

New York Area Plant Molecular Biology  
2004 Organizers

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NYAPMB 2004 Website Design

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*The New York Area Plant Molecular Biology 2004 Meeting schedule is subject to change.*



January 17, 2004  
10 A.M. to 5 P.M.

The Waksman Institute of Microbiology  
Rutgers, the State University of New Jersey

## **New York Area Plant Molecular Biology 2004 Program**

### **9:15 A.M. Registration**

*Register at the Waksman Institute*

*Set up posters in Room 1001*

*Coffee and refreshments in Room 1001.*

### **10:00 A.M. Session I - Waksman Institute Auditorium**

#### **Welcome**

#### **Arvind K. Bharti**

*Waksman Institute, Rutgers University*

High resolution physical mapping of the maize genome and sequencing a part thereof

#### **Jinsheng Lai**

*Waksman Institute, Rutgers University*

Extensive shuffling of gene order in genomes of grass family

#### **Yuling Jiao**

*Yale University*

Transcription analysis of rice chromosome 4 using a genomic fragment-based tiling microarray

#### **Amitabh Mohanty**

*Cold Spring Harbor Laboratory*

Characterization of the Arabidopsis proteome by fluorescent protein tagging

#### **Amy Litt**

*Yale University*

Duplication and diversification in the *APETALA1/FRUITFULL* floral homeotic gene lineage: implications for the evolution of floral development

*A brief meeting of all the Principal Investigators will take place after Session I, just before lunch.*

### **11:30 A.M. Lunch & Poster Session I - Room 1001**

### **1:15 A.M. Session II - Waksman Auditorium**

#### **Vikas Agrawal**

*University of Delaware*

Systems biology approaches to understanding the Arabidopsis hypersensitive response

#### **Mike Li**

*Rutgers University*

Differential gene expression in a disease resistant colonial x creeping bentgrass hybrid relative to its creeping parent

#### **Tessa M. Burch-Smith**

*Yale University*

The requirement for the molecular chaperone Hsp90 in N-mediated resistance to TMV

#### **Rajendra Marathe**

*Yale University*

Plant P58IPK is a suppressor of RNA silencing

#### **Angela G. Peragine**

*University of Pennsylvania*

A role for post-transcriptional gene silencing in vegetative phase change

### **2:45 P.M. Coffee Break & Poster Session II - Room 1001**

### **3:30 P.M. Session III - Waksman Auditorium**

#### **Wolfgang Lukowitz**

*Cold Spring Harbor Laboratory*

A GATA-factor is mediating axis formation in the early Arabidopsis embryo

**Alice J. Paquette**

*New York University*

GA influences the timing of the termination of the root apical meristem and changes in ground-tissue cytoarchitecture

**Fang Qi**

*Queens College, The City University of New York*

*lid2*, a phytochrome signal transduction pathway mutant

**Francine M. Carland**

*Yale University*

*CVP2*-mediated  $IP_3$  signal transduction is essential for closed venation patterns of *Arabidopsis* foliar organs

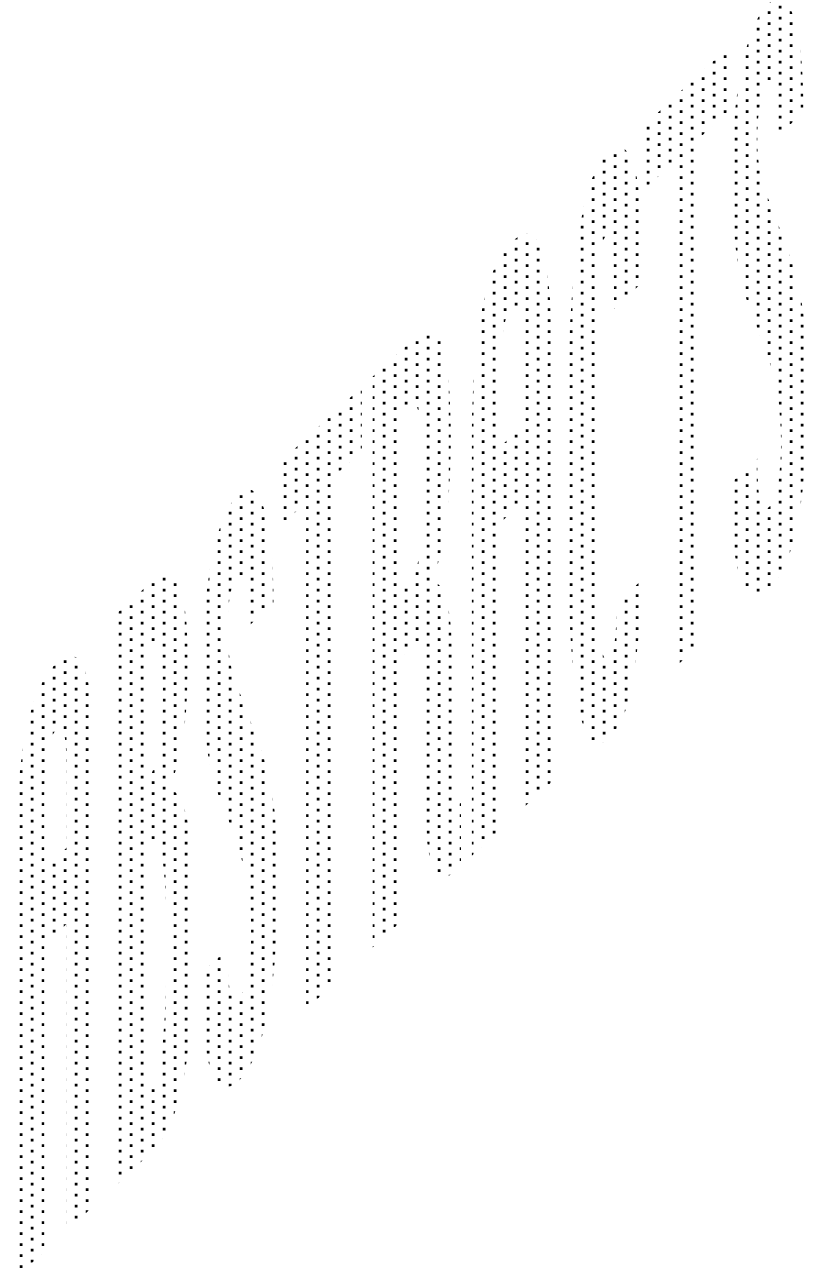
**Michelle T. Juarez**

*Cold Spring Harbor Laboratory*

Adaxial/abaxial specification of the maize leaf

**5:00 P.M. Concluding Remarks & Announcements**

*Posters must be removed by 5:30 P.M.*



## High resolution physical mapping of the maize genome and sequencing a part thereof

Arvind K. Bharti<sup>1</sup>, Fusheng Wei<sup>2</sup>, Ed Butler<sup>2</sup>, Yeisoo Yu<sup>2</sup>, Jose L. Goicoechea<sup>2</sup>, HyeRan Kim<sup>2</sup>, Galina Fuks<sup>1</sup>, Will Nelson<sup>3</sup>, Jamie Hatfield<sup>3</sup>, Heidrun Gundlach<sup>4</sup>, Wojciech M. Karlowski<sup>4</sup>, Christina Raymond<sup>5</sup>, Sarah Towey<sup>5</sup>, David Jaffe<sup>5</sup>, Chad Nusbaum<sup>5</sup>, Bruce Birren<sup>5</sup>, Klaus Mayer<sup>4</sup>, Cari Soderlund<sup>3</sup>, Rod A. Wing<sup>2</sup> and Joachim Messing<sup>1</sup>

