4th Annual Retreat

Saturday, April 4, 2009

Hubert H. Humphrey Institute
Conference Center
Cowles Auditorium

Retreat Schedule

8:30 – 9:05 Breakfast and Poster set up (Atrium)

9:05 – 9:10 *Introductory Remarks* – Neil Olszewski, Co-Director, MPGI

9:10 – 9:30 *"Interdisciplinary Research and Informatics at the University"*- Anne-Francoise Lamblin, Coordinator, Office of the Vice President for Research

9:30 – 10:00 *"Genome-wide Inference of Regulatory Networks in Streptomyces coelicolor"* – Marlene Castro, Graduate Student, PI: Wei-Shou Hu, Chemical Engineering and Material Science

10:00 – 10:30 *"Chemical Ecology of Fungi: A Metabolomic Approach"* – Alma Rodriguez Estrada, Post-Doctoral Associate, PI: Georgiana May, Ecology Evolution and Behavior

10:30 – 10:45 Break (Atrium)

10:45 – 11:15 *"A new approach to fermentation: redox balance through the microbe-electrode interface"* – Jeffrey Flynn, Research Assistant, PI: Jeffrey Gralnick, Biotechnology Institute

11:15 – Noon *"A Highly Interconnected Plant Defense Signaling Network"* – Fumiaki Katagiri, Associate Professor, Plant Biology

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

1:30 – 2:00 *"Loops, rings, and centromere biorientation"* – Laura Burrack, Post-Doctoral Fellow, PI: Judith Berman, Genetics, Cell Biology and Development

2:00 – 2:30 *"Integration of PPI network and expression data for discovery of active subnetworks as biomarkers"* – Rohit Gupta, Graduate Student, PI: Vipin Kumar, Computer Science and Engineering

2:30 – 3:00 *"Phloem protein NaKR1 functions in root development and controls Na+ and K+ accumulation"* – Hui Tian, Graduate Student, PI: John Ward, Plant Biology

3:00 – 3:30 *"Modeling Regulatory Role of Transcriptional Interference in Conjugational Transfer of Drug Resistance in Enterococcus faecalis"* – Anushree Chatterjee, Graduate Student, PI: Wei-Shou Hu, Co-PI: Yiannis Kaznessis, Chemical Engineering and Material Science

3:30 – 3:35 *Closing Remarks* – Neil Olszewski, Co-Director, MPGI

Interdisciplinary Research and Informatics at the University

Anne-Francoise Lamblin, Coordinator, Office of the Vice President for Research

Speaker: Anne-Francoise Lamblin

Genome-wide Inference of Regulatory Networks in Streptomyces coelicolor

Marlene Castro, Salim Charaniya, and Wei-Shou Hu
Department of Chemical Engineering and Materials Science, University of Minnesota

Speaker: Marlene Castro

Streptomycetes, soil-living organisms, are noted for their morphological differentiation and their versatility in the production of secondary metabolites. Regulation of these and other biological processes occurs through dynamic, interconnected networks; however information on the elements integrating those networks is sparse. In this work transcriptome information was combined with genome features to infer regulatory networks.
More than 500 transcriptome data exploring the temporal dynamics of wild-type and mutant strains as well as growth in a variety of media and stress conditions were obtained from in-house and public databases. Gene expression data was combined with intergenic distance, gene function, and synteny to construct a whole genome operon map. Trancriptome profiles at the operon level were used to infer regulatory networks around 692 regulatory elements based on mutual information calculations and data process inequality in ARACNE. The resulting network follows a power law distribution, with a few highly connected regulators. The highest interconnected networks contain more than 50 elements and are constructed around signaling-related elements, like kinases and two-component systems. More than 100 of the inferred networks present significant enrichment in biological functions such as secondary metabolism, chaperones, and defense response. Known interactions were retrieved in more than a dozen networks, including those between the pathway specific regulators cdaR, actII-ORF4, redD and redZ, and their respective antibiotic biosynthesis clusters. Interactions including two-component systems were also retrieved, including the known interactions between absA and the CDA cluster, and vanRS and the vancomycin resistance genes. The interaction between the sense-antisense transcripts scbR and scbA was retrieved. The presence of known consensus sequences in the upstream region of the inferred networks was evaluated and potentially new consensus sequences are proposed. This work integrating genome features and gene expression data will help uncover the cascades regulating secondary metabolism and its interaction with other biological processes. Production of secondary metabolites and morphological differentiation are well known characteristics of Streptomycetes. These soil-living organisms possess some of the largest bacterial genomes sequenced so far and the number of encoded genes in each surpasses that of lower eukaryotes. Few of the more than 900 regulatory genes of Streptomyces coelicolor have been studied so far and of those studied not all the genes they regulate have been uncovered. In this work we combine genome and transcriptome information to infer regulatory networks. More than 500 transcriptome profiles probing temporal dynamics, growth conditions, and mutants were used together with intergenic distance, function and other features to construct a whole-genome operon map. Trancriptome profiles at the operon level were used to infer regulatory networks around 691 regulatory elements, or hubs. More than 100 of the inferred networks present significant enrichment in biological functions such as secondary metabolism, chaperones, and defense response. Literature search for known or suggested interactions resulted in more than a dozen networks with known interacting elements. The retrieval of known interactions allows the assessment of the inferred networks and further filtering for direct interactions. The presence of some known consensus sequences in the upstream region of the inferred networks was evaluated and potentially new consensus sequences are proposed. This work recovered well known regulatory interactions and consensus sequences and unveils potential new targets for both well studied and unstudied regulators.

