6th Annual Retreat

Saturday, November 19, 2011

Room 105

Cargill Building for

Microbial and Plant Genomics

Retreat Schedule

8:00 – 8:50: Breakfast and Poster set up (Atrium)

8:50 – 9:00: Introductory Remarks – Nathan Springer, Director, MPGI

9:00 – 9:20: Identifying the genetic lesion in a Chlamydomonas mutant with elevated expression of hydrogenase and related genes. Xiaoqing Sun, Graduate Student, Plant Biological Sciences Program

9:20 – 9:40: RNA-seq analysis of potato tuber transcriptome dynamics in response to the late blight pathogen Phytophthora infestans. Liangliang Gao, Graduate Student, Plant Biological Sciences Program

9:40 – 10:00: What could make an HIV vaccine effective? Anthony Smith, Research Associate, Microbiology Department

10:00 – 10:20: Use of RNAseq to evaluate gene expression in transgenic alfalfa plants down-regulated for a putative amino acid transporter. Deborah Samac, Adjunct Professor, Plant Pathology Department

10:20 – 10:40: Break (Atrium)

10:40 – 11:00: Fusarium gene expression during infection of pea border cells. Wilfried Jonkers, Post Doctoral Associate, Plant Pathology Department

11:00 – 11:20: Using Illumina Sequencing To Measure Genetic Finesses; One Genome at a Time! Evan Brutinel, Post Doctoral Associate, Biotechnology Institute

Retreat Schedule

11:20 – 11:40: Assessing the role of antisense RNAs in Enterococcus faecalis by genetic approaches and RNAseq. Gary Dunny, Professor, Microbiology Department

11:40 – Noon: Gene expression during the transition to multicellularity. William Ratcliff, Post Doctoral Associate, Ecology, Evolution and Behavior Department

Noon – 1:30: Lunch and Poster Session (Atrium)

1:30-1:50: **The effects of plant cell wall alterations on plant disease resistance.** Gerit Bethke, Post Doctoral Associate, Plant Biology Department

1:50-2:10: Analysis of U.S. Genetically Engineered Crop Regulation and Litigation. Esther McGinnis, Graduate Student, Horticultural Sciences

2:10-2:30: Targeted deletion of tandemly arrayed genes in Arabidopsis using zinc finger nucleases. Yiping Qi, Post Doctoral Associate, Genetics, Cell Biology and Development

2:30-2:50: Co-expression network analysis reveals rewiring of maize transcriptome by domestication. Roman Briskine, Graduate Student, Computer Science and Engineering

2:50-3:00: Closing Remarks - Nathan Springer, Director, MPGI

Identifying the genetic lesion in a Chlamydomonas mutant with elevated expression of hydrogenase and related genes

Xiaoqing Sun, Susan Pribyl, Nancy Haas, Paul A. Lefebvre, and Carolyn D. Silflow
Dept. of Plant Biology, University of Minnesota

Speaker: Xiaoqing Sun

Under anaerobic conditions, the green alga Chlamydomonas reinhardtii produces hydrogen gas in a reaction catalyzed by hydrogenase. The expression of two hydrogenase genes, HYDA1 and HYDA2, is strictly regulated by anaerobiosis, through mechanisms that are still unknown. To identify trans-acting genes in this pathway, we designed a mutant screen using a motility gene reporter driven by a HYDA promoter. The screen of chemically mutagenized cells identified a mutant strain B6-F that, under aerated conditions, is motile and expresses HYDA1 and other hydrogenase-related genes at elevated levels. The mutant phenotype is recessive in stable heterozygous diploids. Genetic mapping placed this mutation to a ~ 1 Mbp region near the centromere of chromosome 1. Analysis of Illumina next generation sequencing data revealed extensive SNPs between the mutant strain B6-F and the strain from which it was derived, B6. Within the 1Mbp region we identified one SNP mutation that disrupted a splicing signal in an unannotated gene encoding a protein of 52 kD. The incorrectly spliced transcript in the mutant strain has been verified by RT-PCR. The cloned wild-type gene is being tested for genetic rescue to further examine the relationship between the mutation and the elevated expression level of HYDA1 and hydrogenase-related genes. The combination of illumina next generation sequencing data and genetic mapping results provides a powerful and efficient solution for identifying mutations in complicated chromosomal regions.

