

Plants and Their Environment: Biology, Molecular Interactions and Homeostasis

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The following is a summary of a breakout session on Plants and Their Environment at the 2012 Genomic Science Contractor-Grantee Meeting held in Bethesda, MD on February 26-29. Authors are the speakers (below) and DOE-BER staff (above).

In a changing climate there is an ever pressing need for hardier plants, both for energy and for agriculture, which will be capable of sustained growth under less than ideal environmental conditions and on marginal lands. To develop such feedstocks, we need to better understand the regulation of mechanisms that make plants resistant to, or tolerant of biotic and abiotic environmental factors that can be detrimental to plant growth. Relationships between plants, microbes and mycorrhizal fungi can also be beneficial to plant health and growth and so we also need to better understand what drives these multitrophic interactions in order to take advantage of their association with plant roots.

In plant biology, scientific challenges of this nature are often approached at a genomic level. However, plant genomics must also be studied in the context of understanding homeostasis within the plant involving interrelationships between the physiological processes, the biochemical reactions and the genes controlling them. The DOE Biological and Environmental Research Advisory Committee 2010 Long-Term Vision Report (DOE/SC-0135) identified the need for new technologies to enable the visualization and quantification of basic homeostatic functions across spatial scales, spanning whole plants (from cellular to tissue levels) to the ecosystems in which they grow, and incorporating temporal scales from milliseconds through to the life-cycle of the organism. These dynamic states of nature are often influenced by extrinsic environmental factors as well as by intrinsic cues controlled by gene expression, and often, these states will influence transcriptional regulation and gene expression in a complex integrated and interactive network involving feedback control. Most particularly, we note that plant hormones and their regulation of resources can be key factors in determining important traits for hardier plants, including optimal tissue architecture in leaves, stems and roots, or enhanced defense mechanisms against pests and pathogens. However, the pleiotropic nature of hormone action has made it extremely difficult to understand exactly how these substrates act either to pattern plant development during normal growth, or to promote resistance during attack. Understanding how hormone homeostasis, influenced by its biosynthesis, transport and metabolic turnover, is regulated in coordination with gene expression and with basic plant functions including the uptake and utilization of essential plant resources and in the context of influences from the environment are key here.

Presentation Summaries

During the 2012 Genomic Science Contractors-Grantees Workshop the breakout session on plant biology, molecular interactions and homeostasis hosted four speakers: Jean Greenberg from University of Chicago, Richard Ferrieri from Brookhaven National Laboratory (BNL), Jocelyn Rose from Cornell University and Leland Cseke from University of Alabama (Huntsville). The common theme of these four talks was that fundamental systems-level studies in plant biology using model systems was

essential to filling gaps in knowledge and that the application of new technologies could facilitate the translation of this new found knowledge to real-world problems.

Systemic Acquired Resistance

In the first talk, Jean Greenberg presented an overview of her research on systemic acquired resistance (SAR) using *Arabidopsis* as a model system because of its substantial genetic resources. By combining imaging and functional genomics technologies, she demonstrated the potential for using radiotracer imaging of intact plants to explore movement of key signal molecules associated with SAR and in response to pathogenic infection by *Pseudomonas syringae*. *P. syringae* is a gram-negative bacterium with polar flagella that can infect a wide range of plant species. A large number of *P. syringae* genes contribute to bacterial survival on and within the host plant, including those involved in bacterial attachment and nutrient uptake. The latter category may include siderophores, which are required for mineral acquisition, most particularly iron. Toxins produced by the pathogen can also contribute significantly to its virulence. For example, coronatine suppresses salicylic acid-mediated defense as well as activating the jasmonic acid signaling pathway through mimicry of jasmonate. An important impact of coronatine action is to induce opening of the plant stoma, permitting entry of the pathogen.