Chemical Ecology of Fungi: a Metabolomic Approach

Rodriguez Estrada, Alma E.
University of Minnesota, Department of Ecology, Evolution and Behavior

Speaker: Alma Rodriguez Estrada

Fusarium verticillioides is a pathogen of maize. However, non-pathogenic isolates of this species grow as endophytes in corn plants (Zea mays) influencing disease severity caused by Ustilago maydis (corn smut). The interaction mechanisms of these fungal species in planta are not understood but it is expected that secondary metabolites (toxins, pigments, antibiotics, etc.) may play important roles. We sought to explore these chemical compounds through a global approach (metabolomics) that would allow a broad understanding of their activities. We have adapted and validated a method to monitor in vitro changes in concentration of chemical compounds (identified and unidentified) when these fungi are grown in single and co-inoculated cultures. An UPLC/TOF/MS (Ultra High Performance Liquid Chromatography/Time of Flight/Mass Spectrometry) instrument was used for data collection and data processing was done with the software MarkerLynks (Waters Corporation). U. maydis chromatograms where highly complex containing approximately 100 peaks while chromatograms of F. verticillioides had less than 40. Thousands of markers (accurate masses that may include fragments and abducts) were detected across samples making data interpretation extremely difficult. Principal Component Analysis (PCA), PLS-DA (Projection to Latent Structures-Discriminant Analysis) and semi-quantitative analysis of identified compounds were also undertaken demonstrating that integration of diverse strategies is mandatory to comprehensively understand complex metabolic data.

A new approach to fermentation: redox balance through the microbe-electrode interface

Jeffrey Flynn, Research Assistant, Microbial Engineering
P.I. Jeffrey Gralnick

Speaker: Jeffrey Flynn

Traditional fermentations are bound by stoichiometric conservation constraining the feedstocks used and products able to be produced effectively. This creates processes where yields are lower than theoretical maximums and costly separations are required to produce pure products. In this study we sought to use Shewanella oneidensis grown on an electrode as biocatalysts allowing for previously unachievable biotransformations. Bacteria from the genus Shewanella are known to transfer electrons to insoluble substrates such as iron oxide and electrodes. The conversion of glycerol to ethanol is not stoichiometrically balanced and requires the removal of a pair of electrons; Shewanella's unique ability to transfer these electrons to an electrode should allow it to balance the fermentation producing a pure ethanol product while generating an electric current. A number of challenges needed to be overcome to test this hypothesis; most notably conferring the abilities to both consume glycerol and excrete ethanol. Through genomic and metabolic pathway study, genes from E. coli and Z. moblis were selected for cloning and introduced into S. oneidensis. Genes were also selected for deletion to ensure ethanol as the sole production product. The strain comprising all of these features is soon to be tested and the success of this experiment will serve as proof that bacteria grown on an electrode can serve as an effective biocatalyst in non-redox balanced bio-conversions.

A Highly Interconnected Plant Defense Signaling Network

Kenichi Tsuda1, Masanao Sato1, 2, Thomas Stoddard1, Jane Glazebrook1, and Fumiaki Katagiri1
1Department of Plant Biology, Microbial and Plant Genomics Institute, University of Minnesota, 1500 Gortner Avenue, St. Paul, MN 55108, USA; 2Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153-8902, Japan.