RNA-seq study in tetraploid potato suggests that pre-priming of key regulators is an important component of R-gene mediated defense against late blight pathogen Phytophthora infestans in the tubers

Gao, Liangliang, Tu, Zheng Jin, Katagiri, Fumiaki , and Bradeen, James

Speaker: Liangliang Gao

Cultivated potato is the world's number one non-grain food commodity. The late blight pathogen Phytophthora infestans has the capacity to attack both potato foliage and tubers. Importantly, foliar resistance against late blight does not guarantee tuber resistance. Most transcriptome studies that have been performed in potato target foliage and very limited studies target tuber-microbe interactions. To understand potato tuber defense mechanisms and to compare tuber and foliage differences in defense, we conducted a time-course RNA-seq study consisting two genotypes (WT and +R-gene line), 2 treatments, 3 time points, and 3 reps for tubers, plus a few samples from the foliage. Over 540 million paired-end Illumina Hi-Seq reads were generated. Each of 87% pass filtered reads were mapped uniquely to one location in the reference genome (a doubled monoploid (DM) line) , representing transcripts from over 30000 potato genes. About one million potential SNPs between tetraploid potato and DM reference genome were detected; of which only 60% are located within known genic regions. We analyzed the transcription levels of potato genes using various software packages. Over 7500 genes were detected to be differentially expressed (DE) among comparisons. We discovered marked differences between the transcriptomes of wild type potato and the transgenic line. Number of up-regulated genes from the wild type potato was substantially higher than that of down-regulated genes during later stage of infection; whereas the transgenic (resistant) line shows more or less equal number of both up and down regulated genes. Various regulatory and metabolic pathways were identified to distinguish organ-specific and transgene-specific responses. Interestingly, transgenic potato shows pre-priming of certain receptor kinases and transcription factors. Timing rather than magnitude of DE seems to be most critical in determining the outcome of battling against late blight.

What could make an HIV vaccine effective?

Speaker: Anthony Smith

Systemic vaccination with the attenuated virus SIVmac239-ΔNef provides sterilizing or partial protection to rhesus monkeys challenged with WT SIV strains, offering important opportunities to study key immunological components of a protective host response. Here we show that intravenous vaccination with SIVmac239-ΔNef provides two potentially crucial immunological barriers localized at mucosal surfaces that correlate with the vaccine's protective effects against WT SIVmac251 vaginal challenge: 1) a conditioned and coordinated response from the mucosal epithelium that blunts the early inflammatory and chemotactic signalling cascade that aids virus propagation and expansion; 2) early on-site generation/diversification of SIV-specific Abs from ectopic germinal center-like lymphoid aggregates. This unique host response to WT SIVmac251 in the female reproductive tract of SIVmac239-ΔNef-vaccinated animals points to a multi-layered strategy for a protective host response during immunodeficiency virus exposure—rapid induction of humroal immunity at mucosal surfaces without the deleterious inflammatory side effects tied to innate recognition of virus. This vaccine-induced host response highlights potential key protective mechanisms needed for an effective HIV vaccine.

Use of RNAseq to evaluate gene expression in transgenic alfalfa plants down-regulated for a putative amino acid transporter

Speaker: Deborah Samac

Controlling diseases in crop plants is critical for increasing yields and reducing potential negative effects of crop chemicals on the environment. Alfalfa (Medicago sativa) is the fourth most widely grown crop in the U.S. It is a highly nutritious feed for livestock and has many attributes that make it very attractive as a biofuel feedstock. The long-term goal of the project is to identify genes that provide protection to alfalfa against foliar diseases. Accessions of the model legume Medicago truncatula, a close relative of alfalfa, were identified that are resistant to several foliar pathogens and microarray technology was used to identify genes specifically expressed in the resistance response. A large proportion of the up regulated genes had only weak similarity to known genes or had no significant matches in the database. Transgenic M. truncatula and alfalfa plants were produced that express interfering RNA (RNAi) constructs of selected genes. Down-regulation of several genes increased susceptibility to the fungal pathogen Colletotrichum trifolii, the causal agent of anthracnose. An alfalfa line down-regulated for expression of a gene with weak similarity to amino acid transporters was selected for expression profiling using RNA-seq and compared with a vector control line, with and without inoculation with C. trifolii. No replications were performed. Sequences were aligned and mapped to the alfalfa gene index using SOAP and normalized by RPKM. Functional annotation was done using MapMan bincodes. Transcript profiles related to pathogen defense and RNAi expression will be discussed.

Fusarium gene expression during infection of pea border cells

Speaker: Wilfried Jonkers

Different Fusarium species can infect pea (Pisum sativum): F. solani f. sp. pisi causes foot and root rot of pea and F. oxysporum f. sp. pisi causes wilting of pea. Germination of the fungal spores depends on the root exudate and in the case of pea roots, also heavily on the border cells. The fungi form a hyphal mantle around the roots and this is the location where specific pathogenicity genes are expressed. It is known that some pathogenicity genes are present in both Fusarium strain and are possibly inherited by horizontal gen transfer, which makes these two strains highly interesting pea pathogens. We will compare the gene expression of these strains, together with a non-host F. oxysporum strain, during germination in root exudate and infection of border cell, and during germination in control buffer without roots. In this way we hope to find genes that are important for the recognition of the root exudate and border cell and other genes that are correlated to pathogenicity gene expression.

