

# Plant Genetics 2003 ABSTRACTS

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## Targets and functions of plant microRNAs - INVITED SPEAKER

Bonnie Bartel, Rice University

MicroRNAs are an extensive class of ~22-nt noncoding RNAs thought to regulate gene expression in metazoans. MicroRNAs are also present in plants, indicating that this class of noncoding RNA arose early in eukaryotic evolution. We identified sixteen Arabidopsis microRNAs, many of which have differential expression patterns in development (Reinhart et al., 2002, *Genes Dev.* 16:1616-1626). Plant microRNA loci potentially encode stem-loop precursors similar to those processed by the Dicer ribonuclease in animals. Mutation of an Arabidopsis Dicer homolog, CARPEL FACTORY (DCL1), prevents microRNA accumulation, demonstrating that similar mechanisms direct microRNA processing in plants and animals. The previously described roles of CARPEL FACTORY in Arabidopsis embryos, leaves, and floral meristems suggest that the microRNAs may play regulatory roles in plant as well as animal development. We predicted regulatory targets for the Arabidopsis microRNAs by identifying mRNAs with near complementarity (Rhoades et al., 2002, *Cell* 110:513-520). Many predicted microRNA targets are members of transcription factor gene families involved in developmental patterning or cell differentiation and, like the microRNAs themselves, are conserved in rice. The near-perfect complementarity between plant microRNAs and their potential targets suggests that many plant microRNAs act similarly to small interfering RNAs and direct mRNA cleavage.

3

## Establishment of polarity in shoots of land plants - INVITED SPEAKER

John L. Bowman, UC Davis Sandra K. Floyd, UC Davis John Alvarez, UC Davis John F. Emery, UC Davis Anat Izhaki, UC Davis Nathaniel P. Hawker, UC Davis Harriette Oldenhof, UC Davis Eric M. Engstrom, UC Davis

Lateral organs of seed plants, such as leaves and floral organs, are formed from the flanks of apical meristems. Therefore, an inherent positional relationship exists between them: organ primordia have an adaxial side adjacent to the meristem, and an abaxial one away from the meristem. Surgical and genetic studies suggest that a morphogenetic gradient, whose source is the meristem, converts the inherent polarity into a functional one. Once an adaxial-abaxial axis of polarity is established within organ primordia, it provides cues for proper lamina growth and asymmetric development. Several key players involved in this process were identified recently, and analyses of these genes support and refine our views of axis formation in plants. The complex relationships between and within various members of these plant-specific gene families (class III HD-ZIPs, YABBYs and KANADIs) may account for a significant portion of the morphological variation in lateral organs of seed plants.

4

## Paramutation, an allele interaction that causes heritable changes in transcription involves long distance interactions and chromatin structural changes - INVITED SPEAKER

Vicki L. Chandler, Regents' Professor; Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Paramutation is an interaction between alleles that causes a directed, heritable alteration in the expression of one allele. The lightly pigmented *B'* allele alters the darkly pigmented *B-I* allele in a *B'/B-I* heterozygote, such that *B-I* becomes *B'* at 100% frequency. *B-I* also spontaneously changes into *B'* at a frequency of 1-10%. The reduced pigment results from 10-fold differences in transcription of *B'* and *B-I*, the reduced transcription state is established in the  $F_1$  and maintained in subsequent generations in the absence of the inducing allele. The extreme stability of the reduced transcription state of *B'* is unusual relative to paramutation at other loci. Recent work on paramutation at *b* (encodes a transcription factor that activates biosynthesis of anthocyanin pigments in maize) will be presented.

Using recombination between *B-I* or *B'* and an allele that does not participate in paramutation, we localized the sequences required for *b* paramutation and increased transcription to a 6 kbp region, located ~100 kbp upstream of the *b* transcription start site. There are no sequence differences between *B'* and *B-I* demonstrating that paramutation is an epigenetic phenomenon, in spite of the stability of *B'*. The region required for paramutation is unique in the genome and contains seven tandem repeats of a sequence present only once in alleles that do not undergo paramutation. Chromatin structure differences between *B'* and *B-I* are limited to within the repeats. Introducing the repeats into other sites in the maize genome demonstrated they are sufficient for paramutation and can function in non-allelic positions. Our current model for the cis and trans interactions mediating paramutation will be presented.

5

## From Genes to Morphogenesis - INVITED SPEAKER

Enrico Coen, John Innes Institute

Although much progress has been made in understanding how gene expression patterns are established during development, much less is known about how these patterns are related to the growth of biological shapes. I will describe conceptual and experimental approaches to bridging this gap, with particular reference to plant development where the lack of cell movement simplifies matters. Growth and shape change in plants can be fully described with four types of regional parameter: growth rate, anisotropy, direction and rotation. A key requirement is to understand how these parameters both influence and respond to the action of genes. This can be addressed using mechanistic models that capture interactions between three components: regional identities, regionalising morphogens and polarising morphogens. By incorporating these interactions within a growing framework, it is possible to generate shape changes and associated gene expression patterns according to particular hypotheses. The results can be compared with experimental observations on growth of normal and mutant forms, allowing further hypotheses and experiments to be formulated. These principles will be illustrated with a study on snapdragon petal growth.

6

## Centromeric chromatin - INVITED SPEAKER

R. Kelly Dawe, University of Georgia Cathy X. Zhong, University of Georgia Christopher N. Topp, University of Georgia

Plant centromeres usually contain thousands of tandemly arrayed repeats and a variety of insertions and other sequences. There is no obvious relationship between sequence and function, suggesting that centromeres attract kinetochore proteins by epigenetic mechanisms. Foremost among known kinetochore proteins is Centromeric Histone H3 (CENH3), which substitutes for histone H3 in centromeres. Its ubiquitous presence at active centromeres and close proximity to centromeric DNA suggest that CENH3 is a key player in centromere specification. In cereal grains such as maize, the centromeres are composed primarily of satellite arrays and transposons known as Centromeric Retrotransposable (CR) elements. Chromatin immunoprecipitation (ChIP) studies indicate that both centromeric satellites (CentC) and CR elements interact with CENH3. Interestingly, substantial quantities of RNA homologous to CR elements and CentC are also co-immunoprecipitated with anti-CENH3 antibodies. RNAs derived from other repeats are not recovered. Since CR elements can be found in the maize EST database, we suggest that centromeric transcripts are initiated by CR elements and extend into CentC arrays. The centromere-associated RNA ranges in size from 40 nt to at least 900 nt; the 22-28 nt class

characteristic of siRNAs were not detected. The results provide clear evidence that centromeric satellite repeats are transcribed, and suggest that maize centromeric chromatin harbors RNA.

## 7

### The evolution of plant form in maize - INVITED SPEAKER

John Doebley, University of Wisconsin

Maize and its ancestor (teosinte) differ dramatically in morphology despite their recent divergence (7,500 years). Using quantitative trait locus (QTL) mapping, we identified several QTL of major effect plus many QTL of modest effect for these morphological differences. The largest effect QTL for plant architecture maps near the *teosinte branched 1* (*tb1*) gene. This QTL largely controls the difference between the long branches of teosinte and the short branches of maize. *tb1* encodes a bHLH transcription factor. From the *tb1* mutant phenotype, we infer that *tb1* protein functions as a repressor of organ growth. Northern blot analysis indicates that the mRNA of the teosinte allele of *tb1* accumulates at about half the level of that of the maize allele. We infer that maize with a higher message level has greater repression of branch growth and therefore shorter branches. We hypothesize that *tb1* represses organ growth in part by down regulating *pcna* and thereby arresting cell division. We have investigated the molecular evolution of *tb1* and demonstrated that its promoter but not its coding sequence show compelling evidence of selection during maize domestication. The evidence for selection extends 58 kb upstream of *tb1* but is absent at 93 kb upstream of *tb1*. The effects of selection do not appear to extend to any neighboring genes. This result suggests that changes in *tb1* regulation underlie morphological evolution in maize. We have also generated a series of maize-teosinte chimeric alleles of *tb1* that we are using to map the portions of the gene that confer specific phenotypic effects.

## 8

### Variation of heterochromatin in Arabidopsis accessions - INVITED SPEAKER

Paul Fransz, Gabi Linc, Federico Tessadori and Roel van Driel Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands

Arabidopsis chromosomes consist of small conspicuous heterochromatic segments that mark the centromere position of each chromosome as well as the NORs of chromosomes 2 and 4. The heterochromatin segments comprise about 15% of the total chromosome, in concordance with the percentage of repetitive sequences. The majority of the chromosome consists of gene-rich euchromatic arms. Variation in content and distribution of heterochromatic aspects has been observed between different Arabidopsis accessions, including number and position of 5S rDNA loci, presence of heterochromatic knobs and relative content of heterochromatin in interphase nuclei. Here we focus on a number of structural features associated with inheritance and polymorphism of heterochromatin in Arabidopsis ecotypes. For example, the heterochromatic knob *hk4S*, which is present in the ecotypes *Ws* and *Col*, has derived from a paracentric inversion. Using different FISH techniques on knob-containing and knobless ecotypes we established the breakpoint of this inversion and examined the nuclear position of knob-flanking gene-regions relative to heterochromatic chromocenters. The data will be discussed within the context of heterochromatin variation in Arabidopsis.

## 9

### Genetic and Epigenetic Control of Seed Development in Arabidopsis - INVITED SPEAKER

Claudia Köhler<sup>1</sup>, Lars Hennig<sup>2</sup>, Charles Spillane<sup>1</sup>, Damian Page<sup>1</sup>, Stephane Pien<sup>1</sup>, Wilhelm Grüssens<sup>2</sup> and Ueli Grossniklaus<sup>1</sup> <sup>1</sup>Institute of Plant Biology & Zürich-Basel Plant Science Center, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland <sup>2</sup>Institute of Plant Sciences & Zürich-Basel Plant Science Center, Swiss federal Institute of Technology, ETH Zentrum, CH-8092 Zürich, Switzerland

In a mutagenesis screen for gametophytic mutants in *Arabidopsis*, we identified *medea* (*mea*) which displays gametophytic maternal effect lethality (Grossniklaus et al. (1998) *Science* 280, 446): Seeds derived from a *mea* mutant female gametophyte abort irrespective of the paternal contribution. *mea* has a strong effect on cell proliferation in the embryo and endosperm, the two fertilization products of flowering plants. This maternal control implies either a prolonged effect of the MEA product or some kind of a cellular memory mechanism. Indeed, *MEA* is involved in the epigenetic control of gene expression at two levels:

First, the *mea* maternal effect is due to genomic imprinting, i.e. only the maternally inherited allele is transcribed after fertilization (Vielle-Calzada, J.P. et al. (1999) *Genes Dev* 13, 2971; Kinoshita et al. (1999) *Plant Cell* 11, 1945; Luo et al. (2000) *Proc Natl Acad Sci USA* 97, 10637). The establishment and maintenance of imprints are poorly understood in both mammals and plants, the only two groups of organisms where gene-specific genomic imprinting has been reported. We are using *mea* to dissect the genetic regulation of genomic imprinting by searching for second-site modifiers. We identified a number of loci elucidating the relationship between DNA methylation and imprinting.

Second, *mea* and other members of the *FIS* class (Grossniklaus et al. (1998) *Science* 280, 446; Ohad et al. (1999) *Plant Cell* 11, 407; Luo et al. (1999) *Proc Natl Acad Sci USA* 96, 296; reviewed in Grossniklaus et al. (2001) *Curr Opin Plant Sci* 4, 21) encode *Polycomb* group (PcG) proteins, which form complexes that repress gene expression by modulating higher order chromatin structure (Pirrotta, V. (1997) *Trends Genet* 13, 314). While PcG genes are best known for their role in regulating homeotic genes in animals, many also regulate cell proliferation, suggesting a functional conservation of between animals and plants. To better understand the function of *MEA* during seed development, we have started to characterize the MEA complex biochemically: a novel component, MS11, was found to be part of the 600 kDa MEA complex. To shed light onto the epigenetic regulation effected by the MEA PcG complex, we used the Affymetrix GeneCHIP Technology to identify potential target genes. The detailed characterization of *PHERESI*, a direct target gene of the MEA complex, will be presented (Köhler et al. (2003) *Genes Dev* 17, 1540).

## 10

### Patterning of leaf shape - INVITED SPEAKER

Sarah Hake, Plant Gene Expression Center, Albany, CA, USA Toshi Foster, HortResearch, Palmerston North, NZ Robyn Johnson, Massey University, Palmerston North, NZ Angela Hay, Oxford University, Oxford, UK

Mature maize leaves have defined cell types along the proximal distal and medial lateral axes. The patterning events that establish these axes take place early in leaf initiation. We have identified a new dominant mutation, *Wavy auricle in blade1* (*Wab1*) that affects both axes. *Wab1* leaves are narrower than normal leaves and displace proximal tissues distally. Analysis of *Wab1* mutants suggest that a lateral domain of the leaf is missing and that this domain lies internal to the domain defined by *narrow sheath1* and *narrow sheath2*. We show that the phenotype is not due to misexpression of knox genes but that *liguleless1* is misexpressed in *Wab1* mutant leaves. Double mutant combinations between *Wab1* and *Ig1* and *Ig2* suggest that the initiation of blade tissue outside of the *Wab1* lateral domain requires the *LG1* and *LG2* gene products. A mosaic analysis shows that both the proximal distal and medial lateral axes are coordinately controlled.

## 11

### Regulation of Intercellular Homeodomain Protein Trafficking. - INVITED SPEAKER

Jae Yean Kim\*, Michelle Cilia, Jing Wang, Zhuang Yuan and Dave Jackson. Cold Spring Harbor Lab, New York, NY 11724. \*Gyeongsang National University, Korea. <http://jacksonlab.cshl.edu>

A fundamental question in biology is how cell fate is determined. The plasticity of plant development and evidence from mosaic analysis indicates that cell fate is

generally determined late in development and controlled by positional information. Cell-cell communication provides the means by which cells determine their position through communication with adjacent cells and distant morphogen sources. In plants, communication can occur by exchange of macromolecules through intercellular channels called plasmodesmata (PDs), which connect most plant cells to their neighbors. PD channels consist of a sleeve of plasmamembrane with a thin tube of appressed endoplasmic reticulum (ER) running through the middle. Diffusion of small molecules and regulated transport of macromolecules is thought to occur through the cytoplasmic space between the ER and plasmamembrane. Although several examples of intercellular transport of transcription factors and mRNAs have been presented in recent years, we still have little idea of whether or how intercellular transport is regulated, nor do we understand the molecular components of plasmodesmata.

Trafficking of the maize KNOTTED1 homeodomain protein between cell layers appears to be responsible for its non-autonomous function in leaf development. We also found that KN1 can traffic between cell layers in the shoot meristem, where it normally functions. Deletion experiments suggest that the homeodomain of KN1 is both necessary and sufficient for intercellular trafficking, yet any deletions within the homeodomain completely abolish this activity. Therefore PD targeting of KN1 may require a structural domain rather than a simple sequence motif. To identify other components of the PD machinery we are also performing genetic screens and results of these will be presented.

This work is funded by the NSF Integrative Plant Biology Program.

## 12

### **Natural variation in Arabidopsis - INVITED SPEAKER**

Maarten Koornneef, Wageningen University Carlos Alonso-Blanco, Centro Nacional de Biotecnología Mark G.M. Aarts, Wageningen University Leonie Bentsink, Wageningen University Emile J.M. Clerckx, Wageningen University Salah E.-D. El-Assal, Wageningen University Mohamed E El-Lithy, Wageningen University Dick Vreugdenhil, Wageningen University

The functional analysis of Arabidopsis genes has been largely based on the phenotypic characterisation of mutants selected by forward and reverse genetics in a very limited number of laboratory 'wild-type' genotypes. As an alternative to induced mutants, an important source of genetic variation can be found among naturally occurring populations of Arabidopsis called, accessions or ecotypes (Alonso-Blanco, and Koornneef, Trends in Plant Sci. (2000) 5: 22-29). Considerable variation has been found for potentially adaptive traits such as resistance (measured as plant survival or damage) to biotic and abiotic stresses, physiological developmental and biochemical traits. The study of natural variation requires quantitative trait loci (QTL) analysis. For efficient QTL analysis sets of recombinant inbred lines have been developed, which show segregation for a large number of traits including those important in crop plants. The variation and genetic analysis of traits such as sugar, starch, phytate and mineral accumulation, enzyme activities, seed dormancy and seed quality, has been analysed. The feasibility to clone individual QTL by a combination of map based cloning, using near isogenic introgression lines has been demonstrated by the isolation of a gene conferring daylength insensitivity. The cloning of this locus identified it to be of a very specific amino-acid change in the cryptochrome 2 gene, that affects the stability of the protein exclusively in short day condition and thereby causes early flowering in short day photoperiods (El-Assal et al. Nature Genetics (2001) 29: 435-440). In case of seed dormancy the combination of fine mapping and a mutant approaches allowed the isolation of a dormancy QTL.

## 13

### **Cytosine methylation and epigenetic stability - INVITED SPEAKER**

Hankuil Yi, Washington University Trevor Stokes, Washington University (currently New York Univ Nicole Riddle, Washington University (currently University of Missouri-Columbia) Barbara Kunkel, Washington University Eric Richards, Washington University

Our group has been studying the regulation and function of cytosine methylation by combining different genetic approaches in Arabidopsis. One arm of this analysis has been characterization of mutations isolated by forward genetic screens for altered DNA methylation. We have identified two loci important for maintenance of cytosine methylation: DDM1, which encodes a SWI2/SNF2-class nucleosome remodeling factor, and the Dnm1-class cytosine-DNA-methyltransferase gene, MET1. Although the morphology of Arabidopsis mutants with reduced cytosine methylation is similar to wild-type, propagation of mutant lines by self-pollination is accompanied by the onset of stochastic developmental abnormalities. These abnormalities are caused by the accumulation of inherited lesions, some of which are epigenetic. We have focused our attention on understanding the molecular basis of one such lesion (bal) that leads to dwarfing and constitutive pathogen responses. More recently, we have begun exploiting natural variation in the different Arabidopsis strains to examine the prevalence, stability, and inheritance of epigenetic variation in cytosine methylation.

## 14

### **Hybridization and ecological divergence in wild sunflowers - INVITED SPEAKER**

Loren Rieseberg, Indiana University

Hybridization is frequent in many organismal groups, but its role in adaptation is poorly understood. In sunflowers, species found in the most extreme habitats are ancient hybrids, and new gene combinations generated by hybridization are speculated to have contributed to ecological divergence. This possibility was tested through phenotypic and genomic comparisons of ancient and synthetic hybrids. Most trait differences in ancient hybrids could be re-created by complementary gene action in synthetic hybrids and were favored by selection. The same combinations of parental chromosomal segments required to generate extreme phenotypes in synthetic hybrids also occurred in ancient hybrids. Thus, hybridization facilitated ecological divergence in sunflowers.

## 15

### **Genetic Variation and Phylogeography in Plants - INVITED SPEAKER**

Barbara Schaal, Washington University Allison Miller, Washington University John Gaskin, US Department of Agriculture

The level of genetic variation in plants is of central importance in determining the evolutionary potential of plant species. The levels of genetic variation are strongly affected by both current patterns of microevolutionary forces, such as gene flow or selection, and by the evolutionary (phylogenetic) history of populations and species. Understanding the interplay of shared history and current evolutionary events is particularly confounding in plants due to the reticulating nature of gene exchange between diverging lineages. The development of rapid DNA sequencing methods has provided an important tool for analyzing the standing distribution of genetic variation within species and for determining the relative importance of adaptive evolution versus other processes. Gene sequences provide historically ordered neutral molecular variation that can be converted to gene genealogies which trace the evolutionary relationships among haplotypes (alleles). Gene genealogies can be used to understand the evolution of specific DNA sequences and relate sequence variation to plant phenotype. For example, in the RPS2 gene in *Arabidopsis thaliana*, resistant phenotypes cluster together on the gene tree. The field of phylogeography examines the distribution of allele genealogies in an explicit geographical context and, when coupled with a nested clade analysis, can provide insight into historical processes such as range expansion, gene flow, and genetic drift. We have used a phylogeographical approach to understand the relative effects of gene flow and historical range expansion in the evolution of endangered cycad species in Asia. Phylogeographic studies can also offer insight into practical issues as well, such as the domestication process in crops and the origin of invasive weeds.

## 16

### Molecular analysis of critically shortened telomeres and telomere fusions in Arabidopsis reveals a Ku-independent pathway for chromosome fusion - INVITED SPEAKER

Michelle Heacock, Elizabeth Spangler, Karel Riha, Jennifer Bullock and Dorothy E. Shippen, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128

We are exploiting Arabidopsis as a model for telomere biology in higher eukaryotes. One important goal is to understand how cells distinguish natural, fully capped chromosome ends from ends that lack a functional telomere. We previously showed that Arabidopsis mutants lacking telomerase are viable for up to ten generations, but progressive telomere erosion leads to the onset of growth and developmental defects by the sixth generation. Later generations are characterized by massive end-to-end chromosome fusions. When a telomerase deficiency is combined with a deficiency in Ku70, a protein essential for double strand break repair via NHEJ (non-homologous end-joining), telomere erosion is dramatically accelerated with double mutants reaching the terminal phenotype by the third or fourth generation. Remarkably, the incidence of anaphase bridges is the same in *tert* mutants as in *ku70 tert* mutants, arguing that NHEJ is either not involved in fusing dysfunctional telomeres, or that an extremely efficient backup mechanism exists. We have developed two novel PCR approaches to follow telomere dynamics in telomerase-deficient plants and to examine the nucleotide sequence at chromosome fusion junctions as a means of elucidating the mechanism of end-joining. We found that telomere uncapping is underway in both in *tert* and *ku70 tert* mutants when the shortest telomeres reached a mean length of ~ 700 bp. Telomeres face an increasing probability of being recruited into fusions as erosion continues in successive generations. In the terminal generation no telomeres shorter than 300-450 bp were detected. Sequence analysis revealed that exonucleolytic processing of the ends occurred prior to fusion. The fusion junctions in *tert* mutants were consistent with joining by NHEJ. However, in the absence of Ku the architecture of fusion junctions was altered displaying an increased frequency of telomere-telomere fusions, and microhomology. Thus, Arabidopsis employs both Ku-dependent and Ku-independent end-joining mechanisms to fuse critically shortened telomeres, with the later mechanism being more homology-driven.