<sup>1</sup>The Plant Genome Initiative at Rutgers, Waksman Institute, Rutgers University, Piscataway, NJ; <sup>2</sup>Arizona Genomics Institute, <sup>3</sup>Arizona Genomics Computational Laboratory, University of Arizona, Tucson, AZ; <sup>4</sup>Munich Information Center for Protein Sequences, Institut für Bioinformatik, Neuberberg, Germany; <sup>5</sup>MIT/Broad Institute Center for Genome Research, Cambridge, MA

Due to its economic significance, maize (>2 Gb) is likely to be the next cereal to be sequenced after rice (0.4 Gb). Sequencing the maize genome will present a new challenge not only because it is 5-times larger but also because it contains many gene families, tandemly arrayed and nested repeat sequences. To establish a framework to sequence maize, we are developing a high information-content fingerprinting (HICF)/BAC sequence tagged connector (STC)-based physical map. This map will be fully integrated with the genetic map and is complementary to previous work by the NSF funded Maize Mapping Project ([www.genome.arizona.edu/fpc/maize](http://www.genome.arizona.edu/fpc/maize)). MMP generated a genetically anchored BAC-based physical map by fingerprinting three deep coverage BAC libraries (*HindIII*, *EcoRI*, and *MboI*) using a lower resolution “agarose” method. The HICF method builds upon the MMP physical map by generating HICF fingerprints of the identical libraries, which allows for a more precise determination of a minimum tiling path of BAC clones across the entire genome. The current success rate of HICF is ~86%, which has already yielded 382,696 successful fingerprints. Preliminary investigation suggests that many HICF megacontigs are likely to join two or more agarose-based contigs and thus should result in the reduction of the total number of contigs. Additional contigs are expected to be anchored based on alignments of STCs to the rice genome. To evaluate the MTP generated by the HICF/agarose physical map, 56 BACs are being sequenced from a ~5 Mb mega-contig, in addition to 100 randomly selected BACs (>10 Mb). All sequence information generated from this project, from both BAC ends and whole BACs, is being annotated, which will provide invaluable data on the sequence organization of the maize genome.

## Extensive shuffling of gene order in genomes of grass family

Jinsheng Lai<sup>1</sup>, Zuzana Swigonova<sup>1</sup>, Jianxin Ma<sup>2</sup>, Jeffrey Bennetzen<sup>2</sup> and Joachim Messing<sup>1</sup>

<sup>1</sup>Waksman Institute, Rutgers University, Piscataway, NJ; <sup>2</sup>Department of Genetics, University of Georgia, Athens, GA

To provide additional insights into the structure and evolution of plant genomes, we isolated and sequenced 24 BAC clones, representing five orthologous region from maize and sorghum and compared them with the orthologous regions from rice. In these regions (more than 4 Mb in length), we have identified putative 212 genes. However, we find that at least 25% of the genes differ in presence/absence in otherwise colinear regions. We further find that most of the non-colinear genes, identified within the five regions in maize and sorghum, are indeed present in non-orthologous positions of the rice genome. They do not cluster in any chromosomal region but appear randomly distributed throughout the rice genome. Several genes were found to be colinear only between maize and sorghum. In contrast to the non-colinear genes, the colinear genes can be determined by a comparison of any two distantly-related genomes (rice-sorghum, or rice-maize). Although there have been many gene losses during the maize diploidization processes from both subgenomes, each colinear gene can be found on at least one of the two subgenomes. We hypothesize that genes in grass genomes can move around the genome as small translocations, often involving only a single gene. Such a dynamic nature of the grass genomes could provide exceptional potential for the evolution of new genetic functions.

## Transcription analysis of rice chromosome 4 using a genomic fragment-based tiling microarray

Yuling Jiao<sup>1</sup>, Peixin Jia<sup>2</sup>, Thomas E. Royce<sup>3</sup>, Paul M. Harrison<sup>3</sup>, Shuliang Yu<sup>2</sup>, Ligeng Ma<sup>1</sup>, Ning Su<sup>1</sup>, Ying Gao<sup>1</sup>, Mark Gerstein<sup>3</sup>, Bin Han<sup>2</sup> and Xing Wang Deng<sup>1</sup>

<sup>1</sup>Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT; <sup>2</sup>National Center for Gene Research, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; <sup>3</sup>Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT

The complete sequence of cultivated rice species *Oryza sativa* genome provides an unprecedented opportunity to study and improve our cereal crops. An essential step towards deciphering the biological information encoded in this nucleotide-level data is to obtain an accurate catalogue of genes and their respective expression patterns. Computational methods are still not sufficiently trained and informed for rice genome annotation, therefore there is a critical need for experimental validation. Here, we report the transcriptional activity of rice chromosome 4 using a genomic DNA fragment-based tiling microarray. Representative organ samples of rice and in vitro cultured cells were examined to catalogue the transcribed regions of rice chromosome 4, and to reveal organ- and developmental stage-specific transcription patterns. Our analysis revealed that 38% of all the genomic fragments with detected transcription lack any previous cDNA or EST support. Among them, about two-thirds also lack computational gene predictions. Further, a high percentage of rice genes that do not have homologues in the Arabidopsis genome are transcribed during rice development, supporting functional roles of that group of genes. Comparison of gene expression among the examined organ types revealed both common and organ-specific expressed genes in chromosome 4.

## Characterization of the Arabidopsis proteome by fluorescent protein tagging

Amitabh Mohanty<sup>1</sup>, Vitaly Citovsky<sup>2</sup>, Georgia Drakaki<sup>4</sup>, David Ehrhardt<sup>3</sup>, David Jackson<sup>1</sup>, Shijun Li<sup>3</sup>, Brigitte Paap<sup>2</sup>, Natasha Raikhel<sup>4</sup>, Sue Rhee<sup>3</sup>, Narasimha Chary Samboju<sup>4</sup> and Guo-wei Tian<sup>2</sup>.

<sup>1</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; <sup>2</sup>Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY; <sup>3</sup>Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA; <sup>4</sup>Department of Botany and Plant Sciences, University of California, Riverside, CA

Arabidopsis has 25,498 predicted genes, however, only about 70% of the genes have been assigned to any functional category, and less than 10% of the genes have been verified experimentally. There are only very limited reports of systematic analysis of genes of unknown function. Moreover, for the majority of the annotated proteins, the sub-cellular location and/or expression pattern is unknown. Thus, a large gap remains in our understanding of the function of a very significant portion of Arabidopsis gene products, many of which are plant specific and likely play fundamental roles in plant biology. Our consortium aims to fill this gap by systematically analyzing a large number of the functionally unassigned genes by Fluorescent Tagging of their Full-Length Protein products (FTFLP). We plan to characterize the Arabidopsis proteome by seeking three specific aims: 1. Selection and sub-cloning of Arabidopsis genes of unknown function with their potential native regulatory sequences. 2. Fluorescent tagging (using Yellow or Cyan fluorescent proteins) of the genes and insertion into Arabidopsis plants. 3. Analysis of the expression patterns and sub-cellular localization of the YFP/CFP-tagged proteins *in planta*. Based on the most recent Arabidopsis genome annotations, more than 8,000 genes are annotated as "unknown" or "putative" protein. We have selected a short list of ~800 for initial characterization.