Using Illumina Sequencing To Measure Genetic Finesses; One Genome at a Time!

Speaker: Evan Brutinel

Tn-seq is a relatively new technique which utilizes Illumina technology to measure genetic fitness on a genome wide scale through massively parallel sequencing. The preparation of samples for sequencing and subsequent data analysis are substantially different than for other Illumina applications. Using the resources of the Minnesota Super-computing Institute (MSI) Tn-seq can be performed in house with a substantial reduction in effort and cost.

Making Sense of Anti-sense in Enterococcus faecalis

G. Dunny, L. Cook, K Frank, S Grindle, A Khodursky, W-S Hu, and A Chatterjee.
Microbiology, Chem Engineering, BBMBB, and BTI

Speaker: Gary Dunny

Enterococcus faecalis is a gram positive bacterium that is a natural inhabitant of the GI tract of humans and many other animals, as well as of many environmental niches. It is non-pathogenic in healthy individuals, but is a leading cause of hospital infections, and plays a major role in the spread of antibiotic resistance. E. faecalis is particularly adept at survival and persistence in many different environments that would be lethal for most of its phylogenetic relatives. Our laboratory is interested in the genetic basis for the robust ability to sense and adapt to different external cues. In a long-standing analysis of a peptide-based cell-cell signaling system that regulates plasmid transfer, we have recently shown that organization of regulatory transcription units in a convergent and overlapping fashion endows the system with multiple layers of posttranscriptional regulatory mechanisms that amplify the modest direct effect of the peptide signals and allow the system to function as a sensitive biological switch. We have been interested in whether similar forms of regulation exist throughout the genome of E. faecalis. Specifically we have employed genetic screens, microarray analysis, and recently RNAseq technologies to examine patterns of differential gene expression in adaptation of E. faecalis from growth in liquid laboratory medium to growth in biofilms in vitro or to growth in a mammalian host. Numerous differentially-expressed genes have been identified, and our genetic screen suggests that extensive anti-sense transcription may occur during growth in an animal. These results are preliminary, but suggest that insights gained from our studies of peptide cell-cell signaling may be relevant to adaptation of the organism to these other environments.

Gene expression during the transition to multicellularity

Speaker: William Ratcliff

We examine changes in gene expression during experimental evolution of multicellularity in the yeast Saccharomyces cerevisiae. Two major steps in this transition are the evolution of cellular clusters, and the evolution of high rates of apoptosis, an adaptation that facilitates cluster-level reproduction. We found that cellular adhesion likely occurred through a large reduction in the expression of an endochitinase required for cell separation after division. High apoptosis strains display upregulation of many mitochondrial genes (known to trigger apoptosis), notably a 46-fold increase in cytochrome c expression.

The effects of plant cell wall alterations on plant disease resistance

Speaker: Gerit Bethke

I am studying the role of plant cell wall alterations on plant resistance to pathogens using the model plant Arabidopsis thaliana and 3 pathogens with different lifestyles (Pseudomonas syringae, Alternaria brassicicola, Botrytis cinerea). So far we tested T-DNA insertion lines in more than 100 genes that are potentially involved in cell wall biosynthesis and modification. Pectin seems to be an important component, since we found several T-DNA lines in genes implicated in pectin biosynthesis and methylesterification with increased susceptibility to the bacterial hemi-biotroph Pseudomonas syringae pv. maculicola ES4326. Preliminary data suggest that total pectin methylesterase activity is induced after pathogen treatment (Pseudomonas and Alternaria). In case of Alternaria this induction seems to be JA dependent, since it is not present in the JA biosynthesis mutant dde2.

Analysis of U.S. Genetically Engineered Crop Regulation and Litigation

Speaker: Esther McGinnis

The commercial potential of genetically engineered (GE) crops has not been fully realized in the United States. Over the past decade, environmental litigation dramatically affected the pace of GE crop development, testing and deregulation. The United States Animal and Plant Health Inspection Service (APHIS) has the responsibility to regulate GE organisms that may pose a risk to plant or animal health. However, recent litigation initiated by nongovernmental organizations such as the Center for Food Safety and the International Center for Technology Assessment has exposed APHIS's vulnerability to lawsuits under the National Environmental Policy Act (NEPA) for failing to adequately assess the environmental risks of GE crops. In these cases, APHIS committed two types of mistakes. APHIS did not recognize and address the legally defined environmental risks of novel GE crops. Furthermore it did not fully appreciate NEPA's sweeping scope and focus on procedural compliance to ensure transparent and thorough environmental decision-making. We conclude that APHIS impeded the development and commercialization of GE crops not by overly strict regulation, but rather through lapses in regulatory oversight that left the agency and genetically engineered crops subject to costly and lengthy litigation.