## 17

### VIRAL SUPPRESSION OF RNA SILENCING IN PLANTS - INVITED SPEAKER

Vicki Vance, Sizo Mlotshwa, Steven Schauer, Xin Ge, Gail Pruss and Lewis Bowman University of South Carolina, Department of Biological Sciences, Columbia, South Carolina 29208, USA, fax: 803-777-4002, email: vance@biol.sc.edu

RNA silencing is a remarkable type of gene regulation based on sequence-specific targeting and degradation of RNA. One key conserved feature of RNA silencing pathways in different organisms is that the process is triggered by dsRNA. The dsRNA is processed into short interfering RNAs (siRNAs) by the activity of a ribonuclease III-like enzyme termed Dicer. The siRNAs incorporate into a large protein complex termed RISC and act as guides to direct the complex to target RNAs and effect their degradation. In plants, RNA silencing may have evolved as a defense against viruses, many of which replicate via dsRNA intermediates. Consistent with this idea, a number of plant viruses encode suppressors of silencing. Here we report the effect of one such suppressor of silencing, the helper component proteinase (HC-Pro) of potyviruses, on features of RNA silencing including transgene methylation, small RNA accumulation and the ability to send and respond to the mobile silencing signal. We show that HC-Pro suppresses several classes of transgene-induced RNA silencing, in each case eliminating accumulation of the siRNAs without blocking either transgene methylation or systemic silencing. Interestingly, the level of endogenous microRNAs in the HC-Pro plants was greatly elevated, suggesting that HC-Pro affects multiple small regulatory RNA pathways, possibly by altering the regulation of one or more Dicer-like enzymes. We have identified several cellular proteins that interact with HC-Pro in the yeast two-hybrid system. Studies of the role of these proteins are providing clues about the mechanism and regulation of RNA silencing and other defense pathways in plants.

## 18

### Genetic dissection of miRNA and siRNA pathways in Arabidopsis - INVITED SPEAKER

Taline Elmayer, Virginie Gasciolli, Isabelle Gy, Franck Vazquez and Hervé Vaucheret Laboratoire de Biologie Cellulaire, INRA, 78026 Versailles Cedex, France

In animals, double-stranded short interfering RNA (siRNA) and single-stranded microRNA (miRNA) regulate gene expression by targeting homologous mRNA for cleavage or interfering with their translation respectively. siRNA are processed from injected or transgene-derived long perfect double-stranded RNA (dsRNA) while miRNA are processed from short imperfect dsRNA precursors transcribed from endogenous intergenic regions. In plants, both transgene siRNA and endogenous miRNA activate cleavage of homologous RNA targets, however one example of translation repression by miRNA has just been described. Here we show that different sets of genes are required for the production of siRNA and miRNA. Mutations in *SGS2/SDE1*, *SGS3*, *AGO1* and *HEN1* impair accumulation of transgene siRNA involved in sense post-transcriptional gene silencing (S-PTGS). Mutations in *SGS2/SDE1*, *SGS3*, *AGO1* and *HEN1* also impair resistance against cucumber mosaic virus, indicating that S-PTGS derives from an antiviral mechanism. Mutations in *SGS2/SDE1*, *SGS3*, *AGO1* and *HEN1* do not interfere with siRNA and IR-PTGS triggered by hairpin transgenes directly producing perfect dsRNA., indicating they act upstream or at the production of dsRNA. Mutations in *HEN1* but not in *SGS2/SDE1*, *SGS3* nor *AGO1* impair the accumulation of endogenous miRNA and the subsequent cleavage of homologous target mRNAs. Our results, together with others showing that mutations in *DCL1* affect miRNA but not siRNA, and that the accumulation of siRNA but not of miRNA is temperature-sensitive, suggest limited connection between siRNA and miRNA pathways in plants.

## 20

### The Oryza Map Alignment Project: Toward a Closed Experimental System for the Genus Oryza - INVITED SPEAKER

Rod Wing, University of Arizona Lincoln Stein, Cold Spring Harbor Laboratory Scott Jackson, Purdue University

With the approaching completion of the International Rice Genome Sequencing Project's penultimate goal of assembling a base-perfect finished rice genome sequence by December 2004, science must now set its sights on a complete functional characterization of rice. Such analyses will include functional annotation of the rice genome using full length cDNAs and mutant knockout lines, proteomic and metabolic profiling and comparative genomics. Ultimately, the knowledge gained from this work will lead to superior rice cultivars that are essential to feed our ever expanding world population. One important area in the functional characterization of rice is in the analysis of the wild relatives of rice. Wild rice species offer an enormous potential to expand the gene pool for cultivated rice as well as help in the identification of conserved regulatory elements. To this end our consortia was just funded by the US National Science Foundation to develop and exploit a set of new tools designed to physically align the genomes of representatives of 12 wild rice species (Table 1) to the sequenced *Oryza sativa* ssp. *japonica* cv. Nipponbare genome – The Oryza Map Alignment Project (OMAP: [www.omap.org](http://www.omap.org)). The long term objective of OMAP is to create a genome-level closed experimental system for the genus *Oryza* that can be used as a research platform to study: evolution, development, genome organization, polyploidy, domestication, comparative genomics, gene regulatory networks, positional cloning, and crop improvement.

The specific objectives are to:

- Construct fingerprint/BAC end sequence physical maps of 12 wild rice species comprising the 10 genome types
- Align the 12 physical maps with the sequenced reference AA genome diploid *Oryza sativa* ssp. *japonica* cv. Nipponbare
- Reconstruct rice chromosomes 1, 3 and 10 across all 12 wild species

- Data mine BAC end sequence/positional information for SNPs, repetitive elements etc.

In my talk I will discuss OMAP, recent progress in BAC library construction of the wild species, and a proposal to determine if the wild species accessions that we are aligning to the sequenced japonica genome can be used as the standard reference wild species for experimental work throughout the world.

## 21

### **Towards the Arabidopsis Haplotype Map using Arrays - SELETED FOR PRESENTATION**

Justin Borevitz<sup>1</sup>, Jonathan Jones<sup>2</sup>, Charles Berry<sup>3</sup>, Joseph Ecker<sup>1</sup> <sup>1</sup> Salk Institute, La Jolla, CA, USA <http://naturalvariation.org> <sup>2</sup> Sainsbury Laboratory, Norwich, UK. <sup>3</sup> UCSD, La Jolla, CA, USA.

High density oligo nucleotide arrays are often used for genome wide transcriptional profiling. Hybridization of total genomic DNA can be used as a control to identify differences between strains. We have extended this highly parallel genotyping tool to complex genome organisms such as Arabidopsis. By testing for statistical differences in hybridization intensity at each 25-mer feature, > 12,000 Single Feature Polymorphisms (SFPs) can be detected between 2 strains (5% error rate by sequencing and permutation testing). SFPs identify potential deletions, can be used for genotyping recombinant inbred lines, and for mapping via bulk segregant analysis. We show that quantitative trait loci can also be mapped using pools of extreme lines, via a method we call eXtreme Array Mapping.

We have applied this high resolution genotyping to > 15 Arabidopsis accessions in order to investigate the patterns of polymorphism genome wide in *A. thaliana*. Over 2,400,000 genotypes were called. The amount and pattern of diversity are highly variable along the genome. A recent selective sweep may purge genetic variation and increase singleton polymorphism levels. High levels of diversity and excess pair-wise diversity are suggestive of balancing selection. These regions are highlighted in this data set against an empirically defined genome wide distribution. Furthermore, linkage disequilibrium is clear, blocks range from 10kb to over 500kb, and is also highly variable. This data defines common haplotype blocks genome wide which is the first step toward association mapping. Analysis of more accessions (200 or greater) will allow quantitative phenotypes to be directly mapped and be a test bed for development of haplotype mapping procedures.

## 22

### **High resolution mapping of of se2.1: A QTL associated with the evolution of mating system in tomato - SELETED FOR PRESENTATION**

Kai-Yi Chen, Department of Plant Breeding, Cornell University; Steven D. Tanksley, Department of Plant Science, Cornell University

The degree to which stigmas are exerted above the stamen in flowers is a key determinant of cross pollination (and hence allogamy) in many plant species. Most species in the genus *Lycopersicon* are obligate or facultative outcrossers and bear flowers with highly exerted stigmas. In contrast, the cultivated tomato (*L. esculentum*) bears flowers with flush or inserted stigmas promoting self-fertilization. It has been observed that a major QTL, *se2.1*, on chromosome 2 is responsible for a large portion of phenotypic variation for this trait and that mutation(s) at this locus were likely involved in the evolution from allogamy to autogamy in this genus. In order to understand the genetic and molecular basis of stigma exertion, we have conducted high resolution mapping at the chromosome region harboring the *se2.1* QTL. The results indicate that this is a compound locus, comprised at least five tightly linked genes, one controlling style length, three controlling stamen length, and the other affecting anther dehiscence, a taxonomic character used to distinguish *Lycopersicon* species from other solanaceous species. This cluster of genes may represent the vestiges of an ancient co-adapted gene complex in controlling mating behavior.

## 23

### **The role of JAGGED in shaping lateral organs - SELETED FOR PRESENTATION**

José R. Dinneny, University of California, San Diego Ramin Yadegari, University of Arizona, Tuscon Robert L. Fischer, University of California, Berkeley Martin F. Yanofsky, University of California, San Diego Detlef Weigel, Max Planck Institute for Developmental Biology, Tübingen, Germany

During the lifecycle of a plant, lateral organs are modified and reshaped to suit changing needs. For example, in flowering plants lateral organs will take the shape of leaves, sepals, petals, stamens and carpels. Each of these organs has a unique form that helps the plant carry out specific functions. While our understanding of the specification of organ identity is advanced, a mechanistic understanding of the processes which sculpt lateral organs has only recently begun to take shape. We have identified and characterized a novel C<sub>2</sub>H<sub>2</sub> Zinc-finger transcription, *JAGGED* (*JAG*), which is involved in maintaining the growth of tissues in lateral organs. Loss of *JAG*-function results in serrated leaves, as well as, short and narrow floral organs with the strongest defects at the distal regions. Using the expression of *Histone 4* to monitor cell cycle activity, we found that, in petals, *JAG* is required to suppress the premature differentiation of tissues necessary for the development of the distal petal. Consistent with these findings, *JAG* is expressed in the growing regions of lateral organs. Furthermore, through the use of gain-of-function *JAG* lines, we find that *JAG* is also sufficient to activate the growth of many different tissues including the cryptic bract, a vestigial leaf-like organ that subtends the flower in many species but is absent from *Arabidopsis*. Unlike other genes expressed in lateral organs, *JAG* is normally not expressed in the cryptic bract, and suggests the possibility that the exclusion of *JAG* from the cryptic bract may be the cause of bractless flowers in *Arabidopsis*. These data show that *JAG* is an important determinant in shaping lateral organs and suggest that regulation of *JAG* may be important for the evolution of plant form.

## 24

### **Variations on a theme: The evolutionary genetics of petal identity. - SELETED FOR PRESENTATION**

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The elucidation of the genetic program controlling floral organ identity has created many new opportunities for understanding the evolution of floral morphology. In particular, it has been suggested that many aspects of floral diversity could have been generated through simple shifts in the functional domains of the homeotic organ identity genes. Members of the lower eudicot family Ranunculaceae are very well suited for testing this hypothesis due to their wide array of perianth types. In many genera, such as *Aquilegia* (columbine), two whorls of morphologically distinct petals are present in the flower, suggesting that two separate petal identity programs may be functioning. In other taxa, only one type of petals are present, although these may occupy the first whorl, as in *Clematis*, or the second, as in *Ranunculus*. The genus *Aquilegia* is especially useful in the study of this phenomenon due to its small diploid genome and the availability of many floral homeotic mutants. We have identified homologs of the *Arabidopsis* genes *APETALA3* (*AP3*), *PISTILLATA* (*PI*), *AGAMOUS* (*AG*), *APETALA1* (*API*) and *SEPALLATA3* (*SEP3*) in *Aquilegia*, and have begun to characterize their expression patterns in both wildtype and mutant plants. It appears that gene duplications which occurred in the *AP3* lineage before the diversification of the Ranunculaceae have facilitated the evolution of multiple forms of petaloid organs in this new model species. This theory is being evaluated through genetic analyses of homeotic mutants that affect the identity of petaloid organs.

## 25

### **The 9DC Cladosporium fulvum resistance gene cluster from Lycopersicon - SELETED FOR PRESENTATION**

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Resistance of tomato to the fungal pathogen *Cladosporium fulvum* is mediated by *Hcr9s* (homologs of *C. fulvum* resistance gene *Cf-9*) and has been introgressed from wild species. Several *Hcr9* clusters have been fully sequenced, including the *Cf-9* cluster. The tandem repeat structure of *Hcr9* clusters allows extensive shuffling

of *Hcr9s* by unequal recombination and gene-conversion events. Active *Hcr9s* may thus be generated. Intragenic recombination in the wild tomato species *Lycopersicon pimpinellifolium* has generated two genes, *Cf-9* and *9DC*, which are both involved in recognition of the *C. fulvum* elicitor AVR9. Southern blot analysis of plants containing *Cf-9* or *9DC* indicated that the composition of their respective clusters is not conserved. In contrast, *Cf-9* and *9DC* are highly conserved, and crosses show they are allelic. This prompted us to isolate the complete *9DC* gene cluster from a single *9DC L. pimpinellifolium* accession. By comparison of this cluster with the known *Cf-9* cluster, the variation present in clusters containing *Hcr9s* with similar specificities was studied. The *9DC* cluster contains five full-length *Hcr9s* and one incomplete *Hcr9*. Surprisingly, the *9DC* gene is perfectly duplicated, and a third *9DC* variant is also present. All three genes mediate recognition of AVR9, though the *9DC* variant is less active than the *9DC* copies. The other two *Hcr9s* in the *9DC* cluster are orthologs of genes in the *Cf-9* cluster. The general structure of the *9DC* gene cluster resembles that of the *Cf-9* cluster.

In *L. pimpinellifolium* at least three different *Hcr9s* mediate AVR9 recognition. We conclude that unequal recombination, gene-conversion and duplication are all involved in the generation of functional *Hcr9s*, but the overall structure of *Hcr9* clusters remains intact.

## 26

### Dissection of pollination syndromes in *Petunia* - SELETED FOR PRESENTATION

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Animal-mediated pollination is essential in the reproductive biology of many flowering plants. A characteristic consequence is the widespread occurrence of pollination syndromes, sets of floral traits that have adapted to a particular group of pollinators. In the genus *Petunia*, pollination syndromes for hawkmoths (*P. axillaris*) or bees (*P. integrifolia*) are found, with profound differences in floral morphology, fragrance, petal color, and nectar. Here we characterize these syndromes using genetic and phenotypic analyses of recombinant inbred line collections carrying wild genome segments in a transposon-rich genetic background. Morphology changes are mainly caused by increased cell numbers in the basal third of *P. axillaris* petals. Distinct physiological aspects of the nectaries modulate volume, concentration and composition of secreted nectar. Fragrance differences are caused by biosynthetic deficiencies and genetically modified circadian control and emission levels of volatile phenylpropanoids. For most floral traits, several quantitative trait loci (QTL) of moderate effect were detected. This work provides a basis to unravel the molecular mechanisms that shape *Petunia* floral evolution.

## 27

### A contradictory GLABRA3 allele helps define gene interactions controlling trichome development - SELETED FOR PRESENTATION

M. David Marks, Jeffrey J. Esch, Margaret Chen, Mark Sanders. University of Minnesota

The development of trichomes in *Arabidopsis* is being used as model system to address questions concerning cell fate and cell differentiation. The products encoded by three key genes, *GL1* (R2R3 MYB), *GL3* (bHLH), and *TTG* (WD40), are predicted to physically interact to form an activation complex that positively regulates trichome development. Previously characterized *gl3* mutants have trichomes that are smaller, less-branched, and undergo less endoreduplication. A new *gl3* mutant, called *gl3-shapshifter* (*gl3-ss1*), has variously shaped trichomes that over expand during early trichome development, that undergoes more endoreduplication, and that have a striking nuclear morphology. The mutant nuclei consist of many interconnected lobes. The Lac OP1-GFP system has been used to show that the complex *gl3-ss1* nuclei contain only a single set of polytene-like chromosomes. The predicted *gl3-ss1* polypeptide reveals a Leu to Phe substitution (position 79) within a region responsible for protein-protein interaction. Yeast interaction assays comparing *GL3* with *gl3-ss1* proteins show that the mutant protein exhibits a 75% and 50% decrease in interaction with *GL1* and *TTG1*, respectively, and no difference in its interaction with *TRY* (R3 MYB, a negative regulator of trichome development). A competitive yeast interaction assay has been used to show that *TRY* has the ability to prevent the *GL1-GL3* interaction and that the *GL1-gl3-ss1* interaction is even more sensitive to *TRY*. Analysis of plants expressing functional GFP-tagged versions of *GL1*, *GL3* and *TRY* show that the proteins are localized in trichome nuclei. These results have been used to model trichome initiation in terms of protein interactions and threshold levels of activator complex.

## 28

### Use of fluorescence-tagged T-DNAs to study interphase chromatin in *Arabidopsis* - SELETED FOR PRESENTATION

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Despite the availability of the *Arabidopsis* genome sequence, little is known about chromosome organization in interphase cells. A new way to study this question by fluorescently tagging different chromosomal regions has proven fruitful in *Drosophila*, yeast and human cells. We have now adapted this approach for plants. Constructs comprising the bacterial tetracycline operator/repressor (tetO/TetR) system and enhanced yellow fluorescent protein (EYFP) (1) as well as the bacterial lac operator/repressor (lacO/LacI) system and red fluorescent protein (DsRed2) have been introduced in *Arabidopsis* to visualize tagged T-DNAs in interphase nuclei of living cells. Different plant lines transformed with the constructs are being used to study interphase chromosome organization. Allelic and nonallelic distance measurements between fluorescently-tagged T-DNAs in hemi- and homozygous material (YY, RR, Y-/R-) as well as results from double homozygous material (YYRR) will be presented.

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1. Matzke, A.J.M., van der Winden, J., and Matzke, M. (2003) *Plant Mol. Biol. Rep.* 21: 9-19, 2003.

## 29

### Genomic Responses to a Century of Phenotypic Selection in Maize - SELETED FOR PRESENTATION

Stephen Moose and Martha Schneerman, Department of Crop Sciences, University of Illinois

The Illinois Protein Strains are the result of the longest continuous genetic selection experiment in higher plants. Initiated in 1896, 104 generations of recurrent selection for grain protein concentration from the open-pollinated maize variety Burr's White has produced Illinois High Protein (IHP, 35% grain protein) and Illinois Low Protein (ILP, 4% grain protein), which are the known phenotypic extremes for grain protein concentration in maize. Beginning at generation 48, reversing the direction of selection within IHP and ILP also created the Illinois Reverse Low Protein (IRLP, 18% grain protein) and Illinois Reverse High Protein (IRHP, 5% grain protein) strains. In addition to grain protein concentration, the selection experiment has produced phenotypic extremes for a variety of correlated traits, particularly kernel starch concentration and whole plant nitrogen metabolism. To gain insights into the response of the genome to more than 100 generations of divergent selection, we have begun to apply genomics approaches to characterize the Illinois Protein strains. These include surveys across populations and time for allelic diversity, transcriptional profiling, and mutagenesis. Though still in progress, our work to date shows that the selection experiment has progressively fixed allelic variants at some loci; whereas others remain dynamically evolving. As expected, the expression of seed storage protein genes has been dramatically altered due to changes in genomic structure, transcriptional control, and post-transcriptional regulation. Despite ample evidence for the quantitative inheritance of the continuous phenotypic variation observed in the strains, variants in single genes can contribute greatly to phenotypic differences. Continued characterization of the unique genetic resource provided by the Illinois Protein Strains will provide additional insights into how plant genomes respond to phenotypic selection, as well as serve as a discovery tool for those genes regulating whole plant carbon/nitrogen metabolism and kernel composition.

## 30

### The pattern of polymorphism in *Arabidopsis* - SELETED FOR PRESENTATION

Tina Hu, University of Southern California; Yoko Ishino, University of Southern California; Christopher Toomajian, University of Southern California; Honggang Zheng, University of Southern California; Jinal Jhaveri, University of Southern California; Erica Bakker, University of Chicago; Elizabeth Massey, University of Southern California; Joy Bergelson, University of Chicago; Martin Kreitman, University of Chicago; Magnus Nordborg, University of Southern California

Surveys of DNA sequence polymorphism in *Arabidopsis thaliana* have revealed that linkage disequilibrium is extensive but decays within 1 cM. When coupled with the high level of variability and the availability of inbred lines, this finding suggests that a "haplotype map" of *A. thaliana* could be extremely useful for mapping loci responsible for natural variation. We are constructing construct such a map by sequencing 1500-2000 short (500-700 bp) fragments distributed throughout the genome in a sample of 96 accessions, chosen so as to include the most commonly used recombinant inbred lines (RILs), as well as to gain insight into population structure in *A. thaliana*. The fragment density means on the order of four fragments per cM, or one fragment every 50 kb. We are also developing a database and bioinformatics tools to make the data available and useful. Seeds from the chosen 96 accessions are available through the stock centers.