As a pre-requisite we have constructed a reference localization map with ~20 Arabidopsis proteins with known sub-cellular localization patterns. This map will serve as a reference for co-localization studies. For each full-length gene, including upstream and downstream native regulatory sequences are being amplified by PCR from genomic DNA. We use these PCR fragments together with YFP or CFP fragments as templates for a second round of PCR (Triple template overlap PCR) for the insertion of the tag into the gene. Under the control of native promoters, the expression levels of some proteins could be too weak for visualization, so we are also using vectors with multiple copies of the 35S enhancer, to enhance the native expression of target genes. Our recent data from this project will be presented.

## Duplication and diversification in the *APETALA1/FRUITFULL* floral homeotic gene lineage: implications for the evolution of floral development

Amy Litt and Vivian F. Irish

Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT

Phylogenetic analyses of angiosperm MADS-box genes suggest that this gene family has undergone multiple duplication events followed by sequence divergence. In order to determine when such events have taken place and to understand the relationships of particular MADS-box gene lineages, we have identified *APETALA1/FRUITFULL*-like MADS-box genes from a wide variety of angiosperm species. Our phylogenetic analyses show two gene clades within the core eudicots, eu*API* (including Arabidopsis *APETALA1* and Antirrhinum *SQUAMOSA*) and eu*FUL* (including Arabidopsis *FRUITFULL*). Non-core eudicot species have only sequences similar to eu*FUL* genes (*FUL*-like). The predicted protein products of the eu*FUL* and *FUL*-like genes share a conserved C-terminal motif. In contrast, the predicted products of members of the eu*API* gene clade contain a different C-terminus that includes an acidic transcription activation domain and a farnesylation signal. Sequence analyses indicate that the eu*API* amino acid motif may have arisen via a translational frame shift from the eu*FUL*/*FUL*-like motif. The eu*API* gene clade includes key regulators of floral development that have been implicated in the specification of perianth identity. However, the presence of eu*API* genes only in the core eudicots suggests that there may have been changes in mechanisms of floral development that are correlated with the fixation of floral structure seen in this clade.

## Systems biology approaches to understanding the Arabidopsis hypersensitive response

Vikas Agrawal<sup>1</sup>, Chu Zhang<sup>1</sup>, Prasad S. Dhurjati<sup>2</sup> and Allan D. Shapiro<sup>1</sup>

<sup>1</sup>Department of Plant and Soil Sciences, <sup>2</sup>Department of Chemical Engineering, University of Delaware, Newark, DE

**Motivation** Cross-talk and feedback regulation in signal transduction networks are often quite complex. The signaling network governing Arabidopsis hypersensitive response to avirulent *Pseudomonas syringae* was analyzed using a systems biology approach. A systems approach uses quantitative data collection and mathematical modeling to understand higher order relationships governing network behavior. The goal was to create a framework for efficient design of experiments by simulating the time course progression of the most important components of the response *in silico*. These predictions were then compared to experimental data for validation (Agrawal, *et al.*, *Biotechnology Progress* 2003).

**Strategy** The most important components of the response were taken as model variables. These included death of individual cells (PCD), salicylic acid (SA) and reactive oxygen (ROS) accumulation, and level of apoplastic superoxide dismutase (SOD) (Zhang, *et al.*, *Molecular Plant-Microbe Interactions*, in press). Initial estimates of kinetic parameters and time delays were made from experimental data and subsequently refined by global fitting of simulated to experimental data. This process resulted in a system of ten delay differential equations governed by expert-system type rules. This system was solved numerically using engineering software (MATLAB). A one-to-one correspondence was maintained between model variables and specific signaling components. The mathematical forms for relationships between model variables also corresponded one-to-one with experimentally observed relationships between signaling components. As such, the assumptions questioned by new data can be readily identified.

**Results** 1) *In silico* simulations of the time course of changes in levels of salicylic acid, PCD and hydrogen peroxide match experimental data. 2) We have used the model to prove that direct negative autoregulation of salicylic acid biosynthesis does not exist in this system. Including terms for this extra negative feedback loop made it impossible for simulated data to match experimental results. 3) Simulations also determined that NPR1-dependent negative feedback on PCD cannot affect the fraction of total PCD seen late in HR progression as a direct consequence of high levels of superoxide. 4) The dynamic profiles of apoplastic superoxide dismutase (SOD) activity and two putative gene induction events have been predicted. 5) "Sensitivity" analysis has been used to predict which model components have the most significant influence on overall system dynamics. These predictions will aid in design of further experiments to test our knowledge of control of the HR.

Our current strategy to improve the explanatory power of the model via explicit modeling of cell-to-cell signaling events using population balance modeling will be described.

## Differential gene expression in a disease resistant colonial x creeping bentgrass hybrid relative to its creeping parent

Huaijun Mike Li, David Rotter and Faith C. Belanger

Department of Plant Biology and Pathology, Rutgers University, New Brunswick, NJ

Creeping bentgrass (*Agrostis stolonifera* L.), an important turfgrass species, is highly susceptible to dollar spot disease caused by *Sclerotinia homoeocarpa* F. T. Bennett. Colonial bentgrass (*A. capillaris* L.), a closely related species, has good resistance. Several interspecific hybrids between creeping bentgrass and colonial bentgrass showed excellent dollar spot resistance in field tests. In order to investigate the mechanism of dollar spot resistance in the interspecific hybrids, we are using suppression subtractive hybridization (SSH) to detect genes unique or over-expressed in either resistant hybrids or the creeping parent following fungal inoculation. Two subtraction libraries were created from resistant hybrid (#15) and its creeping parent cDNAs. Nine hundred sixty colonies from the colonial specific library and 480 colonies from the creeping specific library were screened for differential expression using reverse northern dot blotting. On average, 14% of the colonies screened from both libraries were differentially expressed. Differentially expressed clones were sequenced and compared to the GenBank database. Most of the clones sequenced are homologous to genes related to stress resistance in other plants, and so may be relevant to the disease response of the bentgrasses. Further studies will utilize either Northern analysis or real time RT-PCR to confirm the differential expression of these cDNAs. The sequence of these genes can be used in future mapping project as DNA markers. A disease-responsive gene was identified from two of the clones from the creeping bentgrass-specific library. The full-length sequence of the cDNA was obtained through RACE PCR. The amino acid sequence of the protein was homologous to a wheat benzothiazol-induced protein, a wheat Hessian fly response protein, a maize beta-glucosidase aggregating factor, a barley jasmonate-regulated gene, and a wheat vernalization-related gene. Northern blot analysis confirmed its expression was increased in creeping bentgrass upon dollar spot infection. No expression was detected in hybrid #15 or colonial bentgrass from either greenhouse or field samples. These results validated the effectiveness of the SSH method. Southern blot analysis indicated creeping bentgrass parent and another hybrid (#14) each had a single copy of this gene. Interestingly we didn't detect any homologs from related bentgrass species, such as colonial bentgrass, velvet bentgrass (*A. canina*), or redtop (*A. gigantea*). The apparent uniqueness of this gene to creeping bentgrass is very interesting regarding the evolution of creeping bentgrass.