Speaker: Fumiaki Katagiri

Upon recognition of pathogen attack, plants induce a battery of defense mechanisms to fend off the pathogen. The signaling network that controls the inducible defense is highly interconnected. In such a highly interconnected signaling network, it is often misleading to deduce the function of a wild type gene based on the phenotype of its single mutant. This is because knocking out a single gene deprives not only the effect of the wild type gene but also all the interactions of the wild type gene with other genes in the network at the same time. We used an Arabidopsis quadruple mutant dde2/ein2/pad4/sid2 to show that whereas single mutations have small or no effects, the quadruple mutation can have very large effects on two types of resistance. These results also indicate that different types of resistance extensively share their signaling machineries. By measuring the resistance level of plants with all the possible combination of four mutations and fitting a mixed general linear model to the data, we were able to estimate the contributions to resistance of the effects of single wild-type genes and their interactions. This signaling allocation analysis revealed that each of the four genes can positively contribute to different types of resistance and that different types of resistance have qualitatively different signaling allocation patterns that are quantitatively different between cases of the same type of resistance. Therefore, different types of resistance share the same signaling machineries but how the machineries are used is different among different types. We also noticed that negative genetic interactions among the wild-type genes are very common. This is the basis of resistance robust against perturbations to the signaling network – i.e., with negative interactions, knocking out of one of the genes does not have a strong effect on the resistance phenotype. In another line of research, we investigated relationships among the expression profiles from 21 defense mutants of Arabidopsis. We found that negative regulations between different signaling sectors are prevalent. These negative regulations in combination with the positive contributions of the single sectors at least partly explain the negative genetic interactions among the signaling sectors.

Loops, rings, and centromere biorientation

Dr. Laura Burrack
Postdoctoral fellow
Genetics, Cell Biology and Development
Advisor: Dr. Judith Berman

Speaker: Laura Burrack

In order for proper chromosome segregation to occur, one sister chromatid from each pair must be attached to each spindle pole. Improper microtubule attachments can lead to aneuploidy and cell death. In the budding yeast Saccharomyces cerevisiae, it has recently been shown that looped DNA structures are formed near centromeres. These loop structures are thought to be stabilized by cohesin rings and have been proposed to provide an optimal chromosomal conformation for sister chromatid biorientation. However, S. cerevisiae centromeres are distinct from characterized centromeres in other organisms, including mammals and plants, in that they are sequence-specific point centromeres, rather than epigenetically determined regional centromeres. In this work, we use Candida albicans, an opportunistic fungal pathogen, to ask if loops form near the centromere DNA of an organism with small regional centromeres. Our results indicate that looped DNA structures are found near C. albicans centromeres and suggest that chromosomal conformation may be a conserved mechanism of promoting proper centromere biorientation. We are currently in the process of determining the mechanism of loop formation near C. albicans regional centromeres.

Integration of PPI network and expression data for discovery of active subnetworks as biomarkers

Speaker: Rohit Gupta

In recent years, due to the rapid advancements in high throughput experimental technologies, huge quantities of genomic, genetic and proteomic data sets have become available. Most of these diverse biological data sets provide a different but complimentary view of the genome and functions of its various components. However, it is important to note that complex problems like biomarker discovery and others require more information than provided by any individual type of data source. For example, functional modules or biomarkers identified using combined information from gene expression and protein-protein interaction data are shown to be more reliable and biologically plausible than those obtained from individual data sources. This is because both gene expression data and protein interaction data are noisy and hence the identified groups of genes that are confirmed by both are more likely to be true than random. In this work, we will first review some of the recent work and then will propose data mining based association analysis technique to integrate multiple biological data sets for the task of biomarker discovery. Some preliminary results on biomarkers for breast cancer are reported.

Phloem protein NaKR1 functions in root development and controls Na+ and K+ accumulation

Hui Tian (PBS graduate student), John M. Ward

Speaker: Hui Tian

Potassium (K+) is an essential element for plant growth and development. It is taken up from the soil by means of active transport, and translocated from the root to shoot tissues through the transpiration stream. From the major source rosette leaves K+ is redistributed into young flowers and roots through phloem loading. Sodium(Na+), because of its similarity with K+ and its high abundance in many environments, can be taken up by K+ transporters. As a nonessential element, its accumulation in plants causes toxic effects. In our study, an Arabidopsis mutant accumulating high amount of K+, Na+ and Rb+ in rosette leaves has been identified. The mutant exhibits multiple developmental defects, including short roots, changed root and shoot architecture and late flowering phenotype. By using DNA chip based mapping, a single deletion was detected which cause loss of function of a gene Na+ K+ Root defective-1 (NaKR1). NaKR1 encodes a heavy metal associated domain containing protein. Previous studies indicated this metal binding domain can specifically bind with Cu+/Zn2+/Ni+, and is responsible for protein interaction as well as metal transfer. By studying the expression patterns of NaKR1-GUS fusion protein we found the protein is specifically expressed in phloem tissues. Transient expression of NaKR1-GFP in Arabidopsis seedlings indicated it's a soluble protein localized in nucleus and cytosol. By introducing NaKR1 gene back into the mutant background, both the ionomic and developmental defects are complemented. Further work is being done to characterize the function of the encoded protein to understand how a metal binding phloem protein influences K+ and Na+ accumulation.