Here we report on progress on this project. We have sequenced over 1000 fragments, and are close to finish processing these data. We discuss what preliminary analysis of these data has revealed about the pattern of polymorphism in the *A. thaliana* genome.

## 31

### Dissection of early meiotic prophase events in maize - SELETED FOR PRESENTATION

Wojtek Pawlowski, University of California at Berkeley; Inna N. Golubovskaya, University of California at Berkeley; Ljuda Timofeeva, University of California at Berkeley; Liang Shi, Torrey Mesa Research Institute; Jingqiu Li, Torrey Mesa Research Institute; Waiking Kwan, Torrey Mesa Research Institute; Xun Wang, Torrey Mesa Research Institute; William F. Sheridan, University of North Dakota; W. Zacheus Cande, University of California at Berkeley.

Meiosis and meiotic recombination are key processes for transmission of genetic information from parents to their progeny and for generating genetic variation. We combine cytological, genetic, and molecular approaches to identify proteins and pathways involved in initiation and progression of meiosis and meiotic recombination. The switch from the somatic to the meiotic cell cycle in maize is regulated by *ameiotic1*. In most mutant alleles of *aml*, meiocytes undergo mitosis instead of meiosis or arrest in pre-division interphase. The *aml* gene regulates installation of the meiotic recombination machinery, as meiocytes in *aml* mutants lack chromosomal foci of the recombination protein RAD51 involved in repairing meiotic double-strand breaks. The *aml* gene encodes a novel protein with two conserved domains of unknown function and a coiled-coil domain, frequently associated with protein-protein interactions. Interestingly, the *aml* transcript is constitutively expressed in all plant parts and not limited to meiocytes. These data suggest that *aml* acts as a switch activated by cell cycle-related proteins and triggers a number of downstream events during the meiotic division.

Another key meiotic protein, encoded by the *poor homologous synapsis1* gene, is required for the homology search, a process ensuring that homologous chromosomes find their proper homologs during the meiotic prophase. *phs1* mutants show almost complete chromosome synapsis but homologous pairing and synapsis are completely replaced by synapsis between non-homologous chromosomes. The mutants are also defective in meiotic recombination as they show a delay in repairing double-strand breaks and do not install RAD51 foci on chromosomes. The *phs1* gene encodes a novel meiosis-specific protein with several short conserved domains but with a low overall level of evolutionary conservation. We hypothesize that the PHS1 protein plays a role in ensuring that pairing occurs exclusively between homologous chromosomes and that it may be involved in a multistep process to coordinate pairing and recombination.

## 32

### The Maintenance of *knox* Gene Silencing - SELETED FOR PRESENTATION

Tara Phelps-Durr, Julie Thomas, Phil Vahab and Marja Timmermans, Cold Spring Harbor Laboratory

Development in plants is dependent on a balance between the maintenance of stem cells in the shoot apical meristem (SAM) and the recruitment of daughter cells to form lateral organs. A key step that distinguishes organ founder cells from stem cells in the SAM is the down-regulation of the class I *knotted1*-like homeobox (*knox*) genes. In *Arabidopsis*, there are four class I *knox* genes; *STM*, *KNAT1*, *KNAT2* and *KNAT6*. As daughter cells are recruited from the SAM and lateral organs are initiated, *knox* gene expression must be down-regulated for proper leaf development. One of the genes responsible for maintaining *knox* gene silencing during leaf development is *ASYMMETRIC LEAVES1* (*AS1*). *AS1* is a myb domain transcription factor with homology to the maize *rough sheath2* (*rs2*) and *Antirrhinum PHANTASTICA* (*PHAN*) genes. Null mutations of *AS1*, *rs2* or *PHAN* result in the misexpression of *knox* genes in the developing leaf primordia.

In *rs2* null mutants the misexpression of *knox* genes results in an accumulation of overproliferating cells and displacement of sheath tissue distally into the blade. The misexpression of the *knox* genes occurs in a clonal pattern suggesting that the misregulation arises in the meristem. Since *rs2* is not expressed in the meristem its role is to maintain repression after the initial downregulation of the *knox* genes. To determine what other factors are involved in the repression of *knox* genes during leaf development, a yeast two hybrid screen was performed to identify proteins that interact with RS2.

Eight RS2 interacting proteins were verified. They include the maize homologues of *asymmetric leaves2*, RS2 itself and the chromatin remodeling factor HIRA. We are currently testing these interactions using the *Arabidopsis* homologues and have analyzed publicly available insertion lines to further characterize the interacting proteins. T-DNA insertions in the *HIRA* gene are lethal. Therefore, *Arabidopsis* 35S::*HIRA* lines were constructed. These cosuppression lines have a phenotype similar to *AS1* mutants. RT-PCR analysis confirmed that the 35S::*HIRA* lines are misexpressing *knox* genes. Based on these observations, we believe *AS1* and *AS2* form a complex that recruits HIRA to maintain a silenced chromatin state at the *knox* loci after daughter cells have been recruited from the SAM to form lateral organs.

## 33

### Chromatin modification and phenotypic homeostasis in inter-species hybrids - SELETED FOR PRESENTATION

Richard J. Lawrence, Sasha Preuss and Craig S. Pikaard. Biology Department, Washington University in St. Louis

Decades ago, Barbara McClintock postulated that "genome shock" is a likely consequence of hybridization and/or polyploidization. She suggested that cells have mechanisms to maintain homeostasis in response to such genomic shocks, thereby allowing many hybrids to succeed. We have accumulating evidence that chromatin modifying activities are critical for maintaining uniform phenotypes in inter-species hybrids and allopolyploids. For instance, knocking down the expression of the histone deacetylase HDT2 using RNA interference (RNAi) in *Arabidopsis thaliana* has little phenotypic consequence. However, transforming *A. suecica*, the allotetraploid hybrid of *A. thaliana* and *A. arenosa*, with the same HDT2 RNAi-inducing transgene causes pleiotropic phenotypic abnormalities including altered leaf morphology, altered vernalization requirement, disrupted phase change and abnormal flower development. Importantly, crossing an aphenotypic *A. thaliana* HDT2-RNAi plant with *A. lyrata* results in diploid hybrid offspring that display phenotypic abnormalities similar to those observed in *A. suecica*. *A. thaliana* lines harboring RNAi-inducing transgenes targeting numerous other chromatin modifying activities also show dramatic phenotypes only in hybrids, suggesting that epigenetic mechanisms are important to hybrid success. One well-known response to inter-species hybridization is the silencing of one parent's ribosomal RNA genes, an epigenetic phenomenon known as nucleolar dominance. In *Arabidopsis suecica*, the rRNA genes derived from *A. thaliana* are selectively silenced. Using RNA interference (RNAi) to target 13 genes representing the four families of histone deacetylases (HDAs), we have identified one HDA within the family related to yeast RPD3 and two plant-specific HDAs that are involved in uniparental rRNA gene silencing.

## 34

### A Novel Mechanism of Genetic Reversion at the *HOTHEAD* locus in *Arabidopsis* - SELETED FOR PRESENTATION

Robert E. Pruitt and Susan J. Lolle, Purdue University

A fundamental tenet of classical Mendelian genetics is the principle that alleles are transmitted unchanged from parent to offspring. Several exceptions to this principle are known, including unstable genetic alleles caused by transposons or repeated sequences, as well as epigenetic alleles. The process of gene conversion can also result in alleles that are altered at high frequency as they pass through meiosis during sexual reproduction. Genetic characterization of the *Arabidopsis* organ fusion gene *HOTHEAD* revealed that plants homozygous for many independently isolated, EMS-induced mutant alleles produce wild-type progeny with a frequency of 1-10%. All of these mutations are single nucleotide changes in the coding sequence of the *HOTHEAD* gene and both PCR and DNA sequencing confirm that the mutant nucleotides are specifically changed to the wild-type sequence in these phenotypic revertants. PCR and DNA-blotting experiments fail to identify any cryptic DNA templates within the *Arabidopsis* genome indicating that this represents a novel type of genetic sequence change that is probably not due to gene conversion. Data will be presented concerning the specificity, timing and developmental control of the process resulting in these DNA sequence changes.

## 35

### Chaperone-mediated buffering of genetic variation - SELETED FOR PRESENTATION

Todd A. Sangster 123, Christine Queitsch 14, Catherine McLellan 4, Kurt Schellenberg 4, and Susan Lindquist 3 1These authors contributed equally to this work 2Committee on Genetics, University of Chicago, Chicago, Illinois 60637, USA 3Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA 4Bauer Center for Genomics Research, Harvard University, Cambridge, Massachusetts 02138, USA

Hsp90 plays a central role in the unfolding of genotype to phenotype. As previously shown in *Drosophila melanogaster*, modulation of Hsp90 activity in *Arabidopsis thaliana* by pharmacological inhibition or increased temperature led to an array of aberrant morphological phenotypes, which were dependent upon underlying cryptic genetic variation. Furthermore, in *A. thaliana*, Hsp90 influences morphogenetic responses to environmental cues and buffers normal development from the destabilizing effects of stochastic processes. We have concluded that Hsp90 affects the buffering and release of cryptic genetic variation as a consequence of its biochemical function—chaperoning the maturation of many regulatory proteins. Since the release of the buffered genetic variants is synchronized to environmental stress, the Hsp90 buffer system may influence the rate of evolutionary change or allow the appearance of morphological novelty. In addition, directed manipulation of Hsp90's buffering capacity presents an extraordinary technique to utilize cryptic genetic variation to improve crop plants, potentially bypassing public concerns about transgenic applications.

To explore the implications and possible applications of the Hsp90 buffer, we have targeted the four cytosolic Hsp90 homologs of *A. thaliana* with RNAi constructs, resulting in plants with genetically reduced Hsp90 levels. We have quantitated the Hsp90-dependent phenotypic spectrum in seedlings of Columbia background and have conducted microarray analyses. We have further demonstrated that reduction of Hsp90 levels can strongly affect the penetrance of well-studied recessive mutations; indeed, these can become fully dominant. These plants will be an indispensable tool for further investigation of Hsp90's position in the interplay between genotypes, environments, and stochastic events on the determination of phenotype.

## 36

### Genetic patterning of the *Arabidopsis* perianth - SELETED FOR PRESENTATION

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Floral organs arise in defined numbers and places within the flower primordium. We know much about how floral organs gain their identity, but little about how their location, number, orientation are defined. Based on mutant disruptions, the *PETAL LOSS (PTL)* gene of *Arabidopsis* is involved in controlling the initiation and orientation of petals, and the shape of sepals.

*PTL* encodes a GT transcription factor, one of 28 found in *Arabidopsis*. These were first isolated through their binding to conserved GT elements in the promoters of certain light responsive genes. *PTL* is the first GT factor to be associated with a mutant phenotype, and it is apparently unrelated to light response. The *PTL* protein is transported into the nucleus. *PTL* is expressed in flower primordia from their inception, first in lateral regions and subsequently resolving into four discrete zones between each newly arising sepal. The single large intron is required for this expression.

Ectopic expression of *PTL* using a range of promoters suppresses the growth of tissues in which it is mis-expressed. This suggests that *PTL* normally acts on genes which inhibit growth in the four zones between the sepal primordia, thus helping define the floral blueprint. Modification to these zones in loss of function *ptl* mutants may secondarily influence petal development, perhaps by expanding the space available for them to arise, and/or by weakening signals controlling their initiation and orientation.

*PTL* is also expressed in the margins of developing sepals and leaves. We have some evidence that the abaxially expressed *KANADII* gene represses *PTL* expression in leaves, restricting it to the margins. *PTL* may dampen the growth of the margins of leaves and sepals, thus sculpting their final shape.

## 37

### Adaptive evolution of Centromere Protein C in plants and animals - SELETED FOR PRESENTATION

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Although centromeres perform a conserved function in chromosome segregation, in plants and animals they are typically composed of very rapidly evolving repetitive satellite sequences. Centromere-specific histone variants (CenH3s), which are key centromere determinants, also evolve rapidly, and are under positive (adaptive) selection in both plants and animals. A meiotic drive model for centromere evolution proposes that centromeres compete during female meiosis for preferential inclusion in the sole meiotic product that becomes the egg cell, and that CenH3s continually evolve to suppress the deleterious effects of this meiotic drive. This model predicts that other kinetochore proteins may also be under positive selection in plants and animals, but not in organisms such as yeasts in which all meiotic products survive. Centromere protein C (CenpC) is an inner kinetochore protein found in plants, animals, and fungi that is defined by a conserved 23 amino-acid motif. A second conserved region is found at the C-terminus, although the motif found in plants is dissimilar to that in animals and fungi. We have compared *CenpC* genes from *Arabidopsis* species and from cereals to look for positive selection in this gene, and find three regions that appear to be evolving adaptively. Although the exon/intron structure is largely conserved, we also find that a specific pair of exons has undergone multiple duplication events within the grasses. The lysine/arginine content of the domain encoded by one of the duplicated exons is ~30%, indicating that it may bind DNA. In addition, we find evidence for positive selection of *CenpC* in rodents, but not in yeasts, consistent with the predictions of the meiotic drive model for centromere evolution.

## 38

### The genetic architecture of crop domestication: a meta-analysis - SELETED FOR PRESENTATION

Maria Isabel Chacon and Todd Vision, University of North Carolina at Chapel Hill

Domestication traits can be defined as the chronologically earliest traits to differentiate crops from their wild ancestors. Selection on such traits may result directly from human preferences or from adaptation to cultivation. In recent years, many quantitative trait loci underlying domestication traits, or DQTL, have been mapped in experimental crosses between crops and closely related wild relatives for a variety of crop systems. We have compiled the results of DQTL studies in 14 different systems, including pulses, cereals, and fruit, vegetable, oil and other industrial crops, in order to address the following questions. First, how many DQTL are detected for each trait, what is the distribution of effect sizes, and how much transgressive segregation is observed? We explore the effect of population type and sample size on these measures. Second, what is the distribution of dominance coefficients among DQTL? This is used to address the issue of whether the alleles contributing to domestication are novel mutations or have been culled from standing genetic variation. Third, are the DQTL in each cross randomly distributed along the linkage groups or, as has been suggested by a number of authors, clustered among and within linkage groups? Can this phenomenon help to explain the frequency with which one detects homologous QTL in closely related crops? Fourth, how often do DQTL show antagonistic or epistatic effects, particular when they are linked? The results are interpreted in the context of current theories concerning the interaction of selection, genetic variation, mating system and gene flow on the evolution of loci affecting domestication traits.

## 39

### Molecular evolution of C4-specific gene expression - a case study with the genus *Flaveria* - SELETED FOR PRESENTATION

Peter Westhoff, Udo Gowik, Meryem Akyildiz & Sascha Engelmann Botany, Heinrich Heine University Duesseldorf, D-40225 Duesseldorf, Germany

C4 plants are of polyphyletic origin. This implies that the gene(s) encoding the C4 isoform of phosphoenolpyruvate carboxylase (PEPC) originated from a non-photosynthetic PEPC gene that was already present in the C3 ancestral species. To meet the special requirements of the C4 photosynthetic pathway the expression programme of the C4 progenitor PEPC gene had to be changed to a strong and selective expression in leaf mesophyll cells. Insights into the evolutionary steps involved in these gene expression changes have been possible in the ideally suited dicot genus *Flaveria* (Asteraceae). *Flaveria* contains closely related C3, C3-C4 and C4 species which can be distinguished by their gradual increase in C4 photosynthetic characters. The C4 PEPC gene of *F. trinervia* (called *ppcA*) is expressed at high levels but only in the mesophyll cells, while the orthologous *ppcA* gene of the C3 species *F. pringlei* is expressed at low levels with no apparent cell or organ specificity. This pair of orthologous PEPC genes has been used to identify C4-specific molecular determinants for the mesophyll specific expression of the corresponding gene. By transgenic approaches cis-acting elements were located in the C4 *ppcA* promoter that explain its quantitative and qualitative expression characteristics. A 41 base pair segment (MEM1) was identified in the distal promoter part that was necessary and sufficient for mesophyll specificity. Phylogenetic footprinting allowed to predict an evolutionary model for the C4 *ppcA* promoter.

## 40

### Genes Duplicated by Polyploidy Show Silencing that is Organ Specific

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Plant genomes exhibit high levels of gene redundancy, much of which appears to have arisen from one or more cycles of genome doubling (polyploidy). Polyploidization creates duplicated gene pairs whose expression can be reduced or silenced. Expression of 40 duplicated gene pairs was assayed using allopolyploid cotton as a model system. Silencing or unequal expression was documented for several genes and was variable by organ type. Remarkably, silencing and unequal expression of some duplicated gene pairs are reciprocal and developmentally regulated, with one copy showing silencing in some organs and the other being silenced in other organs. Analysis of three newly-created allotetraploids revealed gene expression and silencing patterns that sometimes mirrored those of the natural polyploid. The genomic extent of gene silencing in a newly-created allotetraploid is currently being investigated. Data suggest that some silencing events may be epigenetically induced during the allopolyploidization process.

## 41

### heritable RNAi in the nematode *Caenorhabditis elegans*

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RNA interference (RNAi) is a sequence-specific posttranscriptional gene silencing mechanism triggered by the introduction of dsRNA. In *Caenorhabditis elegans*, silencing effects of RNAi are generally observable in injected animals and their progeny. For targets expressed in the soma, interference effects are absent in subsequent generations. Grishok *et al.*, (1) first described the transmissibility of RNAi to the F2 generation. However, Grishok's assay was limited by the F2 lethal phenotype resultant from the RNAi effect.

We have developed an assay where we can observe the heritable RNAi (hRNAi) effect over many generations by using a gain of function allele of *oma-1* (oocyte maturation defective). *oma-1* is a non-essential germ line gene initially characterized and kindly provided to us by Rueyling Lin (2). By using this *oma-1* assay, we have detected per durance of the RNAi effect for up to 20 generations. This has enabled us to study the heritable character of RNAi. We have investigated the genetic and biochemical requirements for heritable RNAi, focusing on requirements for the various *rde-* genes, on the optimal molecular character of the trigger, and on the relationship between heritable RNAi and the history of exposure to the trigger.

1) Grishok A *et al.*, Science 2000 Mar; 287: 2494-97

2) Detwiler MR *et al.*, Dev Cell. 2001 Aug; 1(2):187-99.

## 42

### DNA METHYLATION IS MODIFIED UNDER BIOTIC STRESS IN ARABIDOPSIS

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Cytosine methylation represents one of the key processes controlling gene expression in several species. Methylation patterns result from combined action of mechanisms producing methylation (de novo and maintenance) and demethylation. A tight regulation of cytosine methylation state is maintained over different stages of plant development. Seed germination is preceded by global decrease in DNA methylation. Genes controlling flower development are also target of such epigenetic alterations. In addition, this modification has recently been proposed to function as a cellular defensive mechanism preserving the genome integrity from invader DNAs (transgenes, transposons), and affecting gene silencing during viral infections. However, there is still no evidence about alterations in DNA methylation under stress conditions involving the attack by extracellular pathogens. In this work we investigate the occurrence of this epigenetic modification in the Arabidopsis genome in cells infected with *Pseudomonas*. In such pathosystem we monitored the overall methylation states of the plant nuclear genome along infection. Alterations were characterized at the quantitative (HPLC), molecular (AFLP; Southern blots) and cytological levels. Particular features of methylation changes in single copy genes as well as in repetitive sequences are here described.

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### Genetic Variation of *FRI* in *Arabidopsis thaliana*

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The mechanisms controlling flowering is a focus of plant molecular genetics. Natural populations of *Arabidopsis thaliana* show a wide range of variability in flowering time behavior, and allelic variation at *FRI* (*FRIGIDA*) and *FLC* (*FLOWERING LOCUS C*) are major determinants. *FRI* represses flowering by positively regulating *FLC* expression. Two loss-of-function alleles at *FRI* have been reported in previous studies that utilized a relatively small number of accessions. Here we report an extensive survey of genetic variation of *FRI* in *A. thaliana*, sequencing the whole gene and several surrounding loci evenly distributed in a 225 kbp window in 192 accessions representing a wide range of flowering responses. We discuss polymorphism levels in both coding and non-coding regions, as well as linkage disequilibrium and haplotype structure, all in relation to flowering time data measured under different growth conditions.

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### *Athb-12* Is Involved in the Response of *Arabidopsis thaliana* to Beet Severe Curly Top Virus (BSCTV) Infection

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*Athb-12* is an *Arabidopsis thaliana* homeobox gene and a member of the homeodomain-leucine zipper (HD-Zip) family. *Athb-12* has been reported to be induced by ABA-mediated water stress. We also observed that the infection of beet severe curly top virus (BSCTV) induced *Athb-12* expression. The 5' regulatory region of *Athb-12* can drive GUS expression in *Arabidopsis* transgenic plants. *Athb-12* expression was examined using Quantitative RT-PCR. Interacting proteins with *Athb-12* have been identified by yeast two-hybrid technology from a BSCTV infected-*Arabidopsis* cDNA library. Our results indicate that *Athb-12* is one of the regulatory factors involved in biotic as well as abiotic stress cross-talk in *Arabidopsis* plants.

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### ROLE OF CHROMATIN REMODELLING FACTORS DURING FEMALE GAMETOGENESIS IN *Arabidopsis thaliana*.