Jean highlighted her recent work addressing vascular transport of azelaic acid (AZA), a nine-carbon dicarboxylic acid substrate that is considered to play an important role in SAR. Using ^{14}C -AZA applied to leaf discs from *azil-1* mutants and *col-0* wild-type, she demonstrated that substrate movement required protein transporters for active phloem loading. Through collaborations with the BNL group she plans to make use of ^{11}C -AZA in combination with Positron Emission Tomography (PET) to investigate this further at the whole-plant level. Jean also reported on the successful radiolabeling of ^{125}I -flg22 through her collaboration with the Oak Ridge National Laboratory (ORNL) radiochemistry group there. Flg22 is a twenty two amino acid peptide that derives from bacterial flagellum, and hence there is interest in whether this pathogen-derived substrate can influence basic physiological and biochemical functions of the host plant. Jean reported complementary studies using fluorescently labeled flg22. Using multiple approaches, it was possible to associate the long-distance movement of flg22 with a functional receptor for the peptide. Key to the success of the project was the use of defined mutants in *Arabidopsis* that allowed integration of novel approaches with genetic resources. Experiments with poplar from her ORNL collaborators indicate there is likely to be broad conservation of the peptide trafficking mechanism. Finally, Jean addressed key challenges to developing new imaging technologies, most particularly in how to introduce large protein substrates into plants without disturbing their natural processes. She noted that the ORNL group has developed an injector chip that utilizes arrayed nanofilaments suitable for substrate introduction into plants. The group demonstrated in a proof-of-concept that radiolabeled ^{125}I -CmPP16-1 protein can be injected into poplar saplings and its transport measured.

Branch Root Patterning

The second speaker, Richard Ferrieri, presented aspects of his research in root system architecture. Branch root patterning is a critical agronomic trait in plant architecture that can determine crop productivity and stress adaptability. This process is tightly regulated by intrinsic developmental cues in plants that are associated with the biosynthesis and homeostasis of the plant hormone auxin.

Here he described the use of the radioisotope carbon-11 ($t_{1/2}$ 20.4 m) to study the coordination between ectopic branch root patterning and auxin signaling in maize plants that were stressed through damage by *Diabrotica virgifera virgifera*, the western corn rootworm. As an opportunist, this pest not only continues to threaten corn production with annual U.S. crop losses of \$1B, it poses a threat to future bioenergy grasses. Using PET and radiographic imaging of ^{11}C -auxin in combination with radiometric bioassays and other “omics” tools, Richard showed that branch root primordia manifest as sites with elevated levels in carbohydrate metabolic activity, in auxin receptor density and in expression of early auxin response genes. He noted that a particular strength in using radioanalytical techniques coupled with high specific activity PET radioisotopes is the ability to administer non-physiological doses of labeled substrates to plants enabling the measurement of their movement and metabolism without disturbing the natural state of the organism. No other technology can match this. Richard highlighted auxin biosynthesis as an example in plants where the hormone can be biosynthesized via five independent pathways noting that no one has yet to measure their metabolic fluxes under normal or stressed conditions. Using ^{11}C -indole-3-acetonitrile, one of the natural biosynthetic precursors to auxin, he showed that metabolic flux rates for its turnover to auxin were highest in branch root primordial tissues, as compared with regions closer to the root meristem, or to leaves. These sites were also most responsive to root herbivory. Richard pointed out that existing models on auxin transport and hormone bio-distribution in plants may need to be revisited based on new insights provided by radiochemistry.

Function and Evolutionary Diversity in Cutical Structures

The third speaker, Jocelyn Rose, presented aspects of his research on the cuticularized plant cell wall in wild tomato varieties. Like the previous talks, this work centers on using a model system, the cuticle, to understand at a fundamental level the interrelationship between its evolutionary diversity in plants and its biological function. As a waxy surface covering the aerial epidermis of land plants, the cuticle is central to limiting non-stomatal water loss, and acts as the primary interface between plants and their environment providing effective barriers against herbivores and pathogens, protection against UV radiation and biomechanical support. This hydrophobic barrier is contiguous with the polysaccharide cell wall and consists primarily of a lipid polymer, cutin, as well as a mix of organic soluble waxes. Joss utilized a combination of technologies including high resolution optical imaging, chemical analyses of cutin and wax compositions, mapping of cuticle traits using introgression line populations and cell-type specific genetic profiling using laser capture micro dissections to gain insight into the genetic basis for the biodiversity in cuticle structure, composition and biological function. In his high resolution imaging of cuticle morphology he pointed out the need and importance to understanding aspects of the cuticle’s 3-dimensional nature in order to learn how it functions. For example, his imaging work revealed new insights into sub-cavity structures that are thought to maintain water conductance. Further, using functional genomics he showed the feasibility for using mutants in testing mechanisms of action of certain acyltransferases that act at the polysaccharide/cutin interface.