## The requirement for the molecular chaperone Hsp90 in N-mediated resistance to TMV

Tessa M. Burch-Smith, Yule Liu, Michael Schiff, Suhua Feng and S.P. Dinesh-Kumar

Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven CT

The TIR-NBS-LRR R gene, N, confers resistance to the tobacco mosaic virus (TMV) in tobacco and in transgenic *Nicotiana benthamiana*. We performed a yeast three-hybrid screen to identify proteins that interact with the LRR domain of N. This screen of a tomato cDNA library identified LeHSP90 as interacting partner of the LRR of N. Concurrently, we identified LeHSP90 as an interacting partner of NbRar1 in a yeast two-hybrid screen. Rar1 is a conserved signaling component of several R gene pathways and we have previously shown that NbRar1 is required for N-mediated resistance to TMV. On the basis of these two interactions, we checked the interaction of LeHSP90 with NbSGT1, a protein partner of both N and NbRar1. LeHSP90 also interacts with NbSGT1. These yeast interactions were confirmed by *in vitro* pull down and biological significance was demonstrated by coimmunoprecipitating LeHSP90 with N, NbRar1 and NbSGT1 in turn. Further, we show by VIGS of LeHSP90 that it is required for N-mediated resistance. On the basis of our results and considering recently published results on other R genes, we conclude that the highly conserved chaperone HSP90 has an important in R gene signaling. In other efforts to understand the N-TMV interaction, we are also conducting studies to determine the cellular location of the interaction. Preliminary biochemical data indicates that N may be localized to the cytoplasm of uninfected cells.

## Plant P58IPK is a suppressor of RNA silencing

Rajendra Marathe, Radhamani Anandalakshmi and S.P. Dinesh-Kumar

Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven CT

A plant homolog of the bovine P58IPK, a cellular inhibitor of Protein Kinase R (PKR) is a positive regulator of viral pathogenesis. The expression of P58IPK is down-regulated during RNA silencing, an antiviral defense mechanism in plants. When overexpressed, using viral vectors or under constitutive promoter, P58IPK suppresses transgene RNA silencing. The P58IPK-mediated suppression of RNA silencing is correlated with significant reduction but not complete elimination of siRNA. The transgenic plants overexpressing P58IPK show developmental defects.

## A role for post-transcriptional gene silencing in vegetative phase change

Angela G. Peragine, Christine Hunter, Gang Wu and R. Scott Poethig

Department of Biology, University of Pennsylvania, Philadelphia, PA

Genes that regulate the switch from juvenile vegetative to adult vegetative development in *Arabidopsis* have been identified by screening for mutations that cause this transition to occur precociously. Mutations in three genes have identical and fairly specific effects on this transition. One of these—*ZIPPY*—corresponds to *ARGONAUTE7*, a member of the Argonaute protein family. Members of this family have been implicated in the function of both miRNAs and siRNAs. The other mutations we have identified are alleles of *SGS2* and *SGS3*. These genes are required for PTGS and encode, respectively, an RNA-dependant RNA polymerase and a protein of unknown function. Genetic analyses suggest that all three genes act in the same pathway, along with *HASTY*, the *Arabidopsis* orthologue of the miRNA export receptor, Exportin 5. These results suggest that miRNAs or endogenous siRNAs play an important role in vegetative phase change.

## A GATA-factor is mediating axis formation in the early Arabidopsis embryo

Wolfgang Lukowitz<sup>1,2</sup> and Chris Somerville<sup>2</sup>

<sup>1</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; <sup>2</sup> Department of Plant Biology, Carnegie Institution, Stanford University, Stanford, CA

Early development of Arabidopsis proceeds in an almost invariant sequence of cell division and expansion. Axis formation is apparent by the early globular stage, when cells in the lower tier of the proembryo expand along the apical/basal axis. Root formation is initiated at about the same time and involves the uppermost suspensor cell, termed hypophysis, which gives rise to the quiescent center of the meristem. Both processes are dependent on the establishment of polar auxin transport.

Mutations in the *HAN* gene severely disrupt root and axis formation in the early embryo. Cells at the base of the proembryo divide aberrantly producing fewer and irregularly arranged daughters. The cells of the suspensor, including the hypophysis, stop dividing at the 8-cell stage. In contrast to wild type, growth of the mutant embryos is dominated by the upper tier. A variable fraction of the mutants overcomes these early defects and develops into complete and viable seedlings indicating that *HAN* function is not generally required for axis and root formation.

Genetic evidence suggests that *HAN* is a negative regulator of auxin responses. *PIN4*, a presumptive auxin efflux carrier implicated in the formation of an auxin sink, is expressed ectopically in the center of the proembryo. Furthermore, *han* mutations act as suppressors of *monopteros* mutations. *MONOPTEROS* is a positive regulator of auxin responses, and loss of *MONOPTEROS* function results in seedlings without root or hypocotyls. Double-mutant embryos, if they germinate, have a hypocotyl and root.

The *HAN* gene product is similar to transcriptional regulators of the GATA family. In the early embryo, *HAN* transcripts are specifically expressed in the cells of the proembryo. We propose that *HAN* functions in temporarily inhibiting a subset of auxin responses, such as the formation of an auxin sink, to facilitate the establishment of polar auxin transport.

## GA influences the timing of the termination of the root apical meristem and changes in ground-tissue cytoarchitecture

Alice J. Paquette<sup>1</sup> and Philip N. Benfey<sup>2</sup>

<sup>1</sup>New York University, New York, NY, <sup>2</sup>Duke University, Durham, NC

Recent reports suggest that hormones such as auxin and ethylene regulate root growth by altering gibberellin (GA) signaling (Fu and Harberd, *Nature* 2003; Achard, *et al.*, *Plant Cell* 2003). GA responses are repressed by the action of a set of putative transcription factors that make up the DELLA subfamily of GRAS proteins. Two other GRAS family members that regulate root growth are SCARECROW (*SCR*) and SHORT-ROOT (*SHR*). It has been reported that both of these proteins are required for the formation of two layers of ground tissue and for maintenance of the root apical meristem.

While *SHR* does, indeed, appear to be required for these two processes, *SCR* is not strictly essential for either. Analysis of new null alleles of *scr* indicate that, while mutant roots are agravitropic and elongate more slowly during the first week of growth; afterwards, *scr*-mutant roots attain almost wild-type growth rates and exhibit sporadic periclinal division of the ground tissue. This timing parallels a potential phase change in root growth, where the QC becomes two-tiered, and a third layer of ground tissue, the middle cortex, begins to form.