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The formation of the female gametophyte requires the establishment and maintenance of a precise pattern of cell fate. To control cell fate and gene expression in a flexible way, plant cells have developed robust epigenetic mechanisms that play pivotal functions in plant development. In *Arabidopsis thaliana*, several genes encoding chromatin remodelling factors have been shown to be involved in reproductive development; however, their potential role during female gametogenesis remains unknown. We are investigating the gametophytic role of members of the SWI/SNF2 gene family: *PIKLE/GYMNOS*, *SPLAYED*, and *MOT1* as well as the *Arabidopsis* homologues of the human  $\gamma$ *SNF2* and *ATRXL* genes, which belong to distinct clades of the gene family tree (available at [www.chromdb.org](http://www.chromdb.org)). We are also including in our study two other genes: the transcriptional silencing regulator *MOM1* and *FAS2*, a gene encoding a subunit of the CAF-1 complex involved in maintenance of meristem cell fate by regulating nucleosome structure. We have taken advantage of the RNA interference (*RNAi*) strategy to inactivate each of these genes using a promoter specifically acting in the female gametophyte. pFM1 is a 844 bp regulatory sequence that was obtained from an enhancer detector line showing reporter gene expression in the functional megaspore stage on, until full differentiation of the female gametophyte. We modified the dsRNA vectors generated by the group of R. Jorgensen (U. of Arizona) by replacing the 35S promoter with pFM1, and transformed wild-type individuals with palindromic *RNAi* constructions corresponding to each of our candidate genes. A genetic and molecular characterisation of these plants will be presented.

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### *ATX-1*, an *Arabidopsis* Homolog of *Trithorax* Activates Flower Homeotic Genes

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The genes of the *trithorax* (*trxG*) and *Polycomb* groups (*PcG*) are best known for their regulatory functions in *Drosophila* where they control homeotic gene expression. Plants and animals are thought to have independently achieved regulation of their development. Although the main principles of many developmental processes are similar (like the establishment of segmental identity in animals and the formation of floral organs in plants), the two systems employ unrelated sets of homeotic genes. Despite this fact, several plant developmental processes are negatively regulated by plant genes similar to the repressors from the animal *PcG*. Still missing has been evidence for the existence of activating regulators of plant development of the *Trithorax*-type. This is a major gap preventing comparison and parallels between developmental regulations of plants and animals.

We have obtained genetic, molecular, functional, and biochemical evidence that an *Arabidopsis* gene similar to the *Drosophila* *trx*, *ATX1*, plays an important role in the development of floral organs, their spatial arrangement and morphology. The effects are specific: structurally and functionally related flower homeotic genes are under different control. We show that *ATX1* is an epigenetic regulator with a histone H3K4 methyltransferase activity. This is a first example of this kind of enzyme activity reported in plants and in contrast to the *Drosophila* and the yeast *trithorax* homologs, *ATX1* can methylate in the absence of additional proteins. In this regard, it is similar to the human homolog, capable of methylating H3K4 as a recombinant protein. The histone methylating activity of the *ATX1-SET* domain argues that the molecular basis of these effects is the ability of *ATX1* to modify chromatin structure. Both loss-of-function and gain-of-function *atx1* mutant *Arabidopsis* lines have been created and analyzed. Our results suggest a conservation of *trxG* function between the animal and plant kingdoms despite the different biochemical nature of their homeotic genes.

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### Promoter analysis of *Arabidopsis* genes differentially expressed upon exposure to trinitrotoluene.

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2,4,6-trinitrotoluene (TNT) is a nitro-substituted xenobiotic explosive that is toxic to plants and animals. Plants uptake and metabolize TNT but pathways are uncertain and plant response at the molecular level is unknown. We analyzed gene expression in response to TNT in *Arabidopsis* through the use of cDNA microarrays. *Arabidopsis* seedlings were grown on media containing 1 and 10  $\mu$ M TNT. Data analysis revealed that a total of 52 genes were upregulated and 47 genes were downregulated in response to TNT. For each group of differentially regulated genes, the proximal 500 nucleotides from the transcription start site were retrieved from the AGI database and sequence analysis was performed using the computer program MEME (version 3.0). MEME analysis generated a total of 18 conserved motifs for genes differentially regulated by TNT. To elucidate more biologically relevant motifs with higher information content, the motifs found using the MEME program were searched for known regulatory elements in the PLACE database. In most cases, the identified motifs matched known cis-regulatory elements, some of which are known to be associated with oxidative stress. Novel motifs were also discovered that contained ACGT sequences representing a potential binding site of

plant basic leucine zipper (bZIP) family of transcription factors. Another finding was the presence of a conserved CT rich region in the promoter of genes downregulated at 1 and 10 fYM TNT strongly suggesting a regulatory role for this motif.

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### Asymmetries in Arabidopsis development

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Asymmetries arise throughout Arabidopsis development at the level of the organ, tissue, or individual cell. We have followed the generation of asymmetry at two of these levels: (1) the breaking of radial symmetry in the root and (2) the use of asymmetric cell divisions to establish cell fate and pattern in the epidermis.

(1) The Arabidopsis root has a stereotyped radial arrangement of tissues. Superimposed on the radial symmetry is an axis defined by the linear arrangement of xylem elements. The Arabidopsis root has two xylem poles, and lateral roots emerge from pericycle cells adjacent to them. In a screen for mutations that altered the expression of a marker of these specialized pericycle cells, we identified *lonesome highway (lhw)*. *lhw* mutants express the marker in only one file of pericycle cells, produce lateral roots from only one side of the root, and have only one strand of protoxylem. Exogenous IAA cannot rescue the xylem or pericycle phenotypes. *LHW* is a member of a plant specific gene family of unknown function, but expression of several of the family members is upregulated in response to treatments that promote xylem or lateral roots.

(2) In the shoot epidermis, the distribution of stomata is nonrandom, and guard cells obey several patterning rules: they are formed through a stereotyped lineage of asymmetric divisions, and two stomatal complexes are never adjacent to one another. Local pattern likely involves cell-surface mediated signaling based on the identification of a receptor, TMM, and a protease, SDD1, that may process a ligand. We identified 5 additional loci that affect local pattern. One of these, *YODA*, encodes a MAPKKK and acts as a switch to control cell fate. In the absence of *YODA*, the majority of epidermal cells become guard cells. Conversely, when an overactive form of *YODA* is expressed, no stomata are formed. We have used these *YODA* lines to perform a transcriptional analysis of epidermal identity. 365 genes are differentially expressed, and 156 genes mirror the transcriptional profile of *SDD1* and *TMM1*, suggesting they might be additional loci required to be or to become stomata.

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### The contribution of multiple genes to one trait: linolenic acid production in soybean seeds

Kristin Bilyeu, USDA/ARS Paul Beuselinck, USDA/ARS

Research on the model plant *Arabidopsis thaliana* has provided valuable resources to plant molecular genetics which can be utilized in more complex crop species. The streamlined diploid Arabidopsis genome often contains a single locus with a structural gene for a particular trait. Arabidopsis seed linolenic acid levels are largely dependent on the active expression of a microsomal omega-3 fatty acid desaturase enzyme encoded by the *FAD3* gene. The objective of this work was to utilize this information to determine the basis of linolenic acid production in a crop species. Soybean is an important crop which requires additional tools for advanced genetic manipulation of valuable traits. Although soybean functions as a diploid with 20 linkage groups, it has a highly-duplicated genome, suggestive of a polyploid ancestor. Lowering linolenic acid levels in soybean seeds would enhance the value of the oil produced by increasing stability and reducing the need for hydrogenation and production of trans fatty acids. We identified three soybean *FAD3* homologues and are investigating the contribution of each of these genes to soybean seed linolenic acid levels. The results of association experiments with mutant soybean *FAD3* genes indicate an unequal contribution of the *FAD3* genes to the trait, which appears at least in part due to differences in expression of the genes. Two of the three genes appear to be very closely related, and we are also interested in defining the evolutionary changes that have allowed their divergence in contribution to seed linolenic acid levels.

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### New tools for genome navigation: A global expression map of the Arabidopsis root

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A high resolution gene expression map within an organ identifies genes with localized expression, which may have a potential role in cell fate and specialization. Further insight into gene function can be obtained by comparing such detailed gene expression maps between species, correlating gene activity to changes in morphology or function. In the first step of such global expression analyses, we have developed a genome-wide "digital" in situ localization of the *Arabidopsis* root, profiling the expression of more than 22,000 genes. Root expression was broken down into 15 different zones representing almost all cell types and tissues of the root at three developmental stages. In a comparative analysis of gene expression among cell types within the root, we find major patterns that reveal new putative localized hormone activity domains within the root. We also find widespread evidence in plants for chromosomal clustering of co-regulated genes. The next phase of this research will involve developing a similar gene expression map in other species to compare changes in gene regulation over evolution.

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### Partial transmission of paternal DNA during interspecific hybridization in Solanum

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Potato dihaploids are usually produced by pollination of tetraploid *Solanum tuberosum* cultivars using pollen from selected clones of the diploid species, *S. phureja* or, more rarely using pollen of other *Solanum* wild species. The resultant dihaploids were believed to originate by parthenogenesis, largely on the basis of their close morphological similarity with their female parent (Wagenheim et al., 1960). However, molecular and cytology data have both indicated the presence of DNA from the paternal, *S. phureja* parent in at least most dihaploids (Clulow et al., 1991; Waugh et al., 1992; Clulow et al., 1993). Clulow et al (1991) consequently postulated that dihaploids are formed after initial normal fertilization of the egg cell to form a triploid zygote, followed by preferential loss of *S. phureja* chromosomes during subsequent embryo development. This hypothesis, known as the fertilization-elimination theory, fails to explain several key biological observations made on the dihaploid induction process.

Prevost (2000) proposed a new hypothesis (called the pseudo-fertilization theory) based largely on unpublished observations of the potato embryo sac made by Braselton and Wilkinson during dihaploid induction. There, the degrading vegetative nucleus is thought to initiate parthenogenetic development following close membrane contact (pseudo-fertilisation) and at the same time, provide the egg cell with the paternal DNA. Prevost also suggested a competing theory in which two pollen tubes enter the embryo sac, with two sperm cells from one fertilizing the Secondary Endosperm Nuclei and those of the remaining tube pseudo-fertilizing the Egg cell. New evidence is presented to evaluate the validity of each of these proposed models leading to the partial transmission of paternal DNA during dihaploid induction. The broader evolutionary significance of the findings are briefly explored.

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### Phytophthora infestans resistance in wild diploid Solanum species

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Recent insights into the molecular basis of resistance against pathogens suggest that natural populations achieve durable resistance through deployment of multiple *R* genes. In contrast, modern agriculture relies on genetic uniformity to preserve all the agronomic characteristics that are attractive to the consumer. Cultivated potato is clonally propagated by tubers and therefore all individuals in a given field are genetically identical. Resistance based on a single *R* gene is often short-lived, particularly in the case of sexually reproducing pathogens that can spread over long distances, such as *P. infestans*. Deployment of *R* genes as multilines rather than monocultures may promote durability of disease resistance. Overall resistance may also be enhanced in multilines by incompatible races triggering systemic acquired resistance. Our objective is to identify new *Rpi* (Resistance to *P. infestans*) genes through screening multiple individuals in different accessions of wild diploid *Solanum* species, and target them for cloning. This will allow us to test the effectiveness of a mixture of *Rpi* genes deployed in an otherwise genetically uniform crop. At present we have identified several single dominant *Rpi* genes from diverse sources in our diploid *Solanum* collection and have generated the corresponding mapping populations. All these new *Rpi* genes are in different stages of mapping and some of them have been already placed on the potato genetic map. We are also developing virus-induced gene silencing (VIGS) to quickly assess the function of resistance gene candidates. We started testing the system by silencing the phytoene desaturase (PDS) gene in several *Solanum* species and we are now using this approach to silence *R* genes.

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### Heterochronic effects of trichome mutants on trichome initiation.

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The growth and development of trichomes, or leaf hairs, of *Arabidopsis thaliana* has been used as a model for plant cell differentiation, morphogenesis, and cell cycle regulation. Trichomes undergo a process during their development that increases the number of copies of DNA in the cell, called endoreduplication. Various trichome mutants have been discovered in which this process has been altered. *GLABRA3 (GL3)* is a bHLH transcription factor that forms part of the trichome initiation signal. Plants without a functional copy of *GL3 (gl3)* have smaller trichomes, fewer trichomes per leaf, and reduced copies of DNA in their trichomes as compared to wild type plants. *TRYPTICHON (TRY)* encodes a protein that has a myb-like DNA binding domain, but lacks a transcription activation domain. This protein acts as an inhibitor of trichome initiation, and plants that lack a functional TRY protein (*try*) have larger trichomes with increased copies of the genome as compared to wild type plants. We examined the timing of the initiation of the first trichomes on leaves of mutants of *gl3*, *try*, and wild-type plants. Mutant *gl3* plants exhibit a heterochronic delay in the initiation of trichomes. Preliminary evidence suggests that *try* mutants initiate trichomes earlier in leaf development than wild type. This correlation between DNA content of endoreduplicated trichome nuclei and time of trichome initiation suggests that the number of endoreduplication cycles in trichome nuclei is correlated with the number of mitotic cycles in dividing protoderm cells. Thus *GL3* and *TRY* may affect DNA content by altering the time of trichome initiation, rather than by directly regulating endoreduplication.

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### Deletion Null Alleles In Maize Inbreds: Discovery, Analysis of Genetic Diversity and Role in Heterosis

Stephan Brunner, Dupont Crop Genetics

#### Deletion Null Alleles In Maize Inbreds: Discovery, Analysis of Genetic Diversity and Role in Heterosis

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In general, RFLP, low copy number sequence, SSR and SNP haplotype data from individuals of the same species have assumed that an allelic counterpart is found for each gene. Up to now, a significant amount of information on allelic diversity in maize has accumulated. These allelic sequences can vary extensively. But, only one systematic sequence level comparison among maize inbred lines is available over a larger genomic distance [1]. Microcolinearity has been studied over more than 100 kb at the *bronzel* genomic region of the two maize inbred lines McC and B73. In contrast to expectations, the loci between the two inbreds differ not only in the repetitive DNA segments, but also in some cases genes present in one allele are found missing in the other allele and very large deletions/insertions are found as well. It is necessary to verify these observations in other genetic loci and additional inbreds to find out how common are allelic differences consisting of gene deletions and how frequent are large size and sequence differences in intergenic regions. Allelic differences based on gene deletions may tremendously affect breeding because some genes may simply be absent from certain genetic backgrounds and therefore prohibit recombination. If repetitive intergenic regions were highly different, recombination would be mainly restricted to genes and their immediate vicinity, in the individuals carrying divergent alleles. Results of these studies may have significant impact on the understanding of the nature of quantitative variation in maize, on maize breeding and on the methodology of map-based cloning approaches in general. Furthermore, it would allow verifying the hypothesis that heterosis in maize results from the complementation of missing genes between the two alleles in the hybrid.

We are investigating these questions by exploring other genetic loci and additional maize inbreds. We are performing comparative genomic sequencing of several BAC contigs from the same genomic region of both Mo17 and B73, enhanced by PCR based analysis in a larger set of maize germplasm, and by MPSS gene expression analysis.

References:

1. Fu H. and Dooner H.K.: Intraspecific violation of genetic colinearity and its implications in maize. *Proc Natl Acad Sci USA* 2002, 99: 9573-9578.

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### Exploiting transposons for sources of novel allelic variation

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Transposable elements are often utilized to clone and characterize plant genes. However, they can also serve as tools for generating novel and stable allelic variants. Genome insertion of all known DNA transposons is associated with a duplication of sequences at the target site. However, subsequent excisions of the element often fail to remove the duplicated sequences, resulting in the creation of novel excision or 'footprint' alleles. To demonstrate the utility of this process in maize, we have exploited the transposable element *Ac* in a large-scale mutagenesis of the *ps1/yp7* locus. The *ps1* gene encodes lycopene b-cyclase and is necessary for the synthesis of xanthophylls including lutein and zeaxanthin and the production of ABA in maize. Complete loss-of-function alleles of *ps1* result in the accumulation of lycopene as the primary carotenoid and a failure to produce sufficient quantities of ABA to prevent precocious germination. As a result, severe mutant alleles result in seedling lethality. Using simple genetic, molecular genetic and biochemical screens we have identified stable *Ac* excision events that condition *ps1* alleles with partial activity. Importantly, some of these weak mutant alleles permit the accumulation of ABA pools while maintaining high levels of lycopene in seed tissues. These alleles can be maintained as homozygous stocks and have enabled us to manipulate the carotenoid composition of the maize seed. These studies demonstrate the utility of transposable elements in generating an extensive pool of alleles that can be utilized in breeding programs.

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## Apomixis may evolve by genetic stabilization of reticulation-derived asynchronies in ovule formation

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Gametophytic apomixis is characterized at the embryological level by developmental destabilizations of the megasporocyte or surrounding nucellar cells, the egg, and often the central cell (or polar nuclei). Respectively, these destabilizations are followed by unreduced embryo sac formation, parthenogenesis, and often autonomous endosperm formation. We present evidence from *Sorghum*, *Tripsacum* and *Antennaria* that these destabilizations are caused by non allelic gene systems in sexual plants that, from a population standpoint, vary independently of each other: some control onset times and durations of meiotic stages, others control onset times and durations of embryo sac formation stages, still others control onset times and durations of egg maturation, embryony and endosperm formation. In adapted sexual ecotypes, these systems function in harmony resulting in high seed sets; but in certain hybrids, the gene systems do not function harmoniously but rather suffer inadaptive competition often resulting in intermediacy between sexuality and sterility with such anomalies as aposporous or diplosporous embryo sac formation, parthenogenesis, autonomous endosperm formation, polyspory and polarity reversals arising. How the causal alleles from multiple loci among the various gene systems are genetically stabilized (prevention of segregational disruption upon facultative sexual reproduction) in natural apomicts now seems clear. It usually involves structural (karyotypic) heterozygosity. How many genes are involved in the causal systems remains a mystery. We are focusing our efforts on genetically dissecting, in sexual plants, what appear to be the gene systems critical to apomixis.

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### Photoperiod and transgene interactions on flowering time in soybean: A proposal

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Soybean (*Glycine max* L.) is an obligate short-day (SD) species with an absolute requirement of at least 4-6 h of dark period for transition to flowering. Development of latitude-specific cultivars (popularly known as 'maturity groups') has been the key in expanding the cultivation of this major grain legume crop throughout the continental USA. These region-specific cultivars are well-adjusted to the day length differences and optimized to utilize the full-growing season at their respective latitudes. A key resource that is missing in the development of rational strategies is sufficient knowledge of genes involved in the regulation of flowering time in short-day plants such as soybean. In soy, at least seven loci that affect flowering time and maturity have been identified and a few orthologs of *Arabidopsis* candidate flowering genes have also been mapped on its genetic map. However, there have been few studies to elucidate the molecular mechanism of soybean responses to photoperiod. Recent work in rice, also a short-day plant, indicates that key regulatory genes are conserved between rice and the well-characterized long-day (LD) model plant, *Arabidopsis*. However, the interactions between these players appear to result in opposite effects that are specific to the two types of plants (SD versus LD species). We are attempting to use this knowledge in understanding and fine-tuning the photoperiodic responses of soybean, a dicotyledonous SD species.

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### Transcript Profile of Mutant Flower Buds of *Phalaenopsis* by cDNA-RAPD

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Tissue culture has been widely used for mass production of *Phalaenopsis*. However, the occurrence of somaclonal variation during culture is a frequent and important problem. We have collected and observed mutant flowers of *Phal.* Zuma's Pixie and *Phal.* Little Mary which both show lip-like peloric petals among population of tissue cultured plants. Total RNAs were isolated from flower buds of both hybrids. Using the reverse transcription-random amplified polymorphic DNA (cDNA-RAPD) method, we identified 25 amplified fragments differentially expressed in mutant and 32 in normal flower buds. Sequence analysis indicated that 14 fragments are unknown proteins, 10 are metabolism-related proteins, 5 show functions with transcriptional process, 5 have high similarity to viruses, 4 are involved in protein synthesis and degradation, and 3 are homologs of transposons. The others show some homologies with genes involved in cell division, disease resistance, signal transduction, stress response and transport. (e-mail: furechen@mail.npust.edu.tw)

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### DME, a DNA glycosylase domain protein, regulates MEA imprinting and seed viability in *Arabidopsis*

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Genomic imprinting causes genes to be expressed according to their parental origin and is observed exclusively in mammals and flowering plants. In mammals, many of the imprinted genes are expressed in the extraembryonic membranes that transfer nutrients from the mother to the embryo. In plants, the endosperm performs a similar function and is the critical site of gene imprinting. In mammals the mechanism of gene imprinting involves the differential methylation (5-methylcytosine) of alleles. In plants the mechanisms that control imprinting are unknown but both DNA methylation-dependent and -independent mechanisms regulate epigenetic phenomena. We have discovered that the DEMETER (DME) gene mediates endosperm imprinting in *Arabidopsis* (Choi et al., Cell (2002) 110:33-42). Seed viability depends only on the maternal DME allele. DME encodes a large protein with DNA glycosylase and nuclear localization domains. Most DNA glycosylases function in DNA repair and excise modified, damaged, or mismatched bases, creating an abasic site. AP endonuclease and DNA polymerase complete the repair process by nicking the DNA and by replacing the abasic site with a new base, respectively. We find that the DME DNA glycosylase functions in vivo to activate maternal allele transcription of imprinted genes. DME transcription is restricted to the central cell of the female gametophyte, the progenitor of the endosperm. DME is required for maternal allele expression of the imprinted MEDEA (MEA) Polycomb gene in the central cell and endosperm. Ectopic DME expression induces MEA transcription and analysis of the MEA promoter reveals DME-induced nicking at multiple sites. Mutagenesis of the DME DNA glycosylase active site verifies that base excision activity is essential for DME function in vivo. Thus, the DME DNA glycosylase activates maternal expression of imprinted genes in the central cell, a process that is essential for seed viability.