Nutrient Cycling Promoted by Ectomycorrhizal Symbiosis

The last speaker in this session, Leland Cseke, presented yet another interesting model for exploring nutrient cycling promoted by the ectomycorrhizal (ECM) symbiosis between *Laccaria bicolor*, an efficient growth promoting fungus that is dominant in forest ecosystems, and *Populus tremuloides*. Most ECM fungi are epigeous, as is *L. bicolor*, though some are hypogeous. In general, ECM fungi do not show a high degree of host specificity in the colonization of secondary and tertiary root structures of woody species. The fungal hyphae cover the root like a sheath and grow inwards between epidermal and cortical cells forming a Hartig net. Penetration of the root cortex is achieved by a combination of mechanical force and pectinase secretions. However, the hyphae never penetrate into the cell lumens or into the stele. Typically a complex network of fungal hyphae is established serving as the interface for nutrient cycling with the host. Leland's approach to understanding the mechanisms controlling nutrient cycling at this interface was clearly at the systems level where he integrated transcriptomic, proteomic and metabolomic data to arrive at interactive and predictive models linking hormonal defense responses with nutrient transport (including C, N, and P). Since establishment of a symbiotic relationship is key to plant health and growth, a grand challenge is to identify the factors that enable early colonization within the host. Very little is known concerning how potential pathogens establish recognition as symbionts. Temporal profiling of the events leading up to ECM initiation suggests strong ties with auxin signaling and cell wall biogenesis within the host. However, it is unknown whether *Laccaria*-derived hormones facilitate this action. Fundamental studies of this nature will likely have a big impact on regulating and improving carbon sequestration belowground.

Session Discussion and Summary

In the ensuing roundtable discussions certain themes kept re-surfacing. On the one hand, while it is clear that fundamental research in plant science using model systems is essential to attaining new knowledge, it is unclear how this new found knowledge will translate to other biological systems (species) that have relevance to the DOE energy and environment missions. There was considerable discussion of what defines a model system. At the species level *Arabidopsis* continues to reign as the lab rat of the plant world though other species are rapidly emerging. For example, *Setaria viridis* was mentioned as a model grass that may be more suitable as model system of bioenergy grasses than say maize. However, model systems do not necessarily have to be species specific. For example, identifying function of specialized tissues or organs across several plant species or function of specialized interfaces existing between plants and their surrounding environment can certainly be considered equally important as model systems.

A more pressing concern in the roundtable discussions pertained to standardization of techniques for analyzing and interpreting not only bioinformatics data, but data from emerging technologies that were described in this session. In systems biology, no single tool or technology will provide all the answers to biological questions. Therefore, researchers can no longer afford to work in isolation but rather must increasingly rely on collaborations in a "teamed" effort involving technology integration. Unfortunately, data interpretation can be subjective and often misleading. As new imaging tools become available serving systems biology, there will be an increasing need not only to standardize data processing and interpretation, but an increasing need to network this information making it more

readily available to the scientific community. The field lacks a central repository or clearinghouse for such data and there was uniform agreement that this would be of great value.

To summarize some of the findings of this session it is clear that scientists are quickly adapting to the mindset that “teamed” research is a more effective way to approach grand challenges in life science at a systems-level. This is especially true now that scientists are no longer just scrutinizing dynamic states of nature at cellular levels, but are now stepping back to consider how those states transcend form and function at organism and ecosystem scales. To accomplish this task it is clear that new technologies are needed which can fill gaps in knowledge at a fundamental level in model systems and that can translate new knowledge to energy and environmentally relevant systems. This session showcased emerging technologies that rely on recent developments in radiochemistry and in imaging instrumentation to measure movement of key hormones in plants impacting their response to changing environmental conditions. Just as important, there is a need for imaging applied to metabolite movement, particularly as some metabolites have important signaling roles or affect plant growth and fitness. However, to rigorously understand the role of these metabolites and factors that affect their biosynthesis and movement, there is a need for more biochemistry coupled with genetics to enable reconstruction and manipulation of important pathways that synthesize, regulate and mobilize these metabolites. Further, nutrient cycling cannot be excluded from the equation as it is often crucial to regulating these life-sustaining pathways. The need is just as urgent to extent imaging technologies to measuring dynamic uptake of essential nutrients in coordination with gene expression and in coordination with basic plant functions. An obvious outcome of this session is that with emerging technologies such as this, our awareness is heightened of the urgent need to standardize the way we do science; most particularly, in how we process data and interpret results. This is essential if new found knowledge is to become relevant to real-world problems.