Modeling Regulatory Role of Transcriptional Interference in Conjugational Transfer of Drug Resistance in *Enterococcus faecalis*

PI: Wei-Shou Hu
Co-PI: Yiannis N. Kaznessis
Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455 USA

Speaker: Anushree Chatterjee (3rd year Graduate student)

Tetracycline resistance encoding plasmid pCF10 in E. faecalis is highly proficient in transferring mobile genetic elements and drug resistance through conjugation. The key genes prgQ and prgX, involved in regulating conjugation in E. faecalis, are driven by convergent promoters, PQ and PQA respectively. Transcription from PQA gives rise to a long prgX mRNA and a short non-coding Qa RNA, which is antisense to Q, the transcript produced from PQ. In vivo LacZ reporter studies demonstrate that antisense interaction with Qa causes transcriptional termination of an elongating Q, resulting in a shorter Qs transcript under uninduced, conjugation incompetent state. Under induced conditions and in the absence of Qa, a longer Ql transcript is formed, which is required for expression of downstream conjugation genes. The antisense interaction of Qa and Q is affected by their abundance levels, however, the mechanism of this antisense regulation (AR) is not clearly understood. The transcription of convergent PQ and PQA promoters results in distinct probability of aborted transcription due to collision between transcribing RNA polymerases (RNAp), a phenomenon referred to as transcription interference (TI). We developed a discrete mathematical model to quantitatively assess the frequency of RNAp collision and evaluate its effect on transcription efficiency under different relative strength of the promoters PQ and PQA. Our model predicts that in the uninduced state, promoter PQ is negatively regulated by PrgX protein, causing it to behave as the weaker promoter. In the induced state, PQ is de-repressed and acts as the aggressive promoter. The results indicate that, while TI controls the levels of Q, Qa and prgX; AR controls the levels of Qs and Ql. Currently the results obtained from the model are being compared to experimental analysis of the Qa and Q transcripts in vivo.

Poster Session

Wen-Ping Chen, *"High-throughput measurement of protein turnover in plants using stable isotope labeling coupled with LC-MS/MS analysis"*, Post-Doctoral Associate, PI: Jerry D. Cohen, Department of Horticultural Science

Benjamin Clausen, *"Genetic Diversity Within and Among Populations of Tanacetum vulgare (common tansy)"*, Research Assistant, PI: Alan Smith, Department of Horticultural Science

Daniel Coursolle, *"Reduction of soluble and insoluble substrates by Shewanella oneidensis: involvement of MtrA and electron shuttles"*, Graduate Student, PI: Jeff Gralnick, Biotechnology Institute

Junfeng Gao, *"Improving Biodegradation Pathway Prediction"*, Research Assistant, PI: Lynda Ellis, Institute for Health Informatics

Liangliang Gao, *"DArT Based Maps Of Wild Potato Species Facilitate Genome Structure Comparisons In The Genus Solanum"*, Research Assistant, PI: Jim Bradeen, Department of Plant Pathology

Stephanie Gardiner, *"Barley Exhibits a Variety of Resistance Mechanisms to Deoxynivalenol"*, Graduate Student, PI: Gary Muehlbauer, Department of Agronomy and Plant Genetics

Ju-Hoon Lee, *"Metabolic Engineering of Lactococcus lactis for the Development of a One-Step Bioconversion of Lactose into Tagatose"*, Post-Doctoral Associate, PI: Daniel O'Sullivan, Department of Food, Science and Nutrition

Benjamin Millett, *"Wild barley expresses distinct sets of genes in response to pathogens of different trophic lifestyles"*, Research Associate, PI: Gary Muehlbauer, Department of Agronomy and Plant Genetics

Carol Powers, *"Patterns of LD Within a Barley Breeding Program: Impact of Breeding History"*, Graduate Student, PI: Kevin Smith, Department of Agronomy and Plant Genetics

Yiping Qi, *"Purification of resistance protein complexes using a biotinylated affinity tag"*, Graduate Student, PI: Fumiaki Katagiri, Department of Plant Biology

Summer St. Pierre, *"Surveying Genetic and Phenotypic Variation for Response to Plant Density Stress in Barley"*, Research Assistant, PI: Gary Muehlbauer and Nathan Springer, Department of Agronomy and Plant Genetics

Xiaoqing Sun, *"Production of hydrogen by the green alga Chlamydomonas reinhardtii"*, Graduate Student, Graduate Student, PI: Carolyn Silflow and Paul Lefebvre, Department of Plant Biology

Toi Tsilo, *"Molecular Mapping of the Stem Rust Resistance Gene Sr6 on Chromosome 2DS in Wheat"*, Graduate Student, PI: Jim Anderson, Department of Agronomy and Plant Genetics