Levels of GA signaling influence the *scr* and *shr* mutant phenotypes. Increased GA signaling can enhance the phenotype of *scr* mutant roots to resemble that of *shr* mutants with respect to meristem termination and ground-tissue architecture. Conversely, reduced GA signaling appears to rescue the initial slow growth rate and ground-tissue defect of *scr* mutants. For *shr*, while increased GA signaling appears not to exacerbate the mutant phenotype, decreased GA signaling may rescue the meristem-maintenance defect. Reduction of GA signaling does not appear to enable periclinal division of the ground tissue, however, in the absence of *SHR*.

## *lid2*, a phytochrome signal transduction pathway mutant

Fang Qi, Bridgit Goldman and Timothy Short

Queens College, The City University of New York, Queens, NY

Light-regulated morphogenesis and gene expression have long been targets for research in plant biology, and recently molecular and genetic techniques have elucidated numerous signaling components in these pathways. Anthocyanin production and the synthesis of chlorophyll and flavonoids are commonly used for studying interactions of photoreceptor sensory pathways with other stimulus-transduction chains such as carbohydrate availability. We have isolated and characterized a novel mutant designated *lid2* (*light response deficient 2*) that is deficient in some, but not all, far-red light-induced responses. This mutant exhibits reduced chlorophyll accumulation in white light, but lacks the repression of chlorophyll production by far-red light. It also exhibits reduced responsiveness to both blue and red light and therefore the chlorophyll accumulation appears to be uncoupled from normal phytochrome A control. Moreover, far-red inhibition of elongation and cotyledon expansion are unaffected in the *lid2* mutants. Initial mapping data suggest that the *LID2* gene codes for a protein not yet implicated in light responses. Genetic, physiological and biochemical characterization of *lid2* and other mutants will provide for the elucidation of both light- and/or sugar-mediated responses.

## *CVP2*-mediated IP<sub>3</sub> signal transduction is essential for closed venation patterns of Arabidopsis foliar organs

Francine M. Carland and Timothy Nelson

Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT

Vein patterns in leaves and cotyledons form in a spatially-regulated manner through the progressive recruitment of ground cells into vascular cell fate. To gain insight into venation patterning mechanisms, we have characterized the *cotyledon vascular pattern 2* (*cvp 2*) mutants, which exhibit an increase in free vein endings and a resulting open vein network. We cloned *CVP2* by a map-based cloning strategy and found that it encodes an inositol polyphosphate 5-phosphatase (5PTase). 5PTases regulate IP<sub>3</sub> signal transduction by hydrolyzing IP<sub>3</sub> and thus terminate IP<sub>3</sub> signaling. *CVP2* gene expression is initially broad and then gradually restricted to incipient vascular cells in a number of developing organs. Consistent with the inferred enzymatic activity of *CVP2*, IP<sub>3</sub> levels are elevated in *cvp2* mutants. In addition, *cvp2* mutants exhibit hypersensitivity to the plant hormone abscisic acid. We propose that elevated IP<sub>3</sub> levels in *cvp2* mutants reduce ground cell recruitment into vascular cell fate, resulting in premature vein termination, and thus in an open reticulum.

## Adaxial/abaxial specification of the maize leaf

Michelle T. Juarez<sup>1,2</sup> and Marja Timmermans<sup>1</sup>

<sup>1</sup>Cold Spring Harbor Laboratory Cold Spring Harbor, NY; <sup>2</sup>State University of New York, Stony Brook, NY

Specification of adaxial(upper)/abaxial(lower) polarity is responsible for normal outgrowth and patterning of the maize leaf. Establishment of adaxial/abaxial polarity requires signals from the shoot apical meristem as well as from the leaf itself. Through molecular and genetic analyses we have identified several genes involved in setting up adaxial identity. Recessive mutations in *leafbladeless1 (lbl1)* lead to development of radial symmetric abaxialized outgrowths suggesting *lbl1* is required for proper adaxial specification. Semi-dominant mutations in *Rolledleaf1 (Rld1)* develop adaxialized leaves or cause partial inversions of adaxial and abaxial domains in the leaf blade. *lbl1Rld1* double mutants display a mutual suppression of both the single mutant phenotypes suggesting the two mutations act in the same adaxial/abaxial pathway.

Several maize genes were cloned with high homology to two Arabidopsis gene families; *YABBY (YAB)* and *HOMEODOMAIN LEUCINE ZIPPER class III (HD-ZIP III)*, which are known to play a role in abaxial and adaxial cell fate, respectively. In contrast to Arabidopsis, the maize *yab* genes are expressed in the adaxial domain of young leaf primordia. In both the *lbl1* and *Rld1* mutants *yab* expression is altered. This data places the maize *yab* genes downstream of *lbl1* and *rld1* in the adaxial/abaxial specification pathway.

We recently cloned *rld1*, which encodes the homolog of Arabidopsis *REVOLUTA*, a member of the *hd-zip III* family. Members of this gene family are thought to be regulated by microRNAs (miRNA). *rld1* is expressed in the SAM and the adaxial domain of young leaf primordia. Dominant mutant alleles of *Rld1* result from mutations in the miRNA166 complementary site and misexpress *rld1* on the abaxial side. miRNA166 is expressed in the abaxial domain of leaf primordia, consistent with the misexpression of *rld1* in the dominant *Rld1* mutants. miRNA166, thus, has a role in adaxial/abaxial specification by spatial restriction of *hd-zip III*. *rld1* expression is reduced in *lbl1* mutant tissue. This together with the mutual suppressive interaction between *lbl1* and *Rld1* suggests that *lbl1* is upstream of *rld1*. The combination of the genetic and molecular analyses outlines an adaxial/abaxial specification pathway leading to proper development of the maize leaf.



## Comparison of large scale gene expression measurement technologies

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We are in the process of comparing four large scale gene expression technologies, based on expression data from more than 10 tissues/conditions in the model plant *Arabidopsis thaliana*. Data comprising 40 Agilent arrays, 40 Affymetrix arrays and 63 MPSS sequencing runs was obtained using the same mRNA samples for each library. Qiagen/Operon data is forthcoming.

Agilent technology is known to be more reproducible in technical replicates than MPSS than Affymetrix in turn. We have shown that MPSS technology has a dynamic range about 5 fold larger than that of Agilent and 10 times that of Affymetrix. Via global analysis of the data across the libraries we found that MPSS often underestimates the expression levels (relative to the other two) while Agilent overestimates them or vice versa.

We identified 45 genes that were within a 2-fold range in all technologies and all libraries. There were more than 2000 genes that were measured within a factor of 2 across all technologies in each specific library. The overall correlations across technologies for specific libraries were in the range of 0.65-0.75. The measurements of differential expression had a poorer correlation across technologies. There were 6 genes that were expressed above a concentration of 500 transcripts per million (TPM), 592 genes expressed below 10 TPM and 85 genes expressed below 4 TPM in all libraries as measured by all technologies.

Thus we have examined issues of reproducibility, correlation, dynamic range and estimation of the absolute level of transcripts. We are conducting QRT-PCR measurements that will place these comparisons on a stronger foundation. There are issues of the cost to benefit ratios that we have not presented in this study.