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### The complexity of selection at a major effect QTL in maize, *teosinte branched1* (*tb1*)

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The identification of loci selected during crop domestication affords an opportunity to understand the types of sequence changes that lead to phenotypic evolution as well as to characterize the effect of selection on crop genomes. The *tb1* gene in maize underlies a major effect QTL for the morphological transition between maize and its wild ancestor (teosinte). Expression studies have shown that *tb1* transcript levels differ between maize and teosinte, suggesting that regulation of the *tb1* gene was a target of selection. Consistent with this observation, nucleotide diversity immediately 5' to the *tb1* coding sequence, but not in the coding sequence itself, has previously been found to be extremely low. Here we show that a region of approximately 60 kb 5' to *tb1* has very low diversity in maize, and was the target of a selective sweep during domestication. The region of the selective sweep does not appear to include genes 5' to *tb1*, and thus the effect of selection on genomic diversity outside the *tb1* region appears minimal. The relatively large region of low diversity 5' to *tb1* may reflect selection for multiple sites to give a large QTL effect, and also a low rate of recombination 5' to *tb1*. We have also identified intra- and intergenic recombinants between maize and teosinte alleles at and nearby *tb1* that allow us to functionally identify the sites responsible for morphological differences between maize and teosinte. Phenotypic data for recombinants are being gathered in summer 2003, and results will be presented.

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### Functional Analysis of FW2.2 Protein

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*fw2.2* is one of the few QTLs that have been isolated thus far in plant. It is also the first gene known to control fruit size in a crop plant. *fw2.2* encodes a protein with no sequence homology with any protein of known function. Yet it is known to be a regulator (either directly or indirectly) of cell division (Frary et al. 2000, Cong et al. 2002, Liu et al. 2003). Computational modeling had earlier suggested that the FW2.2 protein might have a similar structure to RAS proteins which are found in mammalian cell cycle control and possess the GTP-binding ability (Frary et al. 2000). To shed light on the possible function of FW2.2, we have conducted a series of experiments aimed at elucidating cellular location and function of the FW2.2 protein. To examine whether FW2.2 has the GTP-binding activity, nucleotide exchange assays were performed (Leonard et al. 1994). The binding [<sup>35</sup>S]GTP gammaS ability of the recombinant proteins GST-FW2.2, GST-2TK (as a negative control) and GST-CDC42 (as a positive control) was measured after 10min incubation. The results demonstrated that the amount of CDC 42 protein-bound radioactivity rapidly reached extremely high level (1,350,295 cpm), while that of the FW2.2 protein and the negative control protein GST-2TK binding activity was at very low levels (1,499 and 1,681 cpm, respectively). Thus, FW2.2 is not a GTP-binding protein. Additional analysis of the protein annotation predicted that FW2.2 is a novel protein containing two transmembrane domains and may be coupled with cell membrane. To this prediction, GFP/FW2.2 fusion protein was bombarded into young tomato leaves. The transient expression of GFP/FW2.2 was observed in the plasma membrane under a confocal fluorescence microscope, indicating that FW2.2 is localized at plasma membrane. Further, the yeast two-hybrid screening has revealed that there are about 90 putative positive candidate proteins which might interact with the FW2.2 protein. Among which many proteins, such as Ran-GTP binding protein5, G protein beta-subunit, protein kinase CK2, GSK3/SHAGGY-related protein kinase, arginine decarboxylase, meloidogyne induced giant cell protein, Tfm5 protein, negative growth regulator protein NGR1, tumor related protein, etc., have been found essential in plant or mammalian and yeast cell growth and development by controlling cell division or regulating cell expansion. Further experiments to verify the true interacting partner(s) of FW2.2 will be a key step to better understand how FW2.2 regulates cell division, and thus, controls fruit size.

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### Development of a Transposon Tagging Resource in Barley

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A transposon tagging system in barley (*Hordeum vulgare*) was developed, based upon maize *Ac/Ds* elements. The long-term objective of this project is to generate a set of lines with *Ds* insertions dispersed throughout the genome as a comprehensive tool for gene discovery and reverse genetics. Lines containing *AcTPase* and *Ds* insertions were developed by biolistic transformation in the cultivar Golden Promise and crossed. Subsequent transposition and segregation of the *Ds* away from the site of integration and the *AcTPase* element resulted in a number of lines with stabilized *Ds* elements dispersed throughout the genome. Genomic DNA flanking the *Ds* elements was isolated by inverse PCR and TAIL-PCR. BLAST search results indicate that 82% of the transposed *Ds* elements are found within coding regions of the genome. The principal mapping strategy was to identify sequence polymorphisms in the Wolfe Dominant and Recessive Multiple Marker stocks in the regions corresponding to the genomic DNA flanking the *Ds* insertions and to map the polymorphisms in the Oregon Wolfe Barley mapping population. The 20 transposed *Ds* elements map to six of the seven barley chromosomes. We determined the genome locations of the *Ds* insertions in 19 independent lines using primarily CAPS-based assays of SNPs and PCR-based assays of INDELS. One *Ds* insertion was mapped by RFLP using Southern hybridization. In several instances the mapping results obtained by the PCR-based approach were also confirmed by RFLP analyses. In addition, the cloned genomic DNA flanking sequences are being used to assign corresponding map locations to genomic BAC library inserts, thus integrating the barley genetic and physical maps. Lines containing *Ds* elements inserted into genes of interest may be used directly or by reactivation of the *Ds* elements to tag closely linked genes. Transposon tagging in barley using *Ac/Ds* promises to provide a useful tool for Triticeae functional genomics.

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### Excision of a hybrid *Ds* element induces large genomic deletions in *Arabidopsis*

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Transposon activity is known to cause chromosome rearrangements in the host genome. Surprisingly, no *Dissociator* (*Ds*)-induced chromosome rearrangements have been reported in *Arabidopsis* where *Ds* is intensively used for insertional mutagenesis. We will present three *Arabidopsis* mutants with reduced fertility and propose that excision of a hybrid *Ds* element induced a large genomic deletion flanking *Ds*, ranging in size from 64 to 104 kb. In the mutants *anat* and *haumea*, the deletion mechanism consists of a local replicative transposition followed by *Ds* excision where one end of the newly transposed element and one end of the *Ds* transposon at the original site served as substrate for transposase. Excision of this hybrid element reminiscent of a macrotransposon leads to loss of the chromosomal piece located between the two ends, including one full *Ds* element and the flanking genomic sequence. This mechanism was found to be responsible for several other deletions and occurs at a genetically trackable frequency. Thus, it could be applied to efficiently generate deletions of various sizes in the vicinity of any existing *Ds* element present in the genome. In the mutant *tons missing*, a mechanism that involves endogenous repetitive sequences caused a large flanking deletion at a position unlinked to the starter locus. Our study of *Ds* transposition in *Arabidopsis* revealed new mechanisms that lead to large genomic deletions flanking *Ds* elements, which may contribute to genome dynamics and evolution.

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### Identification and Development of RAPD Markers Linked to CMV-B2 Resistance Gene in Melon

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Melon (*Cucumis melo* L.) cultivar *Yamatouri* was previously demonstrated contained a single dominant gene which confers resistance against *Cucumber mosaic virus* isolates B-2 (CMVB2). In this investigation, 46 RAPD markers were evaluated by RAPD-polymerase chain reaction to detect DNA bands specific linked to CMV-B2 resistant in diverse melons. Two primers (OPE-14 and APB-05) produced specific bands (550-bp and 1050- bp) which were obtained in the 5 CMV resistant cultivars *Yamatouri*, *Miyamauri*, *Mawatauri*, *Sanuki-Shirouri* and *Shinjong* respectively. Among them, bulked segregant analysis of *Yamatouri* (a resistant cultivar) and its family were selected and constructed by crossing between *Yamatouri* and *Vakharman* (a susceptible cultivar) to identify RAPD markers linked to CMV resistance. After screening the DNA from bulked resistant and susceptible plants by PCR with 46 RAPD markers, two specific bands which linked to CMV-B2 resistance were obtained in P<sub>1</sub> *Yamatouri*, F<sub>1</sub>, some F<sub>2</sub> and BCs. These RAPD markers were cloned and sequenced for construction of Sequence characterized amplified region (SCAR) markers for detection of CMV-B2 resistance gene in F<sub>2</sub> population. The derived markers, APB-05 SCAR-1/APB-05SCAR-2 from APB-05 and OPE-14SCAR-1/OPE-14SCAR-2 from OPE-14, yielded a single 1050 bp (designated APB-05) and a 550 bp (designated OPE-14), respectively. The results also showed that SCAR markers could be used as a tool for marker-assisted selection (MAS) linked to CMV resistance in *Yamatouri* melon.

Keywords: CMV, *Cucumis melo*, RAPD, SCAR.

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### Floral MADS box genes and the evolution of dioecy by homeosis in meadow rues (*Thalictrum*, Ranunculaceae).

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In most model species currently used to study dioecy and sex determination, flowers are initially hermaphroditic but abort either stamens or carpels during the course of development. This indicates that sex-determination is acting relatively late in flower development, after floral organ identity has been established. In the genus *Thalictrum* (meadow-rue), however, the flowers of several dioecious species appear to be entirely male or female from inception. Using scanning electron and light microscopy, we have confirmed that *T. dioicum* flowers are unisexual from inception and do not show selective organ abortion. This raises the possibility that in this species, genetic pathways which function at the level of the organ identity genes (or directly upstream) may play a role in the process of sex determination by a homeotic-like mechanism. In order to investigate this possibility, we have identified homologs of the organ identity genes *PISTILLATA (PI)*, *APETALA3 (AP3)* and *AGAMOUS (AG)* from carpellate and staminate individuals of *T. dioicum* and the hermaphroditic *T. thalictroides*. RT-PCR and *in situ* hybridization were used to characterize the differential expression patterns of these genes in male and female flowers of *T. dioicum* and hermaphroditic flowers of *T. thalictroides*. Two of the AP3 loci and one of the AG paralogs were found to have largely sex-specific expression patterns. The implication of these findings for the evolution of dioecy in *Thalictrum* and the potential of this genus as a new model for the study of sex determination are discussed.

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### ATMYBFI PLAYS A POSSIBLE ROLE IN FLORAL DEVELOPMENT IN *ARABIDOPSIS THALIANA*

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*MYB* genes encode for DNA-binding proteins that regulate gene expression at the transcriptional level. They have been shown to be present in most eukaryotes, including plants, in which they form one of the largest known gene families. Plant MYB proteins are highly conserved and are implicated in the regulation of a number of important developmental and metabolic programs. Temporal and spatial expression studies using the *GUS* reporter gene and *in situ* hybridisation have demonstrated that *AtMYBFI* is transiently expressed in early shoot apices at the same time as other genes involved in the regulation of floral development. Various constructs have been transformed into *Arabidopsis* in order to further characterise *AtMYBFI*, resulting in transgenic plants with altered leaf and flower morphology, as well as delayed flowering. Two T-DNA insertion mutants have also been isolated from the KO facility at UW Madison. Observations of these two lines show a severely altered flower morphology along with a significant decrease in the level of expression of *AtMYBFI*. Preliminary studies also indicate altered expression levels of *AtMYBFI* in other flowering mutants such as *apetala (API)* and *Flowering Locus T (FT)*. Like many other genes involved in flowering, RT-PCR blot analysis has demonstrated that *AtMYBFI* is regulated by circadian rhythms. Further analysis of the T-DNA KO plants and other flowering mutants will provide important information in further understanding the role of *AtMYBFI* in controlling floral development.

## 68

### Differential Gene Expression in mediator of paramutation (*mop*) Mutants of Maize

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Paramutation is an epigenetically based allelic interaction. Two mutations that inhibit paramutation have identified two previously uncharacterized loci in maize, *mediator of paramutation1 (mop1)* and *mediator of paramutation2 (mop2)*. Our data suggest that *mop1* and *mop2* encode key players in paramutation, as mutations in these genes are able to inhibit this allelic interaction. In addition to their effects on paramutation, both mutations also have interesting effects on development, including delayed flowering, stunted growth and aberrant inflorescence development (Dorweiler et al 2000, Plant Cell 12:2101). Thus these mutations serve as useful tools for dissecting epigenetic regulation and its connections to development. My research program is identifying those downstream genes whose expression is altered in the *mop1* and *mop2* mutants. We have recently generated preliminary expression profiles of ~10000 maize genes using microarray technology. Furthermore, the delayed flowering and other developmental phenotypes associated with these mutants provide hints about possible target loci whose expression may be affected. We have analyzed the microarray data to identify key genes that are differentially regulated in the mutants relative to normal siblings. A few of the genes that show differential expression include a *CONSTANS*-like gene, and two MADS-box genes. We are currently conducting expression assays to confirm observed differences for these and several additional genes of interest. We have confirmed the differential expression of the *CONSTANS*-like gene that was seen with the microarrays, and are also assaying several candidate target genes such as additional maize homologs of *Arabidopsis* genes controlling flowering time.

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### CMS Ogura mitochondrial protein complex identification.

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Most plant species are hermaphrodites but in many cases a trait called cytoplasmic male sterility (CMS) that lead to female plants can be observed. The molecular determinant of CMS is a mitochondrial gene which is often chimeric due to the highly recombinogenic activity of plant mitochondrial genomes. These genes has been observed in more than 150 species in wild populations and in certain cases it can be induced by intra or interspecific crossings. This trait leads to the coexistence of hermaphrodite and female plants (male steriles). Its maintenance and its expansion are possible thanks to the pollination of the male sterile plants by the hermaphrodite ones and by the slightly higher prolificity of these same male sterile plants. CMS is thus a character with maternal heredity which plays an active role in the phenomena of speciation.

The Ogura CMS is a CMS described for the first time in 1968 by Dr. Ogura in radish (Ogura, 1968). It is characterized by an early degeneration of the tapetum cells of the anther leading to abortion of microspores. Pelletier and coworkers (1983) transferred this trait into Brassica species. This phenotype is related to the expression of a mitochondrial gene encoding a mitochondrial membrane protein called ORF138. It should be noted that this protein is detected in all tissues of the plant whereas the premature degeneration of the tapetum cells is the only phenotype observed to date. The mechanism by which the ORF138 protein leads to the sterility phenotype is still unknown. In order to better understand its action, one of the developed strategies is the search for potential partners within the mitochondrion. Studies on rapeseed plants carrying the sterility inducing cytoplasm show that ORF138 is integrated as oligomers in the inner membrane of the mitochondrion. Immunolocalization of ORF138 following Blue Native PAGE revealed a protein complex of size ranging between 750 and 900 kDa in vegetative as well as in reproductive tissues. The identification of the complex components by mass spectrometry is in progress. We expect that these results will enable us to release relevant working hypotheses for the understanding of the CMS mechanism.

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## 70

### **Analysis of the role of FACKEL, a C-14 sterol reductase essential for normal embryogenesis, in post-embryonic development of Arabidopsis thaliana.**

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Loss-of-function mutations in the sterol C-14 reductase gene *FACKEL* result in a severe seedling-lethal phenotype characterized by disruption of the normal pattern of embryonic cell divisions, the establishment of multiple apical meristems, and failure to develop a hypocotyl. Although *fackel* plants are deficient in the production of brassinosteroid precursors, application of exogenous brassinosteroids fails to rescue the *fackel* phenotype, indicating that the phenotype is not solely a consequence of impaired brassinosteroid synthesis and may result from the loss or reduction of a sterol structure(s) with an uncharacterized role in regulating plant development. Speculation as to the existence of such a novel class of sterol signaling molecules has been fueled by the identification of putative sterol-binding domains (START domains) in proteins that regulate plant development, in particular the class III HD-Zips, implicated in the establishment of adaxial-abaxial polarity of lateral organs. The seedling-lethal phenotype of null *fackel* alleles precludes analysis of the consequences of loss of *FACKEL* in the post-embryonic shoot. To circumvent this limitation, we are attempting to complement the *fackel-x224* mutant with a *FACKEL* gene flanked by lox sites. Removal of the complementing gene within a defined region of the shoot will then be accomplished by expressing the *Cre* recombinase gene under the control of the *Apetela1* promoter, which is active in the inflorescence meristem, and a Heat Shock promoter. Analysis of the phenotype resulting from loss of *FACKEL* in shoot apical meristems should provide new insights in the role of *FACKEL*-derived sterols in plant development.

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### **Genetic variation of heading date among rice cultivars in northern-limit rice cultivation regions**

Kenji Fujino, Hokkaido Green-Bio Institute

Heading date (flowering time) is the most important determinant of adaptation to particular cultivation conditions in rice. Rice, *Oryza sativa* L., is a tropical, short-day plant. Due to a great improvement of heading date, rice is grown and produced under various climatic conditions in latitudes ranging from 53°KN to 40°KS. In northern-limit rice cultivation regions, long natural daylength during the growing period were serious problems for rice. Only extremely early heading cultivars, which have extremely low photoperiod sensitivity, are able to adapt to northern-limit rice cultivation regions. The objectives of this study were to investigate the genetic variation of heading date among the cultivars adapted to northern-limit rice cultivation regions, Hokkaido in Japan and Europe. Also, the study using the cultivars adapted to the specific environmental condition would exploit a wide range of genetic variation for heading date in rice. Based on the results of genetic and physiological analyses of heading date, it was inferred that there are three different genetic bases for heading date: (1) the group of the Hokkaido cultivars; (2) most of the European cultivars, including Arroze Da Terra (Portugal), Dunhung Shali (Hungary) and Italica Livorno (Italy); (3) the type of Fany (France).

Also, epistatic interaction of photoperiod sensitivity was observed. The epistatic interaction exhibited the extremely late heading date, which were similar values of days to heading as an unadapted cultivar. These results suggests that loss of the epistatic interaction might contribute to the adaptation to northern-limit rice cultivation regions.

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### **Genome sequencing and analysis of the rice six chromosomes in RGP**

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Rice is important as one of the major crops worldwide. The rice *Oryza sativa* is suitable as a target for molecular genetics and biology because of its small genome size, extensive genetic and physical map, relative ease of transformation and synteny with other cereal crops. The Rice Genome Research Program (RGP) of Japan is challenging to complete the genome sequence of rice chromosomes 1, 2, 6, 7, 8 and most part of chromosome 9. We have constructed BAC/PAC physical maps of the six chromosomes and carried out sequencing of mapped BACs/PACs with minimum tiling path. The finished BAC/PAC sequences have been annotated by using both Rice Genome Automated Annotation System (RiceGAAS) and manual curation and the results have been submitted to DDBJ. We already reported the completed genome sequence and structure of the chromosome 1, and then moved to the sequencing of the BACs/PACs on the remaining chromosomes for completion. Particularly, sequencing and annotation for most of the BACs/PACs on the chromosome 7 and more than half of the BACs/PACs on the chromosome 8 have been finished so far. In total, we have finished more than 100 Mbp of the rice genome sequences, corresponding to nearly half size of the six chromosomes in our charge. The rice genome sequences are useful resources for genetics and genomic researches of other cereal crops as well as in rice itself. Our sequence data are available via web site at <http://rgp.dna.affrc.go.jp/>. Here we report current status of the rice genome sequencing in RGP and the genome structure revealed by the sequence analysis. This work is supported by the MAFF Rice Genome Project grant GS-1101, GS-1103, GS-1201.

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### **3' Frameshift mutations may induce neo-functionalization in the MADS box gene family**

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The identity of the four floral organs is governed by the combined activity of specific MADS-box floral homeotic genes. Gene duplications followed by functional diversification within the MADS-box gene family have been key processes in floral evolution. Phylogenetic studies of the MADS-box gene family thus have the potential to correlate differences in floral organ morphology with molecular and functional changes in MADS-box genes. Frameshift mutations generally result in loss-of-function changes since they drastically alter the protein sequence, besides creating premature stop codons. Here we present data, suggesting that frameshift mutations in the C-terminal domain of specific ancestral MADS-box genes may have contributed to the structural and functional divergence of the MADS-box gene family. We have identified putative frameshift mutations in the conserved C-terminal motifs of the B-function DEF/AP3 subfamily, the A-function SQUA/AP1 subfamily and the E-function AGL2 subfamily, all involved in the specification of floral organ identity. The newly evolved C-terminal motifs are highly conserved, suggesting a de novo generation of functionality. Interestingly, since the new C-terminal motifs in the A- and B-function subfamilies are only found in higher eudicotyledonous flowering plants, the emergence of these two C-terminal changes coincides with the origin of a highly standardized floral structure, the petal. **3' terminal frameshift mutations might provide an important mechanism to generate novel C-terminal motifs instrumental to the functional diversification of transcription factor families.**

Vandenbussche et al (2003). Structural diversification and neo-functionalization during floral MADS-box gene evolution by C-terminal frameshift mutations. NAR 31-15: 1-9

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### **Tocopherol and carotenoid QTLs in *Arabidopsis thaliana***

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Plants contain a variety of safety mechanisms to limit the production of reactive oxygen species (ROS) or to rapidly detoxify ROS produced and limit the damage they cause. Tocopherols act as physiochemical stabilizers of membranes rich in polyunsaturated fatty acids, physical quenchers of singlet oxygen ( $^1O_2$ ), chemical scavengers of ROS, and chain-breaking antioxidants during lipid oxidation. Tocopherols are collectively known as the essential nutrient vitamin E and play a role in preventing chronic diseases. Carotenoids are accessory light harvesting pigments that absorb at additional wavelengths than chlorophyll and can protect the reaction center from photodamage by directing excess energy away from the sensitive photosystems. Carotenoids, particularly lutein, protect against cancer, heart disease, and macular degeneration. While most genes in the tocopherol and carotenoid synthesis pathways are known, novel genes or alternate regulatory elements of known genes that influence levels of these antioxidants may still be unidentified. We are utilizing the untapped genetic variation between *Arabidopsis* ecotypes to find these genes. Quantitative trait loci (QTL) analysis of recombinant inbred lines has identified several distinct QTLs affecting tocopherol and lutein levels in seeds. We are currently developing near isogenic lines (NILs) for most of these QTLs. A QTL affecting total tocopherol levels on the top of chromosome 3 has been confirmed with a NIL and we are seeking to identify the underlying gene responsible using fine mapping. The identification of genes affecting the level of these antioxidants will further our knowledge of the regulation of enzymatic pathways and have ramifications for traditional crop breeding and metabolic engineering.

