they underpin the timed unfolding of developmental processes and plastic responses to environmental cues that contribute to asthma pathogenesis. We asked whether an epigenetic trajectory to childhood asthma is in place already at birth and what biological pathways are involved. We searched for DNA methylation signatures predictive of childhood asthma (physician-diagnosed with symptoms in the past year reported at least once at age 2-9) in cord blood mononuclear cells (CBMCs) from 36 children (18 non-asthmatics, 18 asthmatics) enrolled in the Infant Immune Study, an unselected birth cohort closely monitored for asthma to age 9. Genome-wide differential DNA methylation was analyzed using NimbleGen 2.1M Promoter microarrays. Differential SMAD3 methylation was assessed by bisulfite sequencing in 56 IIS neonates (28 non-asthmatics, 28 asthmatics). CBMC-secreted TGF-β1 and IL-1β were measured by ELISA. CBMCs harbored 589 asthma-associated DMRs. Network and upstream regulator analysis showed a significant enrichment for DMRs in genes controlled by immunoregulatory TGF-β1 and pro-inflammatory IL-1b. DNA methylation in a subset of these DMRs correlated with TGF-β1 and IL-1β production differentially in neonates who did and did not develop asthma in childhood. Children of asthmatic mothers who developed asthma had higher SMAD3 methylation than did non-asthmatic children. 86% of children of asthmatic mothers who developed asthma exhibited high SMAD3 methylation and high IL-1β production compared to only 25% of non-asthmatic children of asthmatic mothers. Thus, in a proportion of children with childhood-onset asthma, a distinctive methylome exists already at birth and alters responsiveness to innate immunoregulatory and pro-inflammatory signals, thereby promoting a trajectory that may ultimately lead to clinical disease. Maternal asthma strongly influenced the relationship between SMAD3 methylation and cytokine production.

PS-5

Epigenetic Mechanisms Associated with Plant-Pathogen Interaction and Small RNA Mobility. J. GOHLKE, W. Chen, A. Seifi, T. Kendall and R. A. Mosher. School of Plant Sciences, University of Arizona, Tucson, AZ. Email: gohlke@email.arizona.edu
Silencing by small RNAs is a gene regulatory mechanism that is important for development, responses to stress and defense against pathogens and foreign genetic elements. Small RNAs are able to induce epigenetic modification in recipient cells by a process called RNA-directed DNA methylation. We found that DNA methylation plays a dual role during plant-pathogen interaction in the *Agrobacterium-Arabidopsis* model system. On the one hand, siRNA-mediated DNA methylation can inactivate *Agrobacterium* oncogenes, thereby preventing development of *Agrobacterium*-induced plant tumors. On the other hand, it induces transcriptional changes that influence development and physiology of plant tumors. Mobile small RNAs most likely play a role during this process as they have increasingly been recognized as regulatory molecules with the potential to transmit information between cells, organisms and species. ARGONAUTE4 (AGO4) is known to bind siRNAs and direct DNA methylation to target loci. Although this process of transcriptional gene silencing occurs in the nucleus, AGO4 might also contribute to non-cell autonomous functions of siRNAs in the cytoplasm. Indeed, we found that despite their nuclear functions, both AGO4 and siRNAs are also present in the cytoplasm. As movement of nuclear siRNAs requires cytoplasmic transit, this finding raises the possibility that AGO4 is involved in small RNA mobility. It might participate in intra- or intercellular siRNA transport or reception, thereby playing an important role in transmission of RNA signals. Various aspects of siRNA mobility between cells or organisms will be discussed.

**PS-6**

*Arabidopsis* MSH1 Mutation Alters the Epigenome and Produces Heritable Changes in Plant Growth. KAMALDEEP S. VIRDI1,*, John D. Laurie2,*, Ying-Zhi Xu2, Jiantao Yu2, Mon-Ray Shao2, Robersy Sanchez2, Hardik Kundariya2, Dong Wang3, Jean-Jack M. Riethoven4, Yashitola Wamboldt2, Maria P. Arrieta-Montiel2, Vikas Shedge2, and Sally A. Mackenzie2. 1School of Biological Sciences, University of Nebraska, Lincoln, NE 68588; 2Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68588, 3Department of Statistics, University of Nebraska, Lincoln, NE 68588; 4Center for Biotechnology, University of Nebraska, Lincoln, NE 68588. Email: smackenzie2@unl.edu

Plant phenotypes respond to environmental change, an adaptive capacity that is at least partly transgenerational. However, epigenetic components of this interplay are difficult to measure. Depletion of the nuclear-encoded protein MSH1 causes dramatic and heritable changes in plant development, and here we show that crossing these altered plants with isogenic wild type produces epi-lines with heritable, enhanced growth vigour. Pericentromeric DNA hypermethylation occurs in a subset of msh1 mutants, indicative of heightened transposon repression, while enhanced growth epi-lines show large chromosomal segments of differential CG methylation, reflecting genome-wide reprogramming. When seedlings are treated with 5-azacytidine, root growth of epi-lines is restored to wild-type levels, implicating hypermethylation in enhanced growth. Crafts of wild-type floral stems to mutant rosettes produce progeny with enhanced growth and altered CG methylation strikingly similar to epi-lines, indicating a mobile signal when MSH1 is downregulated, and confirming the programmed nature of methylome and phenotype changes. *These authors contributed equally to this work.*

**PS-9**

Beyond Big Data: Lessons for the Lab Taken from the Human Toxome Project. R. A. FASANI, C. B. Livi, and M. Rosenberg. Agilent Technologies, Inc., 5301 Stevens Creek Blvd., Santa Clara, CA 95051. Email: rick.fasani@agilent.com

The Human Toxome Project is part of a long-term vision to modernize toxicity testing for the 21st century. In the initial phase of the project, a consortium of six academic and commercial organizations has partnered to comprehensively map pathways of toxicity, using endocrine disruption in cultured cells as a model system, along with a range of experimental and computational tools. Of course, effectively gathering, managing, and analyzing the data from high-throughput experiments is a challenge in its own right—doing so for a growing number of -omics technologies, with ever-larger data sets, across multiple institutions, complicates the process at every step. Here, we present an overview of Big Data and the Cloud in plain English, and outline the major data challenges we have faced on the Human Toxome Project. We describe the solutions we have implemented, including The Human Toxome Collaboratorium, a shared computational environment hosted on third-party cloud services. The Collaboratorium provides a familiar virtual desktop, with a powerful mix of commercial, open-source, and custom-built applications. As such, it shares some of the challenges of traditional information technology, but with unique and unexpected constraints that emerge from the cloud. Indeed, the greatest challenge may not the size of the data but the complexity of the collaboration. Ultimately, the lessons we have learned and the solutions we have employed can be used in any lab, whether the data is big or small.