## Cloning a gamete fusion gene using insertional fusion-defective mutants

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Our goal is to isolate and analyze the gene responsible for gamete fusion in *Chlamydomonas reinhardtii*, a unicellular, eukaryotic green algae. To this end, we have generated fusion-defective insertional mutants and are using these mutants to clone the gene. The plasmid pSP124S, containing the *ble* gene, was inserted as a random mutagen using the acid-washed beads transformation technique. Mutants were selected using a streptomycin selection procedure. Approximately 700 non-mating colonies were isolated and analyzed by phase-contrast microscopy to determine their mating deficiencies. Among the mutants isolated were non-agglutinating mutants, non-motile mutants with low fusion capacity and one motile, fusion-defective mutant, *clone 9-5 (cl 5)*. *cl 5* forms 15 % pairs when mated with the opposite mating type (similar to the pair formation in *gam* mutants). Southern Blot analysis showed 1 insertion for *clone-45* (a previously isolated fusion-defective mutant), and 2 insertions for *cl 5*. While we continue to screen for new mutants, we are now using LMS-PCR to identify the genomic sequences flanking the insertion in the genome of *clone-45*. After PCR, we will sequence this flanking DNA and compare it to the gamete/zygote EST library available from the *Chlamydomonas* Genome Center as well as to the full genomic sequence that is now available as a blast database. We then will isolate the wild type gene by screening a *Chlamydomonas* BAC library.

## Characterization of species within the green algal genus *Dunaliella*.

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Halotolerant unicellular green algae of the genus *Dunaliella* are among the few commercially used microalgae. Currently, there are about 28 unicellular green algal species described that fall within the genus *Dunaliella*. 5 freshwater and 23 saltwater species are recognized for the genus *Dunaliella*. Until a few years ago, morphological and physiological characteristics have been the major criteria for species distinction. Unfortunately, cell morphology depends largely on environmental factors such as salinity and age of the cell culture. Because of great morphological variation even within one species, there has been confusion as to the correct classification of various described species and subspecies. Only recently has additional molecular analysis of the number of introns within the 18s rDNA gene as well as sequence analysis of the nuclear internal transcribed spacer 1 and 2 regions (ITS1 and ITS2) been used to classify species of the genus *Dunaliella*. Here we present the current status of sequence analysis to further delineate *Dunaliella* species and subspecies of the sectio *Viridae* from each other.

## Quantifying and interpreting maintenance costs of phenotypic plasticity using recombinant inbred lines of *Arabidopsis thaliana*

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Like many plant species, *Arabidopsis thaliana* shows phenotypic plasticity for many traits and in response to diverse stimuli. Yet it has not evolved to a point where it survives and reproduces in all environments. Such observations motivate theory about fitness costs associated with plasticity, such as “maintenance costs” for sustaining sensory and response pathways and “genetic costs” associated with pleiotropic or epistatic effects of the genes involving in detection and transduction of signals. Using the segregating variation found in the Columbia x Kashmir Recombinant Inbred Lines, we quantified plasticity costs of vernalization-induced plasticity. Replicates of each RIL were grown in both brief and long vernalization treatments. For each RIL we quantified (1) environment-specific fitness, (2) environment-specific flowering time, and (3) plasticity induced by the contrasting treatments. A multiple regression model examined dependence of a RIL’s mean fitness on its mean flowering time and its plasticity. We verified that some RILs are Kashmir-like and plastic, showing accelerated flowering after vernalization; others are Columbia-like and lack plasticity. When rosettes were chilled only briefly, we detected significant plasticity costs. When rosettes were exposed to lengthy vernalization, or when plants were vernalized at the seed stage, costs were not detected. This is a novel approach to the study of plasticity costs, because costs are interpretable. This is because the roles of the *FRIGIDA*, *FLOWERING LOCUS C*, and other vernalization-sensitive genes are well-characterized, and because there is allelic variation for *FRIGIDA* in this particular recombinant inbred population.

## Expression of the yeast L3 and the pokeweed antiviral protein genes confers resistance to trichothecene mycotoxins

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Trichothecenes are a highly diverse class of toxic, sesquiterpenoid secondary metabolites that are produced mainly by plant pathogenic fungi. The contamination of important agricultural products, such as wheat, barley or maize with the trichothecene mycotoxin, deoxynivalenol (DON) due to infection with *Fusarium graminearum* and *F. culmorum* is a worldwide problem. Trichothecene mycotoxins interact with the peptidyltransferase site of eukaryotic ribosomes and inhibit protein synthesis. Ribosomal protein L3 (*RPL3*) participates in the formation of the peptidyltransferase center. Mutations in the *RPL3* gene (called *TCMI*) were initially identified by conferring resistance to trichodermin, a trichothecene mycotoxin that inhibits the peptidyl transferase reaction. To determine if expression of the yeast *RPL3* gene will confer resistance to trichothecene mycotoxins, we generated transgenic tobacco plants expressing either the wild type or mutant forms of the yeast *RPL3* alone or together with pokeweed antiviral protein (PAP), a ribosome inactivating protein that inhibits viral and fungal infection. Transgenic plants containing the wild type yeast *RPL3* and PAP or a mutant form of the yeast *RPL3* (L3D) and PAP were phenotypically normal. Similarly, transgenic lines expressing the yeast *RPL3* or L3D alone were indistinguishable from wild type plants. To determine if transgenic tobacco plants expressing the yeast *RPL3* genes are resistant to trichothecenes, seeds from transgenic and wild type plants were germinated on MS medium, containing 1  $\mu$ M DAS (4,15-diacetoxyscirpenol) or 10  $\mu$ M of DON and their root length was measured at the end of six weeks. Plants from all transgenic lines showed resistance to DAS and DON compared to the wild type plants. However, the highest level of resistance was observed with transgenic plants expressing the yeast *RPL3* genes together with PAP. To confirm that yeast *RPL3* genes were expressed in these plants, we carried out real-time PCR analysis using primers specific for the yeast *RPL3* genes, which do not hybridize to the tobacco L3 genes. The results confirmed the expression of the yeast *RPL3* genes in the transgenic lines. These results demonstrate that we can obtain phenotypically normal transgenic plants that show high levels of resistance to DON and DAS by coexpressing the wild type or mutant forms of the yeast *RPL3* together with PAP in transgenic tobacco plants.

## Tissue-specific expression and dynamic organization of SR splicing factors in *Arabidopsis thaliana*

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The organization of the pre-mRNA splicing machinery has been extensively studied in cultured mammalian and yeast cells. However, far less is known about its organization and dynamics in living plant cells and in different cell types of an intact living organism. Here we report on the expression, organization and dynamics of pre-mRNA splicing factors (SR33, SR1/atSRp34 and atSRp30) in *Arabidopsis thaliana*. Distinct tissue-specific expression patterns were observed, and in addition, differences in the distribution of the respective proteins within the nuclei of different cell types were identified. These factors localized in a cell type-dependent speckled pattern as well as being diffusely distributed throughout the nucleoplasm. Upon examination by immunoelectron microscopy, these speckles correspond to interchromatin granule clusters (IGCs). Time-lapse microscopy revealed that speckles move within a constrained nuclear space and their organization is regulated during the cell cycle. Fluorescence recovery after photobleaching (FRAP) analysis revealed a rapid exchange rate of splicing factors in nuclear speckles. The dynamic organization of plant speckles is closely related to the transcriptional activity of the cells and can also be modulated by the phosphorylation state of the splicing factors. The organization and dynamic behavior of speckles in *Arabidopsis* cell nuclei provides significant insight into understanding the functional compartmentalization of the nucleus and its relationship to chromatin organization within various cell types of a single organism.