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### **Homeobox gene regulation of secondary growth and wood formation**

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Secondary growth and wood formation involve the maintenance of a stem cell population in the vascular cambium, and recruitment of daughter cells into nascent vascular tissues. Similar processes occur in the function of apical meristems, where homeobox genes have been identified that regulate specific stem cell functions. We have cloned the poplar orthologs of three *Arabidopsis* homeobox genes, *SHOOT MERISTEMLESS*, *BREVIPEDICELLUS*, and *BELLRINGER*, which have been shown to regulate distinct apical meristem functions. Like the *Arabidopsis* genes, the poplar orthologs are expressed in shoot apical meristems. We show that these genes are also expressed in stems undergoing secondary growth, suggesting they play a role in the function of the stem cells in the vascular cambium. We present overexpression phenotypes for each gene in poplar trees, including morphological defects associated with stem morphology. We discuss the possible role of genes and mechanisms regulating apical meristems in the evolution of secondary growth and wood formation.

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### **Two new loci are involved in control of endosperm development by the FIS pathway**

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The seed of flowering plants is composed of three genetically distinct compartments: the maternal integuments, the diploid embryo and its surrounding triploid endosperm. *Arabidopsis thaliana* endosperm is emerging as a suitable model for studying early developmental events, such as antero-posterior polarity establishment, gene imprinting, DNA methylations. An enhancer-trap line from Jim Haseloff's library, KS117, was characterized in our lab. In this line, the GFP expression pattern reports endosperm polarization process. A screen based on KS117 GFP misexpression allowed us to isolate 11 mutants that develop ectopic posterior structures. The genetic control of all these 11 mutations is maternal gametophytic: the maternally inherited allele determines endosperm phenotype, independently of the paternal copy. 9 of these mutants are allelic to *fis1*, *fis2*, *fis3* and *demeter* mutants, which our group already described as polarity mutants. FIS proteins are Polycomb group proteins, involved, in drosophila and mammals, in epigenetic controls of transcription domains. They are involved, in *A.thaliana*, in endosperm development after fertilization, and *FIS1* has been shown to be a paternally imprinted gene. We have now shown that *FIS2* is paternally imprinted as well. A fascinating aspect of *fis* phenotype is the development of an autonomous endosperm without fertilization. Both the new mutants also develop autonomous seeds, and they map to distinct loci. Thus, our screen revealed the existence of at least 2 new loci involved in endosperm development. The identification of the affected genes will permit further description of maternal control of seed development in *A.thaliana*.

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### **AP2 domain-containing transcription factors and disease resistance: a phylogenetic and genomic analysis**

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The large AP2/EREBP transcription factor gene family is found only in the plant kingdom. Four major groups have been defined: DREB, ERF, RAV and AP2L2. The ERF group can be further resolved phylogenetically into 10 subfamilies, based on analysis of the *Arabidopsis* family. Several ERF subfamilies include genes that are involved in mediating disease resistance, as suggested by induction of expression following pathogen challenge, or by overexpression-mediated resistance. The ERF subfamily of the AP2/EREBP family plays an important role in mediating resistance responses. We describe here 1) ERF subfamily phylogenetic structure in *Arabidopsis* and across species, and 2) conserved and divergent features of one subfamily having ERF members that mediate disease resistance. Overall, there is high conservation within the AP2 DNA binding domain, and little conservation outside the domain, throughout the ERF group.

One of these subfamilies (B-3a) includes the well-known tomato *Pti-4* and *AtERF1* genes. Both of these transcription factors confer tolerance to multiple pathogens when overexpressed constitutively in *Arabidopsis* (Gu et al. *Plant Cell* 14: 817, 2002). We have identified motifs unique to this relatively small subfamily that are conserved amongst eudicots and monocots, as well as motifs unique to the monocots. This suggests that defense response pathways controlled by members of the B-3c subfamily are highly conserved in the eudicots and monocots, but that specific interactions with other regulators has diverged.

The second of these subfamilies (B-3c) includes *ERF1*, which when overexpressed in *Arabidopsis* confers resistance to necrotrophic pathogens (Berrocal-Lobo et al. *Plant J.* 29: 23, 2002). We have identified another member of the B-3c subfamily which confers resistance to multiple pathogens when overexpressed in *Arabidopsis*.

We have identified motifs unique to the B-3csubfamily.

Although each of these two subfamilies shares unique structural features across species, there are essentially no common motifs between them. Thus, ERF transcription factors controlling defense responses have diverged substantially, perhaps indicating control of different sets of defense-related genes or interactions with different co-regulators.

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### **Trial for Positional Cloning of a Segregation Distortion Gene in a Japonica and Indica Rice Cross**

Yoshiaki Harushima and Nori Kurata, Plant Genetics Lab., National Institute of Genetics

Genetic mechanics for isolation of "species" are called as reproductive barriers and these include hybrid incompatibility, hybrid inviability, hybrid sterility, hybrid breakdown, etc. The distortions of allele frequencies from Mendelian expectation in progeny of inter- or intra- species hybrid due to reproductive barrier genes have been often observed. We previously mapped all reproductive barriers causing allele frequency distortions from Mendelian expectation in three F<sub>2</sub> populations from Japonica-Indica hybrids by a regression analysis. The aim of this study is isolation of the most prominent barrier on chromosome 3 detected in a F<sub>2</sub> of Nipponbare-Kasalath hybrid by positional cloning, and elucidation of the molecular nature of the reproductive barrier. We have clarified the target gene was a male gametophyte gene and it interact with maternal locus on chromosome 6. That is, the pollen with Kasalath genotype on the barrier locus preferentially fertilized by 94% probability in maternal plant that is heterozygote or Kasalath homozygote on the interactive locus of chromosome 6. This gene combination helps inbreeding in Kasalath. For detailed mapping of this gametophyte gene, we selected plants with recombination in 2.3 cM interval from 5689 F<sub>2</sub> and 473 backcross plants, the genetic map of the male gametophyte gene was constructed by the dosage analysis using the bulked DNA pools of selfed progenies of the selected plants. The physical map of this region was constructed using Nipponbare BACs, PACs and Kasalath BACs. To narrow candidate region, PCR markers have been developed using genome sequences of Nipponbare and Kasalath. Analysis of expressed genes from the candidate region and phenotypic analysis of near isogenic lines are in progress.

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### **Timberline genes – how trees climb high mountains**

Berthold Heinze, Austrian Federal Office and Research Centre for Forests

Many tree species have a wide distribution range not only in a geographical sense, but also vertically, covering a range of altitudes with diverse habitat conditions. The high mountain ranges in Europe, which is generally poor in forest tree species, are a striking example. Here, species like e.g. Norway spruce (*Picea abies*) span a range of 1500 m in altitude. Heritable differences in growth characteristics between seed provenances of such diverse habitats have long been recognized. However, the genetic basis of these severe growth differences is not known to date. This is due to many factors, among which the slow growth and extra-long generation time of high-elevation tree provenances play a major role. It is practically impossible to conduct multi-generation field experiments in one researcher's lifetime. A number of hypotheses have been developed over the past decades for the causes of heritable adaptation to extreme mountain environments. These have invoked e.g. overall genome size, severe selection and reduced genetic variability, specific genes or gene combinations traced by isoenzyme markers, or the existence of different genetic pools for high and low elevation populations during the glacial past. Others have emphasized a strong influence of the maternal environment on traits in the offspring. However, generally speaking, none of these hypotheses have stood a rigorous genetic test with available markers and methods, and are not broadly accepted. In this contribution, I want to critically review the concepts developed in the past, and suggest possible ways for resolving some of these issues with newly available genomics technology.

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### **INVUNCHE, AN ISWI-LIKE CHROMATIN REMODELING FACTOR THAT REGULATES SEED DEVELOPMENT IN ARABIDOPSIS THALIANA.**

Wilson A Huanca and Jean Philippe Vielle-Calzada. Laboratory of Reproductive Development and Apomixis; CINVESTAV - Unidad Irapuato, MEXICO.

Recent studies identified some of the mechanisms responsible for altering chromatin structure and allowing interactions between regulatory proteins and genomic DNA in plants. We are interested in elucidating the function of the Imitation of Switch (ISWI) class of ATPases during megagametophyte formation and seed development in *Arabidopsis thaliana*. Members of the ISWI class of ATPases regulate chromosomal assembly and transcriptional regulation during at least two flowering pathways. We determined the pattern of expression of 3 members of the *ISWI* family by reverse transcription PCR (RT-PCR). All 3 genes are expressed in most vegetative and reproductive tissues (flower buds, mature flowers, unpollinated and pollinated gynoecia, and siliques). To determine if *ISWI* genes have a specific role during female reproductive development, we modified the pGSA1131 dsRNA vector (kindly provided by Jorgensen's group, U. of Arizona) and replaced the 35S promoter by pFM1, a 844 bp regulatory sequence that drives expression only in the megagametophyte and the young seed. The original and modified vectors were used to clone palindromic coding sequences and silence each of the *ISWI* genes by RNA interference (RNAi). Transformants for a particular *ISWI*-like gene (*INVUNCHE;INV*, an infant male divinity venerated in Chiloé, Chile) showed reduced vegetative growth and aborted seed formation with variable degrees of penetrance. Aborting seeds contain defective embryos arrested at the mid-globular stage. These embryos have aberrant patterns of cell division in the embryo-proper, poor differentiation of the protoderm, and additional cells in the suspensor. The endosperm does not form following fertilization and the central cell contains a single large nucleus. *In-situ* hybridization shows that *INV* is expressed after fertilization in both the embryo and the endosperm. Our results indicate that *INV* regulates both early embryo patterning and the initiation endosperm development in Arabidopsis.

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### **Endosperm specific suppression of Wx gene expression by RNA interference**

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*Wx* encodes granule bound starch synthase (GBSS) which plays a key role for amylose synthesis in cereal grains. *Wx* is a single copy gene in rice and is specifically expressed in pollen and endosperm. The gene is one of the model systems for genetic studies, and we have been reported pollen specific and endosperm specific gene silencing of *Wx* (Itoh et al., 1997; Itoh et al., 2003)

Efficient RNA silencing by intron containing hairpin RNA (ihpRNA) vectors have been reported and those vectors are widely used for functional analysis of plant genes. To study for RNA silencing of *Wx* gene and splicing effects on RNA silencing, we developed two types of vectors, pWRI-A and pWRI-B. Both two vectors had

*Wx* promoter, sense and antisense sequences of 1st exon (5'UTR region), and 1st intron between sense and antisense sequences. Features of two vectors as follows; pWRI-B has a +1G to T mutation at 5' splice site of the 1st intron, whereas pWRI-A has the wild type sequence. Therefore, the splicing efficiency on the *WRI-B* pre-mRNA was extremely lower than *WRI-A* pre-mRNA. Both two genes can transcribe *ihp* RNA, but the amount of the spliced *ihp* RNA are different between two genes. Both two vectors were introduced into rice cultivar Kinmaze (*Wx*), and then regenerated plants were analyzed.

Over 100 transgenic rice plants were tested and 95~100 % of those WRIA/*Wx* and WRIB/*Wx* plants showed strong suppression of endogenous *Wx* in the endosperm, and a weak suppression occurred in pollen. RNA analysis showed that 23nt short RNA was detected among those silenced endosperms. These results indicated efficient RNA silencing occurred in endosperm of both two lines. Although there are no major differences in the proportion of silencing occurrence between both two lines, endogenous *Wx* suppression levels of WRIB/*Wx* was weaker than that of WRIA/*Wx*. Effect of intron splicing on *Wx* silencing is discussed.

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### Comparative genetics and genomics of disease resistance in the Solanaceae

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The plant family Solanaceae defines the broadest comparative genetic systems in the dicots. In our lab, we have used comparative approaches to study the structural and functional divergence of resistance genes. This talk will summarize recent results from our comparative genetic and genomic studies of disease resistance genes. Results from studies of NBS-LRR-containing R genes and the *Pto* gene family in *Capsicum*, *Lycopersicon* and *Solanum* species will be described. For NBS-LRR R genes, specificity, that is which pathogen taxa are controlled, diverges much more rapidly than general resistance function. Based upon comparative sequence and map data, we can propose extended R gene families and in some cases evolutionary lineages which can be tested as additional sequence data become available. Furthermore, dominant R genes often occur at or near positions that coincide with karyotypic rearrangements during speciation, perhaps defining a mechanism for the radiation of these gene families throughout plant genomes. In contrast to NBS-LRR R genes, *Pto* still defines a unique resistance gene subclass. We have isolated functional homologs of this tomato gene from two *Capsicum* species considered non-hosts to *Pseudomonas syringae* pv. *tomato* that show 99% amino similarity to *L. pimpinellifolium Pto*, along with *Capsicum* homologs of most of the known tomato homologs. *In vitro* interaction assays indicated the possibility of functional conservation. Transient expression of AvrPto confirmed that both *Capsicum* species specifically recognize this molecule resulting in a characteristic hypersensitive response. The implications of these results for prevailing models of genome evolution and R gene mechanisms will be discussed.

## 86

### Target-based prediction of miRNA precursors within the Arabidopsis genome

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Our laboratory is performing localized saturation mutagenesis of Arabidopsis using a Ds targeted tagging approach. Apart from the annotated genes within chromosomal regions, there was also the possibility that miRNA precursor genes were present as targets for knock out studies. As only a limited number of miRNAs have been identified molecularly, this stimulated a systematic computational based search for miRNA precursors within the Arabidopsis genome. Previously reported computational based approaches in animal systems relied upon conservation of the approximately 21 nt miRNA sequences between two or more genomes in order to identify initial candidates for more thorough analysis. In this study we have designed an mRNA-target based algorithm that relies on finding sufficient complementarity between an 18 to 25 nt transcript subsequence and a corresponding intergenic subsequence to identify initial candidates. Sequences immediately adjacent to these potential miRNA sequences are then assessed for complementarity that would allow suitable base pairing for the corresponding RNA to be processed by the RNAi machinery. This approach requires only one sequenced genome and may detect miRNA precursor genes that have diverged too much to be detected by comparative genomics prediction methods. The results from this algorithm adds to the number of known miRNA precursors in Arabidopsis by detecting a significant number of precursors that are related to previously identified families, as well as predicting new miRNAs, some of which appear to be conserved in rice. Preliminary experimental data support this computational approach to miRNA identification.

## 87

### 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.

## 88

### Regulation of pathogenesis regulated (PR) genes by TGA transcription factors in Arabidopsis thaliana

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Systemic Acquired Resistance (SAR) is a long lasting induced resistance response after infection with an avirulent pathogen. Several pathogenesis related (PR) genes are induced during SAR which are responsible for resistance to pathogens. The PR gene expression is blocked in *npr1* mutant. NPR1 is a novel nuclear protein and has been shown to interact differentially with different family members of the TGA (bZIP) transcription factors. The TGA transcription factors can bind to the PR gene promoters. We have screened T-DNA insertion Knockout lines of strong interactors TGA2 and TGA3. *tga3* mutant shows reduced expression of PR1 gene after induction with SAR inducer. On the other hand *tga2* knockout do not show very significant change in PR gene expression after induction. However, Over-expression of another TGA; TGA6 in *tga2* causes embryo lethality and reduced seed set. The embryo lethality is associated with constitutive expression of PR1. This phenotype is slowly recovered in subsequent generations, suggesting an epigenetic control. Further experiments to find out the possible causes of embryo lethality and the epigenetic regulation will also be presented.

## 90

### Trapping and characterization of novel chromatin and nuclear matrix protein genes showing tissue specific expression

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Our attempt to elucidate nuclear proteins which correlate with developmental programs through the association with chromatin or nuclear matrix function has found several novel proteins. Kinds of chromatin remodeling factors have been clarified as factors of chromatin modification and those affect gene repression which consequently modify developmental programs. A lot of transcription factors have being identified as well. However, other plant genes affecting developmental steps have not been analyzed yet. We started from finding of novel nuclear protein genes by trapping them using nuclear transportation trap (NTT) system in yeast. Rice

cDNAs collected from three developmental stages were used. Of above 2,000 trapped genes, 523 unique genes were identified. These genes were classified into nuclear/nuclear-related, nonnuclear and unknown groups. Localization analysis of these proteins performed by the introduction of GFP-fused genes into onion cells revealed about 80 % trapped genes localized to the nuclei, even using non-nuclear and unknown protein groups. These were consist of chromatin-associated, nuclear matrix-associated and foci or speckles forming proteins in the nuclei. Proteins localized both in nucleus and cytoplasm in a single cell or in separate cells were also observed. Simultaneous application of these genes for expression analysis by microarray showed tissue/stage specific expression in some members. Several genes were confirmed to express in the stages of reproductive organ development. One of these, an unknown protein gene, preferentially localized on the nuclear matrix and expressed in the male gamete, tapetum and female nucellus. Functional analysis of this gene is undergoing by RNAi and GFP-fusion transformation and by a two-hybrid assay as well.

## 91

### **Genetic variation in perennial grasses: genomic analysis of divergent adaptations between two North American *Leymus* wildrye species**

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Basin wildrye (*Leymus cinereus*) and beardless wildrye (*Leymus triticoides*) are distributed in diverse environments of western North America, including virtually all of the Great Basin. A robust, densely tufted bunchgrass, *L. cinereus* frequently exhibits culms up to 20 dm tall and large prolific spikes 11-20 cm long with 3-6 spiklets per node. The strongly rhizomatous *L. triticoides* exhibits culms less than 7 dm tall and relatively short slender spikes less than 7 cm long with 1-2 spiklets per node, often forming extensive sod-bound vegetative clones. *Leymus triticoides* also manifests high levels of seed dormancy and salt tolerance that are absent or wanting, respectively, in *L. cinereus*. Although *L. cinereus* and *L. triticoides* hybridize in nature, these species occupy distinct niches. Whereas *L. triticoides* is especially well adapted to saline meadows of valley bottoms of California, Nevada, and Oregon. The more abundant *L. cinereus* is widely adapted to dry places throughout much of the sagebrush (*Artemisia* spp.) vegetation zones and beyond. Divergent growth habit, seed dormancy, and salt tolerance traits probably account for apparent departures in adaptation and distribution. Initial genetic studies consistently detected one major plant height gene and two chromosome regions controlling growth habit. Thus, distinct morphogenetic differences in plant height and growth habit evolved by divergent selection at independent loci. The two most consistent growth habit gene effects, in these allotetraploid species, were detected in homoeologous regions of Triticeae chromosome 3. Evaluations of seed dormancy and salt-tolerance are also underway. Future objectives aim to identify genes and mechanisms controlling morphogenetic variation in growth habit of perennial grasses.

## 92

### **Analyses of transgressive QTL affecting epidermal markers of vegetative phase change in maize identified a regulatory locus that acts at the same level as *glossy15***

Nick Lauter, University of Illinois Urbana-Champaign Steve Moose, University of Illinois Urbana-Champaign

The intermated B73 X Mo17 recombinant inbred lines (IBMRILs) were used to identify QTL that affect the timing of the onset of adult pubescence and the loss of juvenile wax, both of which are markers of vegetative phase change in maize. Although B73 and Mo17 have essentially the same phenotypes for these traits, substantial phenotypic variation among the IBMRILs allowed the detection of several QTL for each trait, including two loci that pleiotropically affect both traits. One of these maps to *glossy15* on chromosome 9, a gene known to regulate both hairs and wax. The second maps to bin 2 of chromosome 7, where no locus affecting these traits had previously been known. The remaining QTL detected affect only one or the other of the phase change markers, revealing the positions and actions of novel candidates for downstream targets of *glossy15* and the 7.02 QTL. The interaction effect between these two QTL as well as their individual genetic effects were assessed using multiple large populations that were derived from IBMRILs with particular combinations of genotypes at the loci affecting these traits. The QTL differences at *glossy15* and the 7.02 locus both make dosage- dependent contributions and interact in a purely additive manner, suggesting that the 7.02 QTL might be acting in a separate pathway. However, QTL analyses of other traits that might be affected by loci with broad effects on vegetative phase change did not detect the 7.02 QTL. We are investigating the possibilities that the 7.02 locus could encode a microRNA that antagonizes *glossy15* or a paralogue that can augment its function. These experiments have revealed that multiple loci can be modulated in this pathway, such that the same phenotypic end can be achieved by alternate genotypic means.

## 93

### **Arabidopsis Centrin-2 Function in Homologous Recombination and Nucleotide Excision Repair**

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The function of Centrin family EF-hand Ca<sup>2+</sup>-binding proteins has been implicated in different cellular processes such as cell division, early response to stresses, and DNA repair. Through a genetic screen, Arabidopsis mutants exhibiting high homologous recombination (HR) have been isolated. A hyper-recombination mutant was impaired in centrin expression. This Arabidopsis Centrin contains four well-conserved EF-hand Ca<sup>2+</sup>-binding motifs with 58% sequence identity to that of HsCent2, a protein with known function in nucleotide excision repair (NER). It however differed from other members in the family by its unique N-terminal ATP-binding motif. Using biochemical approaches, we have been able to show that AtCen2 protein, similar to HsCent2, was stabilized through binding to Ca<sup>2+</sup>. It bound to ATP in vitro in a structure-dependent manner. Using proteomic approaches, we have identified at least two proteins co-immunoprecipitated with AtCen2, one of which is DD11 (DNA damage-inducible I). DD11 protein contains a ubiquitin homology domain (UBQ), a retroviral aspartyl protease domain (rvp), and a ubiquitin-associated domain (UBA), a motif also presented in Rad23 and SNF1-like kinase. The interaction between AtDD11 AtCen2 has been confirmed in vitro by using affinity pull-down assay. Furthermore, we have been able to show that AtCen2 protein could stimulate NER, while addition of EGTA and recombinant DD1-1 protein affected NER efficiency. Based on these observations, we propose that destabilization of AtCen2, probably via DD11 and/or Ca<sup>2+</sup>-signalling, leads to a disassociation of the XPC complex, thus decreasing NER efficiency. Our data suggest that AtCen2 is probably playing a direct role in controlling NER, in response to DNA damage and cellular signaling.