Targeted deletion of tandemly arrayed genes in Arabidopsis using zinc finger nucleases

Speaker: Yiping Qi

Tandemly arrayed homologous genes pose a big challenge for plant genetic studies. Here we present a novel strategy to delete tandemly arrayed gene clusters using site-specific zinc finger nucleases. The success of this approach demonstrates this method is very useful not only in basic research, but also in agriculture application.

Co-expression network analysis reveals rewiring of maize transcriptome by domestication

Roman Briskine, Ruth Swanson-Wagner, Robert Schaefer, Peter Tiffin, Nathan Springer, Chad L Myers

Speaker: Roman Briskine

Traditional methods of comparative genomics exploit the abundance of sequence data to extend our knowledge about gene functions and evolutionary processes. However, gene expression profiling represents an attractive alternative for the development of complementary methods. One of the particularly interesting questions addressed by comparative genomics concerns the effects of domestication on wild progenitors. Standard sequence-based methods identified several thousand candidate genes that underlie the process of maize domestication from its wild ancestor, teosinte. Nevertheless, further research is necessary to narrow the list of candidates and extend the characterization of targeted genes. In this study, we built co-expression networks based on high-quality expression data from the diverse varieties of maize and teosinte. Global comparisons of the co-expression networks revealed evidence for transcriptome rewiring due to domestication. We also developed a novel bootstrapping approach to identify specific candidate genes that presumably were direct targets or downstream effects of selection during domestication. We show that our candidate gene list overlaps considerably with domestication genes identified through previous independent sequence-based analyses, an enrichment that was not obtained using extant approaches for comparative expression analysis. Our study demonstrates the utility of expression analysis applied to the question of maize domestication and provides new insights into robust methods for comparing expression patterns across species.

POSTER SESSION

Are photomorphogenic responses to UV-B the consequences of DNA damage? Jessica Biever, Graduate Student, Plant Biological Sciences, PI: Gary Gardner

Discovery of Biuret Hydrolases in Diverse Protein Families Detect Food Toxicant, Cyanuric Acid. Stephan Cameron, Graduate Student, Biochemistry, Molecular Biology and Biophsyics, PI: Lawrence Wackett.

Optimizing functional genomics screening strategies for drug target prediction. Raamesh Deshpande, Graduate Student, Computer Science, PI: Chad Myers

From Arabidopsis to Camelina: Translating our knowledge of trichome development. Kevin Dorn, Graduate Student, Plant Biological Sciences, PI: David Marks.

Heritable epigenetic variation among maize inbreds. Steve Eichten, Graduate Student, Plant Biological Sciences, PI: Nathan Springer

Death, Disease, and Defective Plants. Rachel Hillmer, Graduate Student, Plant Biological Sciences, PI: Fumiaki Katagiri

The mar Locus of Escherichia coli is Modulated Through Acquisition of a Multidrug Resistance-Encoding IncA/C Plasmid. Timothy Johnson, Assistant Professor, Veterinary Biological Sciences

Sythetic Ecology: Engineering a Cooperative System Between Shewanella and Geobacter. Aunica Kane, Graduate Student, Biochemistry, Molecular Biology and Biophsyics, PI: Jeffrey Gralnick

POSTER SESSION

Characterization of an O-GlcNAc Transferase in the cyanobacteria Synechococcus elongatus. Kerry Sokol, Graduate Student, Molecular, Cellular and Developmental Biology, PI: Neil Olszewski

Genetic Control of Maize Shoot Apical Meristem Architecture. Addie Thompson, Graduate Student, Agronomy and Plant Genetics, PI: Gary Muehlbauer

Interactions between genotype and chemical environment in Saccharomyces cerevisiae. Benjamin Vandersluis, Graduate Student, Computer Science, PI: Chad Myers

Alternative pathways for generating medium chain alcohols from amino acid precursors. Jiashi Wei, Graduate Student, BMBB, PI: Brett Barney.

Primitive multicellularity of Escherichia coli. Xiao Yi, Graduate Student, Ecology and Evolutionary Biology, PI: Antony Dean

Population genetic structure across the genome of Medicago truncatula. Jeremey Yoder, Post Doctoral Associate, Plant Biology, PI: Peter Tiffin

Population-Scale Deep Sequencing Reveals Extensive Structural Variation in Medicago species. Peng Zhou, Graduate Student, Plant Pathology, PI: Nevin Young

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