## Mutagenesis of *Brassica oleracea*

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A rapid cycling strain of *Brassica oleracea* was mutagenized by exposure to EMS. The resulting population (1000 pools of seed each containing self-pollinated seed from 10 mutagenized plants) was screened for mutants with visibly altered phenotype. Putative mutants were identified in which the following traits were affected: pigmentation (albino, yellow-green, purple, variegated), plant stature (dwarfed, spindly), wax deposition (absent or abnormal wax deposition), leaf morphology (serrated, lobed leaves), phyllotaxy, flowering time (late flowering, early flowering), flower color, flower morphology (*apetala*-like, *agamous*-like), male sterility and silique morphology. The abundance of mutations suggests that many traits in *B. oleracea* are determined by single loci. Mutant lines will be deposited with the Crucifer Genetics Cooperative and made available for research and education.

## Developing tools for genomics education using *Arabidopsis thaliana* and *Brassica oleracea*

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A laboratory activity (MapPlants) has been created that will introduce undergraduate students to modern genetics by allowing them to grow and analyze populations of *Arabidopsis* plants segregating for a mutant phenotype and then identify the chromosomal location of the affected gene using genomic resources.

Large-scale mutagenesis of the rapid-cycling *Brassica oleracea* line, TO1000 has led to the identification of many mutants. The *B. oleracea* mutants are being characterized genetically and mapping populations are being generated. The large flowers and seeds of TO1000 make this plant well suited for genetic experiments in the classroom. As sequencing of the *B. oleracea* genome proceeds, genomics education tools using *B. oleracea* will be developed.

## Elucidation of the lysine biosynthesis pathway in plants focusing on the genetic model *Arabidopsis thaliana*

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The exact pathway by which plants synthesize the essential amino acid lysine is unknown. Yet, the nutritional value of the major crop plants is limited by the low lysine content. Strategies to improve the lysine content must begin with the characterization of the biosynthetic pathway. The published literature indicates that in plants, the lysine pathway proceeds via the intermediate diaminopimelate (DAP), as it does in bacteria, but only two of the 7 possible enzyme activities have been identified. The availability of the complete genome sequence of *Arabidopsis thaliana* offers the chance to characterize the genes for lysine biosynthesis. Homologs of bacterial lysine genes have been identified for dihydrodipicolinate synthase, dihydrodipicolinate reductase, diaminopimelate epimerase and diaminopimelate decarboxylase. Homologs could not be identified for tetrahydrodipicolinate acylase, acyldiaminopimelate aminotransferase and acyl-diaminopimelate deacylase, or meso-diaminopimelate dehydrogenase, suggesting that *Arabidopsis* uses novel genes to carry out the conversion of tetrahydrodipicolinate to LL-diaminopimelate. In an ongoing effort each of the *Arabidopsis* genes will be functionally characterized. Three genes were identified with homology to dihydrodipicolinate reductase. The cDNA for one of them was able to functionally complement the diaminopimelate auxotrophy of a *dapB* strain of *Escherichia coli*. A single gene was identified with homology to diaminopimelate epimerase. A cDNA derived from the gene was used to express the protein as a recombinant enzyme in *E. coli*. The protein was partially purified and demonstrated to have diaminopimelate epimerase activity *in vitro*. By systematically characterizing the genes in the lysine pathway, we hope to elucidate the anabolism of this essential amino acid in the model organism *Arabidopsis thaliana*.

## *KANADI* genes and leaf enations

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The leaves of most plants exhibit striking differences between their adaxial (dorsal or upper) and abaxial (ventral or lower) surfaces. This sidedness of leaves represents an axis of asymmetry that corresponds to the thickness of the leaf. Cell-types within the leaf are polarized along this adaxial-abaxial axis. Loss-of-function mutations in *KANADII* (*KANI*) result in the ectopic appearance of adaxial traits on the abaxial sides of leaves. The *KANI* gene product is a member of a plant-specific family of transcription factors recently dubbed the GARP domain family. Plants carrying mutations in both *kan1* and a closely related gene *kan2* display dramatic polarity defects that include the formation of leaf-like outgrowths on the abaxial surface of leaves. We are exploring the formation of these outgrowths or enations in order to further understand the role of *KAN* genes in the specification of abaxial fate.

## Deciphering the roles of xyloglucan galactosylation in the assembly of a functional plant cell wall

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Xyloglucans are the principal hemicellulose in the primary walls of the most flowering plants. These polysaccharides consist of a  $\beta$ -(1,4)-linked glucan backbone, which is substituted by xylose residues in an “XXXG” repeat pattern. Some of the second and third xylosyl residues within this core structure carry galactosyl and fucosyl-galactosyl side chain, respectively. The main function of xyloglucan is the binding and cross-linking of cellulose microfibrils, which leads to the establishment of a load-bearing three-dimensional network believed to play a key role in cell wall assembly and remodeling during expansion growth.

We recently found that the xyloglucan of *mur3* mutant of Arabidopsis lacks the fucosyl-galactosyl side chain because of a defect in xyloglucan galactosyltransferase I, which converts XXXG to its galactosylated derivative XXLG (Madson, *et al. Plant Cell* 2003). While chemically induced *mur3* alleles do not lead to obvious visible phenotypes, a T-DNA insertion line is slightly stunted suggesting a defect in cell wall assembly.

The recent isolation and characterization of a knockout mutant in the *MUR3* paralog *AtGT18* revealed that it contains only small amounts of galactose on the central xylose residue within the xyloglucan repeat unit. This suggests that the *AtGT18* gene encodes xyloglucan galactosyltransferase II, which converts XXXG to XLXG. The *atgt18* plants show a “droopy” phenotype and abnormal patterns of lignin deposition presumably reflecting a defect in xyloglucan-cellulose interactions.

To obtain plants with a very low degree of xyloglucan galactosylation, we conducted crosses between *mur3* and *atgt18* lines. Preliminary results on the F2 population indicate segregation for plants with very severe growth defects, which could be double mutants. We hope that a comparative analysis of the various mutant lines will lead to meaningful insights into the functional significance of xyloglucan galactosylation.

## MAP-kinase signaling in the Arabidopsis embryo

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Upon fertilization, the Arabidopsis zygote elongates more than 2-fold and divides transversely into a small apical and a large basal cell. The daughter cells of the zygote subsequently establish two lineages with fundamentally different developmental fates. Cells of the basal lineage continue to elongate and to divide transversely producing the mostly extra-embryonic suspensor. Cells of the apical lineage switch to a more isodiametric growth pattern and produce the proembryo.