## 95

### **A Selective-Mapping Strategy to Identify *OVATE*-Interacting QTLs Involved in Pear-Shaped Fruit Formation in Tomato**

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With normal segregating F2 populations, no major or minor quantitative trait loci (QTLs), other than *OVATE*, were detected as determinants for the formation of pear-shaped fruit in tomato. The *OVATE* locus has been cloned and transgenic studies suggested that the wild-type *OVATE* functions as a suppressor for the formation of pear-shaped fruit. Further evaluation on advanced backcross populations and different tomato accessions with homozygous mutant *OVATE* alleles revealed an unbalanced phenotypic effect of the wild-type (round) and the mutant (pear-shaped) *OVATE* alleles on tomato fruit shape: the wild-type alleles are always associated with round-fruit shape regardless of their genetic background, while fruit with the loss-of-function mutant alleles display a wide range of shape variation among different genetic background. These results indicated that, at least in the studied cases, the mutant alleles of *OVATE* is necessary, but not sufficient, for pear-shaped fruit formation, and the *OVATE* locus genetically interacts with other QTLs in determining pear-shaped fruit formation. On the basis of these observations, we propose that 1) several parallel genetic pathways are directly involved in pear-shaped fruit formation; 2) *OVATE* functions as a master regulator to suppress the functions of these genetic pathways and, thus, the formation of pear-shaped fruit; 3) in the presence of *OVATE* mutations, the different combinations of these *OVATE*-mediated pathways might determine the degree of the pear-shaped phenotypes in different tomato lines. To test these hypotheses, we need to identify other QTLs involved in pear-shaped fruit formation. However, strong QTL effects from *OVATE* might be the reason for the un-detection of other minor QTLs from previous mapping populations. To exclude the strong phenotypic effects caused by genotypic variation at the *OVATE* locus, we have developed a selected F2 mapping population in

which the segregating F2 individuals with homozygous mutant alleles at the *OVATE* locus are kept for further QTL analysis. Our preliminary results show that fruit from this population exhibit a whole range of shape variation from round to strong pear-shaped. Further analysis is undergoing to identify other QTLs through this selected population.

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### Light signal transduction genes and regulation of pigmentation in tomato

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#### Light signal transduction genes and regulation of pigmentation in tomato

Yongsheng Liu, Jim Giovannoni

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Due to the inability to move towards favorable or away from unfavorable environmental conditions, plants have adapted a high degree of developmental plasticity to optimize their growth and reproduction in response to their surroundings. Fleshy fruit ripening is a developmental process which has evolved to allow plants to use animal vectors for seed distribution and ripening-associated color development is linked to animal interest and resulting seed release and dispersal. The most extensively studied system of fruit color change and ripening is tomato in part due to the rapid and dramatic color change associated with ripening of tomato fruit (due to the degradation of chlorophyll and accumulation of colored carotenoids) and the availability an array of pigmentation mutants. The major pigments of ripe tomato fruit, beta-carotene and lycopene, play important roles in human nutrition due to pro-vitamin A and antioxidant activities. While recent gene isolation efforts and metabolic engineering have primarily targeted genes involved in carotenoid biosynthesis, factors that regulate flux through the carotenoid pathway remain largely unknown. In an effort to better understand the underlying molecular regulation of fruit pigmentation, we have employed forward (positional cloning) and reverse (RNAi-mediated gene repression) genetics approaches to identify critical regulatory components involved in tomato fruit pigmentation. We have isolated a candidate gene for the *high pigment-1 (hp-1)* locus, previously shown via mutation to represent an important regulator of tomato fruit pigmentation through altered responsiveness to light. Recessive *hp-1* mutations (loss of function) result in increased accumulation of lycopene and beta-carotene during fruit ripening and elevated levels of chlorophyll in leaves and green fruit. In addition, targeted disruptions of a tomato *COP1-like* gene and a *DIM* homologue (whose Arabidopsis counterparts negatively regulate light signaling) were shown to result in similar phenotypes. In contrast, down-regulation of the tomato homologue of the Arabidopsis *HY5* gene positively influencing light responses, caused reduced pigmentation. These results suggest genes encoding components of light signal transduction machinery also influence fruit pigmentation and represent powerful genetic tools for manipulation of fruit quality and nutritional value.

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### A histone H3-like centromere protein CENP-A homolog in the primitive red alga *Cyanidioschyzon*

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In most eukaryotic chromosomes, the centromere contains highly repetitive DNA elements. Recent studies revealed, however, that the centromere determinant is not such repetitive elements but the evolutionarily conserved histone H3 variant, CENP-A. We present that the primitive red alga *Cyanidioschyzon merolae* may possess centromeres and that a homolog of CENP-A, designated CmCENPA, has been identified from *C. merolae*. Western blot analyses indicated that CmCENPA was regulated by the cell cycle-dependent expression mechanism. Indirect immunofluorescence microscopy using anti-CmCENPA antibody revealed that intense foci colocalized with the nucleus throughout the cell cycle and were transmitted to the daughter cells. Coimmunostaining of anti-CmCENPA and antitubulin antibody showed the colocalization of CmCENPA with spindle poles, implying that CmCENPA plays an important role in centromere segregation. Availability of CmCENPA for determining the yet-known centromere positions on the *C. merolae* chromosomes is discussed.

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### Molecular Characterization of Heteromorphy in *Primula*: New insights from a Classic Model system

Angela Smith and Andrew McCubbin, Washington State University

Heteromorphic self-incompatibility (HSI) systems in plants, promote out-breeding through a physiological recognition system combined with a set of morphological characteristics. In *Primula* there are two floral morphs, which exhibit reciprocally positioned anthers and stigma. Pollen number and size, and size and shape of stigmatic and stylar cells also differ between morphs. Since the pioneering studies of Darwin and Hildebrand, HSI has been extensively used as a model system to study genetic and evolutionary biology. HSI is controlled by a diallelic *S*-locus, the short style (thrum) morph is determined by the dominant *S* allele being *S*<sub>s</sub> in genotype and the long styled (pin) morph is homozygous recessive (*ss*). Studies have shown that this *S*-locus is in fact a 'supergene' of tightly linked genes held in complete linkage disequilibrium with alleles of at least 7 loci linked and inherited as a single unit. To date there has been little characterization of the molecular genetics of this system. Identification and characterization of the genes regulating HSI has the potential to make a significant impact on our understanding of floral biology, in the functioning of HSI and also the developmental regulation of floral morphology (cell size/shape) and male reproductive capacity (pollen number). As a first step we have constructed reciprocally subtracted cDNA libraries of floral tissues between mating types. To date 15 classes of cDNA have been confirmed to exhibit differential expression between morphs. Several classes have homology to genes potentially involved in signaling and/or developmental regulation including a RALF-like putative peptide hormone, a putative allene oxide synthase, a DEAD-box RNA helicase and an RNA binding protein.

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### Functional Genomics of Maize Chromatin Genes

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Chromatin level control of gene expression is an important regulatory component for growth and development in all living organisms. To better understand this regulation in plants, we are studying the function of the entire known complement of chromatin proteins in maize. The goal of the NSF-funded Plant Genome Research Project on Functional Genomics of Plant Chromatin Genes is to provide information and tools to the research community to enhance the study of the role of chromatin structure and modification. Our website ([www.chromdb.org](http://www.chromdb.org)) provides sequence information, phylogeny, mapping data, and mutant status for 294 Arabidopsis and 255 maize genes that were identified by homology to known chromatin proteins in other species. In maize, 130 of these genes have been targeted for RNAi mediated silencing using a transgenic approach. This required 105 unique constructs, which have been completed and bombarded into maize embryonic callus. To date, we have analyzed 303 T1 events, representing 55 of these unique constructs. Quality control testing is underway to determine that the target gene mRNA has been reduced in transgenic lines. Additionally, genetic assays have been completed on many of the transgenic lines to determine whether the targeted gene participates in specific epigenetic phenomena, like paramutation at the *B* gene and stable transgene silencing. This poster presents an update on our progress toward creating transgenic lines deficient in chromatin related proteins, and also the most recent data from our genetic assays.

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### Characterization of the Mechanism of Glyphosate Resistance in Horseweed (*Conyza canadensis*).

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*Conyza canadensis* is a winter or summer annual indigenous to North America that is readily dispersed and is now represented in both the New and Old World's in a wide variety of agricultural systems. The appearance of glyphosate resistant horseweed in 2001 in Roundup Ready® soybean and cotton has focused a high level of attention on this normally easily controlled weed. Resistance is not due to over-expression of EPSPS or glyphosate metabolism or differences in glyphosate uptake. There are three isozymes of EPSPS in horseweed mutually identical in sensitive and resistance lines. This removes the possibility there is a target site mutation involved in resistance. One isozyme is not fully expressed and is a nonfunctional enzyme or pseudo-gene. The other two functional EPSPS's are very sensitive to glyphosate. Genetic studies show that glyphosate resistance in horseweed is dominant and transmitted by a nuclear gene. The principal measurable difference corresponding to resistant horseweed is decreased translocation of glyphosate from treated leaves with an additional decrease in the production of shikimate in source and sink leaves. This implicates a sequestration or exclusion mechanism for glyphosate resistance.

## 101

### Ecological Genetics of Drought Adaptation in *Arabidopsis thaliana*

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Drought stress is ubiquitous, often severe, and an important selective force in the evolution of plant growth, development, and physiology. Despite its importance, we know surprisingly little about natural genetic variation underlying drought adaptation. Our goal is to understand effects of alleles on whole plant physiology, growth, and fitness. Many  $C_3$  annuals show a tradeoff between two drought adaptation mechanisms – dehydration avoidance and drought escape. Dehydration avoidance (reduced stomatal opening reflected by  $\delta^{13}C$ ) is correlated with slower growth and longer time to flowering. To test for a genetically-based tradeoff we used a greenhouse screening of 39 genotypes of *Arabidopsis thaliana* native to a wide range of climatic conditions. We found a highly significant positive genetic correlation between  $\delta^{13}C$  and flowering time. We then examined pleiotropy, using a combination of mutants and near-isogenic lines of naturally polymorphic loci, to test for positive mutational covariance between  $\delta^{13}C$  and flowering time. We found strong evidence for pleiotropy using near-isogenic lines of *FRIGIDA* and *FLOWERING LOCUS C*, cloned loci known to be responsible for natural variation in flowering time. We conclude that correlated evolution of  $\delta^{13}C$  and flowering time is explained in part by the fixation of pleiotropic alleles that alter both  $\delta^{13}C$  and time to flowering. We also report on genome-wide screens for other drought adaptation loci (QTL). These data provide some of the first information on genes that function in natural drought adaptation, such as their genetic architecture, and their effects on whole plant physiology. This addresses why, as well as how, the *Arabidopsis* genome has evolved functional variation in traits important in drought adaptation.

## 102

### Mechanisms of tolerance to drought and freezing in *A. thaliana*: an association study approach

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We are interested in exploring mechanisms of tolerance to abiotic stresses (cold/freezing, drought) through the exploitation of natural genetic diversity in *Arabidopsis thaliana*. Abiotic stress represents a serious threat to plants. Plants are nonetheless able to respond to these stresses with a number of biochemical and physiological changes. Complex networks of genes are induced or repressed in response to freezing/cold and drought. It is thought that the upstream regulators of gene expression may serve as important targets for modifying plants' ability to tolerate stress.

Natural genetic variation in the model plant *Arabidopsis thaliana* is a resource for both basic and applied research. Accessions (ecotypes) have been collected from sites around the world with different environmental conditions. Important functional diversity has been demonstrated in a wide range of agronomically important traits (Alonso-Blanco, C. & Koornneef, M. (2000) Trends Plant Sci. 5, 22-9) that reflects the underlying genetic diversity. These studies represent a mine of information for examining *A. thaliana* growth and development under environmental stresses.

The successful exploitation of genetic diversity requires a basic knowledge of the extent of the variation present in a species. We have recently defined a nested core collection that maximizes genetic diversity in *A. thaliana*. Here we present our initial results on the phenotyping of the core collection with respect to drought tolerance, freezing tolerance and cold acclimation ability. In addition, variation was found in the sequences and expression of several genes (e.g., CBF1, 2, 3) considered to be important in controlling the response to freezing and drought. By correlating genotypes with phenotypes (association studies), it may be possible to identify the role of a given gene in a response, such as tolerance.

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### PP2C-type phosphatase inactivates MAPK

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Protein phosphorylation is important in eukaryotic signal transduction and is mediated by concerted action of protein kinases and protein phosphatases. So far major interest was expressed for protein kinases and protein phosphatases were considered as comprising little specificity. In contrast to other eukaryotes, plants have an unusually large family of 76 different PP2C genes in *Arabidopsis*. At present, little is known about their functions and substrates.

The evidence is provided that PP2C display exquisite substrate specificity for precise down-regulation of wound-induced MAPK activity. The data on MAPK as a substrate for PP2C and their specificity, focusing primary on the alfalfa member of PP2C family MP2C will be presented that reflects the mechanism for direct MAPK inactivation through dephosphorylation. Mutant analysis indicated that inactivation of MAPK SIMK depends on the catalytic activity of MP2C. Comparison of MP2C with two other PP2Cs, ABI2 and AtP2CHA, revealed that only MP2C is able to dephosphorylate and inactivate SIMK. In agreement with the data that MP2C directly interacts with SIMK in vivo and in vitro, the MAPK was identified as an interaction partner of MP2C in a yeast two-hybrid screen. Wound-induced PP2C expression correlates with the time window when SIMK is inactivated, supporting the proposed model that MP2C is involved in resetting the SIMK signaling pathway.

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Meskiene I., Bögre L., Glaser W., Balog J., Brandstötter M., Zwerger K., Ammerer G., Hirt H. (1998) MP2C, a plant protein phosphatase 2C, functions as a negative regulator of mitogen-activated protein kinase pathways in yeast and plants. Proc. Nat. Ac. Sci. USA 95: 1938-1943

## 104

### Natural Variation in *Arabidopsis thaliana* – Biomass, Stress Tolerance and Heterosis

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Natural genetic variation in the model plant *Arabidopsis thaliana* is an important resource for both basic and applied research. We have established 159 homogenised natural accession-derived *A. thaliana* lines and performed a large crossing program that yielded 396 F1 and 348 F2 populations from crosses to the three reference lines Col-0, C24, and Nd. Furthermore, four recombinant inbred line (RIL) populations (Col-0/C24, Ak-1/C24, Beh-1/C24, Nd/C24) and a series of genetic substitution lines (Col-0/C24) have been / are being created. A set of 112 evenly spaced SNPs was used to establish a framework marker system for high-throughput genotyping. A subset of the accession-derived lines and crossbred progenies have been evaluated for biomass accumulation, water loss, photosynthetic efficiency, metabolic content, and salt and freezing tolerance. Significant phenotypic variation has been observed for all these traits. We detected significant heterosis for shoot biomass in a number of F1 hybrids, and analysed the combination Col-0/C24 in more detail. Heterosis for biomass was enhanced at higher light intensities: we found 51 to 66% MPH at low and intermediate light intensities (60 and 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and 161% at high light intensity (240  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). While at the low and intermediate light intensities relative growth rates of the hybrids were higher only in the early developmental phase (0-15 DAS), the hybrids showed increased RGR over the entire vegetative phase (until 25 DAS) at high light intensity.

## 105

### A primitive Y chromosome controls sex determination in papaya

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Diverse systems for sex determination in eukaryotes have evolved many times among groups of plants and animals. One system involves physically distinct (heteromorphic) sex chromosomes (X and Y, or Z and W) that are homozygous in one sex (usually female) and heterozygous in the other (usually male). We report a papaya primitive Y chromosome with a small male-specific region, the MSY, that is about 10% of the chromosome showing severe suppression of recombination and degeneration of DNA sequences. The MSY consists of a mosaic of conserved, X-degenerated, and ampliconic sequences. High frequencies of sequence duplications and transposable element insertions contributed to the degeneration of the MSY resulting in low gene density. One unique feature of the incipient Y chromosome in papaya is the small physical size of the MSY region. The small size and the mosaic structure of sequence degradation in the MSY region suggest a recent origin of the papaya sex chromosomes. Hermaphrodite and male plants of papaya share identical DNA sequences in most parts of the MSY region, suggesting that divergence between male and hermaphrodite is a second step of sex chromosome evolution after recessive mutations resulted in producing the female sex. This finding provides direct evidence for the origin of sex chromosomes from autosomes.

## 106

### Genetic Diversity of Endangered Endemic *Magnolia stellata* in Central Honshu of Japan

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*Magnolia stellata* (Magnoliaceae) is endemic to central Honshu of Japan. Over the last three decades, activities such as road construction have resulted in local extinction and habitat fragmentation, leading to a rapid decrease in population, which can result in reduced genetic variation. Populations with low genetic variation are likely to become extinct because the lack of genetic diversity limits their potential to adapt to a changing environment. Although *M. stellata* is endangered, little is known of its genetic structure, which is important information that is required for its conservation. To discuss the conservation genetics of this species, we examined the levels and patterns of allozyme variation at ten loci encoding five enzymes in 19 populations across the range of habitats. The mean level of genetic variation was high in the *M. stellata* 19 populations ( $H_e = 0.186$ ). By contrast, the levels of genetic variation were lower ( $H_e = 0.060$  and  $0.086$ ) in two geographically isolated populations than in less-isolated populations ( $H_e = 0.116 - 0.280$ ). We also found significant genetic differentiation among all populations ( $F_{ST} = 0.207$ ,  $P < 0.05$ ), but not among less-isolated populations ( $F_{ST} = 0.190$ ,  $P > 0.05$ ). Furthermore, cluster analysis of 12 populations, growing in close proximity suggested that there were three distinct groups of populations associated with the geographical distribution. Finally, the allozyme data suggest that *M. stellata* has different pattern of genetic variation among populations, so each population may need to be conserved.

## 107

### An in Silico Approach Towards Identifying Transcription Factor Targets

Michael Nodine, University of Arizona

Transcription factor binding motif, temporal and spatial gene expression and RNAi phenotype databases offer not only a centralized resource, but also the potential to generate new hypotheses by integrating these different data-sets. Using these databases I wrote algorithms, and then used these to predict gene targets of three well-characterized transcription factors, *Caenorhabditis elegans* *Homeobox-22* (*CEH-22*), *defective pharynx development-4* (*PHA-4*) and *uncoordinated locomotion-86* (*UNC-86*), which play central roles in *C. elegans* development. A number of novel targets of these transcription factors were predicted, including a high frequency of putative transcription factors and genes encoding proteins predicted to be involved in signal transduction. The functional classifications of the gene targets recovered are consistent with previous hypotheses regarding the specific roles of *CEH-22*, *PHA-4* and *UNC-86* during *C. elegans* development. This study provides a paradigm for combining large amounts of information from different data-sets in order to identify novel genes involved in developmental pathways. Furthermore, as more transcription factors and genes in the *Arabidopsis* genome are characterized, similar methods can be developed.

## 108

### Genetic Architecture of Apomixis in Erigeron (Asteraceae)

Richard Noyes, University Of Colorado

Elucidation of the identity, number, distribution, and interactions of genes controlling asexual seed formation (apomixis) in flowering plants promises insight into sexual developmental pathways and brings us closer to the manipulation of reproductive mode for agronomic purposes. Previous genetic mapping for apomictic triploid Erigeron (fleabanes; Asteraceae) has shown that the trait is controlled by two independent loci. One locus is associated with the production of unreduced megagametophytes (diplospory), while the second locus is associated with autonomous embryo and seed formation (parthenogenesis). These two apomixis loci segregate independently resulting in F1 progeny that include diplosporous plants that produce unreduced eggs (but no seed), and individuals bearing markers linked to parthenogenesis (but lacking capacity for parthenogenetic seed production). To test for the functional additivity of these distinct apomictic loci in Erigeron, two diplosporous (but non-apomictic) plants were crossed with a plant possessing parthenogenesis-linked markers to form two F2 populations of consisting of 235 and 172 individuals. Analyses show that ca. 5% of F2s are apomictic (combining diplosporous and parthenogenetic functions) and demonstrate the additivity of diplospory and parthenogenesis-linked markers. Recombinants are extremely variable in the expression of apomixis. However, quantitative analysis shows that seed production closely covaries with diplospory, and, consequently, that variability in overall apomictic seed production is due mostly to variation in expression of diplospory and not parthenogenesis. This result is consistent with the hypothesis that diplospory but not parthenogenesis is strongly affected by gene interactions or modifier loci.

## 109

## **Proteomic and Genetic Analyses of Genome Reprogramming during Cell Dedifferentiation in Arabidopsis**

zhaohua peng, Mississippi State University

Plant cells are totipotent, meaning that most of the differentiated cells are capable of undergoing cell dedifferentiation to regain morphogenic potential and thus regenerating new plants like zygote cells. The concept of totipotency includes a two-step process. The first is acquiring, in response to appropriate stimuli, stem cell morphogenic potential (dedifferentiation), and the second is expressing this potential during morphogenesis (regeneration). At molecular level, the genome undergoes reprogramming to restore the stem cell status during dedifferentiation, and then the genes in the reprogrammed genome are expressed following the orderly pattern of a zygote cell during regeneration. To investigate the molecular mechanism of genome reprogramming, chromatin and chromatin associated proteins were isolated and analyzed with 2-Dimensional Gels. Substantial differences have been observed in the chromatin proteomes isolated from explants and the dedifferentiated suspension culture. Mass spectrometry analyses have revealed the molecular identities of many chromatin-associated proteins. In addition, several mutants of the cell dedifferentiation pathway have been identified. One of the mutants is insensitive to both auxin and cytokinin, suggesting that it is a cellular component that integrates the signals from auxin and cytokinin to regulate cell fate switch. Molecular characterization of the gene product is underway.