We have identified mutations in three genes that affect this process in similar ways, and have characterized one of these genes, designated *YODA*, in detail. Mutant zygotes elongate only about 30% before dividing. While development of the apical cell is initially not affected, cells of the basal lineage are much smaller than in wild type and their division pattern is irregular. No visible suspensor is formed, and a molecular marker for suspensor development is absent in 80% of the mutants. Despite these defects, *yoda* embryos can occasionally form all pattern elements of a seedling and develop into dwarfed and sterile plants.

*YODA* encodes a MAPKK kinase expressed throughout development in all tissues analyzed. All nine alleles we recovered are predicted to abolish or reduce kinase activity. Manipulations in the presumptive regulatory domain of the protein result in artificial gain-of-function alleles which have opposite phenotypic effects as loss-of-function alleles. Growth of the suspensor is exaggerated, and growth of the proembryo suppressed. In the most severe cases, the zygote develops into a file of cells without recognizable proembryo. We propose that *YODA* identifies a MAP-kinase signaling pathway that modulates a fundamental cell-fate decision in the early embryo. *YODA* signaling promotes a developmental program characterized by elongation, transverse divisions and, ultimately, extra-embryonic fate. After the asymmetric division of the zygote, *YODA* activity is down-regulated in the apical lineage which becomes free to adopt an embryonic fate.

## Comparative molecular and morphological study on the halotolerant unicellular green alga *Dunaliella*

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The unicellular green algae of the genus *Dunaliella* living in saltwater are known to be extreme halotolerant. Some species have been reported to grow even in environmental conditions containing saturated concentrations of NaCl. At the same time described species such as *Dunaliella salina* and *Dunaliella bardawil* are used commercially to produce β-carotene. The species *D. salina* was described by Teodoresco in 1905. The name *D. bardawil* was given to a new isolate of the genus *Dunaliella* found in North Sinai in the late 1970's. Although both species are morphologically very similar the species name *D. bardawil* persisted to exist in literature. Recent molecular analysis has shown that both species are phylogenetically closely related. However, *D. bardawil* is still recognized as a separate species. In a combined molecular and morphological approach the species *D. salina* and *D. bardawil* have been compared to re-evaluate the current classification of *D. bardawil*. Our results strongly suggest that *D. bardawil* is not a separate species but belongs to one subspecies of *D. salina*.

## The Arabidopsis *dep1* and *dep2* mutations act synergistically to alter phytochrome-regulated growth responses

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A genetic screen of *Arabidopsis thaliana* seedlings yielded a series of mutants that display slightly longer hypocotyls under white light than those of wild type. Two of the complementation groups, designated *dep1* and *dep2* for deficient photomorphogenesis, showed marginally longer hypocotyls than those of the wild type under continuous white light, red light, and far-red light. Although their apical hooks open in all light regimes, a greater proportion of the cotyledons remained appressed under these light conditions. Both mutants exhibit slightly delayed flowering times in long days compared with that of wild type. Neither *dep1* nor *dep2* mutants exhibit any detectable abnormal phenotype in darkness, indicating that the deficiencies are light dependent.

Double mutants harboring both *dep1* and *dep2* exhibit much more pronounced phenotypic deficiencies, more comparable to those of a *phyA* null mutant with respect to germination, hypocotyl elongation, cotyledon opening in far-red light, far-red-induced anthocyanin accumulation, far-red-preconditioned greening responses and late flowering. The *dep1* and *dep2* single mutants and the double mutant exhibit normal levels of phytochrome A apoprotein, and the receptors apparently bind chromophore as they undergo normal light-dependent degradation.

However, extracts of the double mutant may contain reduced amounts of spectrally active phytochrome. Furthermore, the *dep* mutants show additional deficiencies more characteristic of phyB pathway mutants, including loss of red-dependent gravitropic sensitivity and longer hypocotyls in red light. Therefore, Dep1 and Dep2 play pleiotropic roles in signaling downstream of multiple phytochromes. The synergistic effects observed in the double mutants suggest either partial redundancy or parallel pathways at work at this stage of phytochrome signaling.

## Role of the Arabidopsis DDB Protein in photomorphogenesis and regulated protein degradation

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The dramatic developmental transition that occurs when a seedling encounters light is called photomorphogenesis. Genetic screens have identified four biochemical entities that are critical for this process in *Arabidopsis thaliana*. These are COP1 (for *constitutively photomorphogenic*), COP10, the COP9 signalosome (CSN), and DET1 (for *de-etiolated*). COP1, COP10, and the CSN all act in the ubiquitin-proteasome pathway of regulated protein degradation. COP1 is an E3 ligase, while COP10 is similar, at least in amino acid sequence, to E2 ubiquitin conjugase variant proteins. The CSN, in part, regulates the activity of cullin-based E3 ligases by controlling the level of RUB1 modification of cullin molecules. These protein degradation-related activities of COP1, COP10, and the CSN result in changes in gene expression by controlling the abundance of key transcription factors. DET1, on the other hand, is hypothesized to be involved in chromatin regulation through its interaction with histones.

Interestingly, two groups have now reported that an unexpected protein -- DDB (for damaged DNA binding) -- interacts with several key players in photomorphogenesis. First isolated in a mammalian system, DDB is a heterodimer composed of DDB1 and DDB2. DDB1 shuttles between the cytoplasm and the nucleus, while DDB2 is constitutively nuclear. The evidence that DDB functions in the same pathway as genes that control photomorphogenesis is the following. First, DDB1 binds to DET1 (Shroeder, *et al.*, *Curr. Biol.* 2002). Second, in a mammalian system, a ubiquitin ligase activity attributed to the DDB1-DDB2 complex is regulated by the CSN (Groisman, *et al.*, *Cell* 2003).

I am utilizing a combination of genetic and biochemical techniques to address the role of DDB in photomorphogenesis and in regulated protein degradation. Arabidopsis contains two copies of the DDB1 gene, DDB1a and DDB1b. While T-DNA knock-out lines of DDB1a have no phenotype, a lack of DDB1b is lethal and results in defective embryogenesis. The expectation is that these studies will yield insight into the role of the DDB protein in the life of the Arabidopsis plant as well as the more general question of how DDB functions in regulated protein degradation in eukaryotic cells.

## Evolution of *ndhF* in *Dioscorea*

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*Dioscorea* is a genus of monocots that comprise the “true yams.” *Stenophora* is a well-supported section within this group. Our phylogenetic investigations reveal that some members of *Stenophora* possess a second copy of the chloroplast gene, *ndhF*. This second copy of *ndhF* is truncated, and is more closely related to other members of Dioscoreales and outgroup taxa. Phylogenetic evidence suggests that an ancient duplication may have been followed by losses within *Dioscorea*.

## Novel ubiquitin-conjugating enzyme variant COP10 composes of a complex

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COP10 (COstitutive Photomorphogenesis 10) has been initially identified as a negative regulator of photomorphogenesis in Arabidopsis. Interestingly, COP10 was revealed to be a novel ubiquitin-conjugating enzyme variant, which composed of a complex, named COP10 complex. Here we show the biochemical purification data of the COP10 complex.