## **110**

### **Single Nucleotide Polymorphisms as a tool to elucidate the mode of inheritance in sweetpotato using the Cold Induced Gene as a model**

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Single Nucleotide Polymorphisms (SNPs) are being used to identify allelic differences, and as tools for genetic mapping. Since SNPs are abundant, they are suitable for high-throughput genotyping as is used in marker-assisted breeding, EST (expressed sequence tag) mapping, and the integration of genetic and physical maps. By searching for the presence of SNPs, especially in the 3' untranslated region of an EST that forms part of a gene of interest, alleles can be detected, and the gene can be genetically mapped. Genetic analysis of sweetpotato is complicated by its high level of heterozygosity and its complex system of self-incompatibility. As an out-breeding species, sweetpotato shows wide variation at the level of its geographic subpopulations. Therefore the possibility to discriminate between individual alleles of many genetic loci would be a big help to understand the genetic background of sweetpotato.

The present project aims the identification of SNPs in selected genes in the hexaploid sweetpotato, in order to use them as markers for the discrimination of alleles in constructing linkage map. The 'cold induced' protein gene has been selected for the study since this gene provided the highest number of SNPs and its cDNA was also fully sequenced. Altogether 134 putative SNP sites representing single mutations in the alleles were identified. SNaPshot assays were conducted using two sweetpotato cultivars ('Tanzania', and 'Beauregard'). Thirty seven SNP sites out of the putative 134 yielded polymorphisms.

A total of 15 SNP specific primers were used to analyze the inheritance of the 'cold induced' gene in the Tanzania x Beauregard mapping family. The type of ploidy was analyzed based on simplex markers by comparing the observed progeny genotypic distribution with the expected distribution for three cytological hypotheses in sweetpotato Autohexaploid (hexasomic), Tetradiploid (tetra-disomic) and Allohexaploid (disomic).

## **111**

### **The rRNA gene dosage control mechanism of diploids controls nucleolar dominance in hybrids**

Richard Lawrence and Craig S. Pikaard, Biology Department, Washington University in St. Louis, USA

Eukaryotes have hundreds of nuclear ribosomal RNA genes but express fewer than half of them. Likewise, allopolyploids and hybrids often express rRNA genes inherited from one parent but silence the other parental set, an epigenetic phenomenon known as nucleolar dominance. Using Arabidopsis, we find that promoter DNA of the active subset of rRNA genes is unmethylated and is associated with histone H3 methylated on lysine 4 ( $H3^{\text{trimethyl}K4}$ ) in both pure species and hybrids. By contrast, promoter DNA of silenced rRNA genes is heavily methylated and is associated with  $H3^{\text{dimethyl}K9}$ . Chemical inhibitors of DNA methylation or histone deacetylation induce promoter DNA de-methylation, a switch to association with  $H3^{\text{trimethyl}K4}$  and derepression of silenced genes. Furthermore, knocking down the activity of three histone deacetylases, HDA6, HDT1 and HDT2 using RNAi causes the derepression of silenced rRNA genes subjected to nucleolar dominance coincident with promoter DNA de-methylation and a switch from  $H3^{\text{dimethyl}K9}$  to  $H3^{\text{trimethyl}K4}$  association. These data reveal that nucleolar dominance and rRNA gene dosage control in pure species (non-hybrids) utilize a common mechanism in which switching transcriptional states requires a concerted switch in both promoter cytosine methylation and histone methylation state. The fact that blocking DNA methylation or histone deacetylation results in the same chemical changes indicates a circular pathway of mutual reinforcement between cytosine and histone modification.

## **112**

### **Characteristics of Hsp90 buffered genetic variation and implications for evolutionary capacitance**

Todd A. Sangster, Committee on Genetics, University of Chicago; Kurt Schellenberg, Bauer Center for Genomics Research, Harvard University; Justin Borevitz, Salk Institute; Julin Maloof, University of California Davis; Susan Lindquist, Whitehead Institute for Biomedical Research; Christine Queitsch, Bauer Center for Genomics Research, Harvard University

We have demonstrated that manipulation of Hsp90 results in the expression of altered phenotypes, which are due to uncovering of normally hidden genetic variation. Such a mechanism has empirically testable implications for the pace of, nature of, and constraints on evolution. The evolutionary effects of the Hsp90 buffer will depend on several parameters, two of which are the nature and frequency of the Hsp90-buffered polymorphisms.

To this end, we have performed a QTL analysis of a developmental response, hypocotyl length in the dark, using geldanamycin to pharmacologically inhibit Hsp90. Measures of the additive genetic variance of this trait dramatically increased when compared to a control sample with uninhibited Hsp90. That is, underlying genetic factors contributing to this response were more faithfully exposed when Hsp90 was inhibited. Our study revealed two novel QTLs unobserved in control conditions. Fine mapping revealed that one of these is linked to the red-light photoreceptor PhyB, known to be involved in a host of plasticity responses. Both QTLs displayed cytoplasmic epistasis effects; the generality of this phenomenon and its implications for mapping analyses will be discussed.

The argument has been raised that Hsp90 cannot act as an evolutionary capacitor as any advantageous effects of revealed genetic polymorphisms would be outweighed by a simultaneous decrease in developmental stability. However, our experimental data demonstrate that the phenotypic effect of a revealed polymorphism affecting hypocotyl length in the dark is an order of magnitude larger than the effects of increased non-genetic variance. Moreover, we find that loci influencing non-genetic variance map to different positions than those affecting trait mean. Therefore, in this case, these Hsp90-dependent polymorphisms may be selected upon when revealed, suggesting the feasibility of chaperone-mediated evolutionary capacitance.

## **113**

### **Evolutionary and expression analysis of chloroplast small heat shock proteins from a diverse group of plants**

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It has been demonstrated that the chloroplast small heat shock proteins (Cp-sHSP) are correlated with increased thermotolerance in a number of plant species. They

appear to improve heat tolerance by protecting photosystem II during high temperature stress. Our research is focused on determining the functional significance of the qualitative and quantitative variation in Cp-sHSPs in plant species and ecotypes with differing levels of heat tolerance. To accomplish this we are isolating and characterizing the Cp-sHSP genes from two heat-sensitive species *Agrostis stolonifera*, *Chenopodium album* (New York and Mississippi ecotypes), two moderately heat-tolerant species, *Spartina alterniflora* and *Amaranthus retroflexus*, and two very heat-tolerant species, *Agave americana* and *Ferocactus wislizenii*. Gene-specific primers were designed from the methionine-rich region of the Cp-sHSP that directed the amplification of 300 bp fragments, which were cloned and sequenced. These sequences showed significant similarity with Cp-sHSPs from other closely related species. The fragments were used for PCR-based genome walking so that the complete genes could be isolated. By characterizing these Cp-sHSPs and their expression, we will determine if those found in the very heat-tolerant plants have unique characteristics.

## 114

### A genetic approach to studying mitochondrial retrograde regulation in plants

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Several lines of evidence suggest that plant mitochondria sense abiotic and biotic stresses and then influence nuclear gene expression in response. Mechanisms through which plant organelles transmit their status, including the effects of various stresses on metabolism, to the nucleus to control gene expression (retrograde regulation) are just beginning to be elucidated. Our main focus has been on altered nuclear gene expression following perturbation of metabolism in plant mitochondria. The alternative respiratory pathway (AP) branches from the cytochrome respiratory pathway (CP) at the ubiquinone pool in the plant mitochondrial electron transport chain (mtETC). In several plant species, inhibition of the CP or the TCA cycle causes increased expression of alternative oxidase (AOX), the nucleus-encoded terminal oxidase of the AP. Several lines of evidence suggest that the regulatory pathways between 1) inhibition of the CP and altered nuclear gene expression and 2) inhibition of the TCA cycle and altered nuclear gene expression are distinct. Inhibition of the CP or TCA cycle causes increased expression of the *AtAOX1a* gene in *Arabidopsis* leaves and in suspension-cultured *Arabidopsis* cells. We produced an *Arabidopsis* line (AOX1aP::Luc-25) with an *AtAOX1a* promoter-5'UTR::firefly luciferase transgene and demonstrated that the transgene responds to disruption of the CP or the TCA cycle like the endogenous gene. We are employing genetic screens to identify genes that are involved in these mitochondrial retrograde pathways and have mutagenized seeds from our AOX1aP::Luc-25 line. Screening and analyses of mutagenized plants is ongoing and has resulted in the isolation of several putative mutants that lack transgene induction following CP inhibition. True mutants will allow us to 1) identify signal transduction pathway components; 2) test our hypothesis that there are multiple mitochondrial retrograde pathways; 3) test the hypothesis that mitochondrial retrograde regulation is part of the overall responses of plants to stresses.

## 115

### Re-targeting of a duplicated gene product and the evolution of novel gene function in maize

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The maize genome encodes duplicate genes for the enzyme coproporphyrinogen III oxidase (coprogen oxidase) which catalyzes a step in the synthesis of tetrapyrroles. This enzyme activity has been found in exclusively in the plastids in higher plants studied. The maize genes, *cpx1* and *cpx2*, encode almost identical enzymes, but with different targeting information. GFP fusions with CPX1 are localized to the plastids, but fusions with CPX2 localize to the mitochondria. This change in location is due to a deletion of the target peptide information from *cpx2*, and small alterations in more 5' sequences that create an open reading frame producing a signal peptide. Complementation of a yeast mutant demonstrates that both genes encode an active enzyme. The *cpx* genes are expressed ubiquitously at similar levels, but based on their mutant phenotypes, they are diverged in function. Null mutations were obtained by reverse genetic screening. A null mutation in the *cpx1* gene has a yellow seedling-necrotic homozygous phenotype, but does not display the dominant lesion mimic phenotype observed for other mutants in tetrapyrrole synthesis. Some *cpx2* mutant homozygotes have a normal phenotype under standard growth conditions, while others are very stunted. Teosintes and other *Zea mays* subspecies also carry the two types of coprogen oxidase genes, but only the chloroplast targeted form is found in the rice genome. We discuss models for the evolution of the *cpx* genes in the grasses and possible roles of mitochondrion-localized coprogen oxidase activity in maize.

## 116

### The role of prenylation in plant development

Mark Running, Donald Danforth Plant Science Center Qin Zeng, Donald Danforth Plant Science Center Kevin Lehnbeuter, Donald Danforth Plant Science Center Sarah Hake, USDA/ARS PGEC and UC Berkeley Naomi Ori, Hebrew University of Jerusalem

Protein prenylation is a post-translational lipid modification conserved among eukaryotes. Prenylation is necessary for the function of a wide variety of proteins involved in cell division, cell growth, and signalling. Protein prenylation is catalyzed by 3 enzymes, of which farnesyltransferase (PFT) and geranylgeranyltransferase-I (PGGT-I) share a common  $\alpha$  subunit but have distinct  $\beta$  subunits. Mutants in PFT/PGGT-I  $\alpha$  are lethal in yeast and have not been described in animals. The *Arabidopsis pluripetala* (*plp*) mutants are defective in the PFT/PGGT-I  $\alpha$  subunit and lack PFT and PGGT-I activity. *plp* plants are viable and fertile but show specific developmental defects. Shoot meristems of *plp* plants are larger and contain more cells than wild type, and sometimes show fasciation. Root meristems show a progressive loss of organized cell division, leading to premature termination. Electron microscopy studies of *plp* cells suggest that *plp* is required for proper cell shape and subcellular organization. Flowers of *plp* plants contain more organs, particularly petals, with a loss of restriction of organ initiation positions. Double mutant studies suggest that *PLP* acts in the same pathway as *ERAI*, and also indicates roles for prenylation-mediated signalling in additional aspects of leaf and flower development. *plp* mutants provide a unique opportunity to study the functional role of prenylation in eukaryotes.

## 117

### Characterization and QTL analysis of the Est-1/Col RIL population for light dependent responses.

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We are utilizing natural variation present in different accessions of *Arabidopsis thaliana* to identify genes involved in light responsiveness. Plants are capable of responding in a variety of ways to specific light cues. We have created a RIL (Recombinant Inbred Line) population between Est-1 (Estland) and the common lab strain Col (Columbia). We have characterized a population of 280 RILs for light responsiveness by analyzing both hypocotyl elongation and flowering time. QTL (Quantitative Trait Loci) analysis has revealed major QTL in white, blue, red, and far-red light conditions for hypocotyl elongation, and flowering time QTL in both short and long days. Two candidate genes for a red light hypocotyl elongation QTL and a long day flowering QTL are *PHYB* and *FT*, respectively. Novel QTL affecting light responses were also detected, and all large effect QTL are currently in the process of being confirmed in NILs (Near Isogenic Line).

## 118

### Regulation of Wound Signal Transduction in Arabidopsis by PP2C Type Phosphatase

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Reversible protein phosphorylation is a major mechanism of biological signal transduction. This process is mediated by protein kinases and phosphoprotein phosphatases in diverse signal transduction pathways, including wounding, salt stress and cold.

Previously we identified mitogen-activated protein kinases (MAPK) that mediate wound signal transduction and a PP2C-type phosphatase, MP2C, a negative regulator of MAPK in alfalfa (Bogre et al., 1997; Meskiene et al. 1998; 2003).

A related PP2C type phosphatase from Arabidopsis, termed AP2C was isolated. A MAPK was identified as interacting protein in yeast two hybrid screen of Arabidopsis cDNA library with AP2C as a bait. AP2C shows specificity in interaction with two MAPKs in yeast and inactivates them in a transient expression assay in plant cells. Molecular and biochemical analysis of T-DNA insertional lines and transgenic plants over expressing this phosphatase indicate that PP2C phosphatases are negative regulators of MAPKs in stress induced signal transduction pathways.

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Meskiene, I. et al. (1998). "MP2C, a plant protein phosphatase 2C, functions as a negative regulator of mitogen-activated protein kinase pathways in yeast and plants." PNAS 95(4): 1938-43.

Bogre, L. et al. (1997). "Wounding induces the rapid and transient activation of a specific MAP kinase pathway." Plant Cell 9: 75-83.

## 119

### Molecular Population Genetics of the Arabidopsis CLAVATA2 region

Kristen Shepard, Barnard College; Michael Purugganan, North Carolina State University

The *Arabidopsis thaliana* *CLAVATA2* (*CLV2*) gene encodes a leucine-rich repeat protein that regulates shoot meristem development. Sequencing of *CLV2* from multiple accessions indicates that this gene is unusually polymorphic, with ~10 fold greater nucleotide diversity than previously studied *Arabidopsis* developmental regulatory genes. This elevated variation might be caused by maintenance of a balanced polymorphism at *CLV2*, by effects of selection at neighboring loci, or by a high rate of mutation in this region of the genome. To distinguish among these hypotheses, we have conducted molecular population genetic analyses of *CLV2* and 10 flanking genes that span 40 kb on Chromosome I. The levels and patterns of sequence diversity vary widely among these 11 genes, and adjacent genes appear to be subject to contrasting evolutionary forces. *CLV2* shows the highest nucleotide diversity in this region. The presence of several distinct *CLV2* haplotypes and an excess of intermediate frequency mutations suggest that *CLV2* may harbor a balanced polymorphism.

## 120

### LUH, A Homolog Of A Transcriptional Co-repressor LEUNIG, May be Involved In ABA signaling

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LEUNIG (LUG) has been shown to be a negative regulator of AGAMOUS (AG) and shares structural motifs with yeast Tup1 and drosophila Groucho transcriptional corepressors. LUG has only one homolog in *Arabidopsis* named LEUNIG HOMOLOG (LUH). LUH is 44% identical to LUG at the amino acid level, with 7 WD repeats at the C-terminal end similar to LUG. Unlike *lug*, the null mutant of *LUH*, *luh-1*, does not show any floral phenotype unlike *LUG*. However, *luh-1* is delayed in flowering and is defective in germination. The rate of germination of *luh-1* is about 75% of that of wild type. Also, the primary root length of *luh-1* seedlings is only about 70% of that of wild type. In addition, LUH mRNA expression is increased in *abi-1-1* (abscisic acid insensitive 1) mutant and is induced by Abscisic acid (ABA). This indicates that *LUH* might play a role in ABA signaling. The elongation of primary root in *luh-1* mutants seem to be less sensitive to inhibition by ABA, compared to the wild type. Studies are underway to test the expression of ABA responsive genes in *luh-1* mutant plants. Preliminary studies suggest a role for *LUH* in positively mediating ABA signal transduction.

## 121

### Characterization of genes associated with guard cell differentiation and function in Arabidopsis thaliana

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Guard cells are highly specialized and differentiated cells that are crucial for regulating water loss, while optimizing CO<sub>2</sub> uptake for photosynthesis. The objective of our research is to gain a better understanding of the unique aspects of guard cell differentiation and stomatal regulation by identifying guard-cell-specific genes from the model plant, *Arabidopsis thaliana*. To accomplish this, we have screened several thousand lines from two T-DNA enhancer trap tagged collections of *Arabidopsis* for guard-cell-specific reporter gene expression. We have identified seven lines, which we have named GCA (guard cell associated), in which the reporter gene (GUS) is expressed in guard cells or guard mother cells. We have localized the site of the T-DNA insertion for four of those lines and are working toward identifying those of the other three. In line GCA2, the T-DNA construct has interrupted a novel gene and homozygotes for this insertion are characterized by abnormally shaped stomatal complexes in cotyledons. The only other obvious morphological phenotype among the tagged lines is dwarfism in line GCA4. Current progress in verifying the expression patterns, elucidating the subcellular localization, and analyzing knockout mutants for each of the tagged genes will be presented.

## 122

### Interaction of PENNYWISE and BREVIPEDICELLUS regulates early internode patterning events

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Plant architecture results from activity of the shoot apical meristem (SAM), which initiates leaves, internodes and axillary meristems. *KNOTTED1*-like homeobox (*KNOX*) genes are expressed in specific patterns in the SAM and are involved in patterning mechanisms important for plant architecture. *KNOX* proteins interact with *BEL1*-like (*BELL*) homeodomain proteins, and together bind a DNA target motif with high affinity. To gain more insight into *KNOX* function, we characterized a mutation in a *BELL* gene, *PENNYWISE* (*PNY*), which has similar defects in inflorescence patterning as mutations in the *KNOX* gene *BREVIPEDICELLUS* (*BP*). Both *bp* and *pnv* have randomly shorter internodes and display a slight increase in the number of axillary branches. The double mutant shows extremely short internodes interspersed with long internodes and increased branching, suggesting that the two gene products function together. The expression of *PNY* and *BP* overlap in a discrete domain in the inflorescence meristem at the boundaries of developing floral primordia. Physical association of *PNY* and *BP* suggest that they form a heterodimer that regulates early internode patterning events in the inflorescence meristem.

## 123

### Mechanisms Generating Phenotypic Variation at the *Rp1* Rust Resistance Locus of Maize

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The *Rp1* rust resistance locus of maize consists of a cluster of NBS-LRR genes. Different *Rp1* haplotypes can be very different structurally due to mispairing and recombination in meiosis. Different maize haplotypes may carry from one to more than 40 *Rp1* genes, most or all of which have no detectable phenotypes. The phenotypically undetectable genes may be functional but simply do not recognize any of the currently prevalent rust biotypes. We are characterizing recombinant haplotypes with novel phenotypes to identify the genes controlling the phenotypes and to characterize the types of recombination events that give rise to them. We have generated and characterized novel haplotypes with nonparental race specificities. Comparisons of these haplotypes to the parental haplotypes has indicated most of the novel race specificities are due to the reassortment of the *Rp1* genes into novel combinations. We have also characterized haplotypes with more unusual phenotypes. Two of these confer defense reactions to any rust isolate and confer lesion mimic phenotypes under normal growing conditions, and a third induces defense responses spontaneously. These phenotypes are controlled by genes derived from recombination events in their LRR-coding regions. We have constructed genes similar to some of these in vitro and are now testing them in transgenic plants to examine the structural basis for their novel regulation of defense responses. We have also tested them in transient transformation assays in maize and other cereals to determine the extent to which *Rp1* genes can function in different taxa, which indicated some *Rp1* genes do not function in heterologous species. The identification and characterization of genes with novel phenotypes will shed light on how complex resistance genes function and evolve.

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### Control of axillary branching in petunia

Kimberley Snowden, HortResearch Bart Janssen, HortResearch Kerry Templeton, HortResearch Jo Simons, HortResearch Toshi Foster, HortResearch Carolyn Napoli, University of Arizona

A mutational approach has been used to study the genetic control of axillary branch development in *Petunia hybrida*. Mutations at three *Dad* (*Decreased apical dominance*) loci act primarily to increase the growth of axillary meristems into branches during vegetative development. This leads to a highly branched phenotype for *dad1* and *dad2* mutants and a moderately branched phenotype for *dad3*. Graft analyses of the *dad* mutants with wild-type plants suggest that a factor that promotes branching is produced in *dad1* and *dad3* mutant roots and is transported to the shoot system.

We have cloned the *Dad1* gene which encodes a putative carotenoid cleavage dioxygenase, and is orthologous to the branching genes *MAX4* from Arabidopsis and *RMS1* from pea. The *Dad1* gene is expressed in root and shoot tissue, consistent with the observation that a small piece of grafted wild-type shoot tissue is sufficient to revert a *dad1* mutant to near wild-type branching. However growth of mutant *dad1* roots above the graft union results in branch development, indicating that the mutant *dad1* roots produce a branch promoting substance.

The *dad2* mutant phenotype is unaffected by grafting, indicating that this gene's activity is restricted to the shoot, and may encode a later step in branch initiation. We are currently using transposon tagging to isolate the *Dad2* gene. In addition, the three different *dad* mutants have been isolated and characterised. This work, combined with graft analysis between the different mutants, indicates that the *Dad2* gene acts within the same pathway as *Dad1* and *Dad3*, and the *Dad1* and *Dad3* genes may work on the same step in the pathway controlling branch production.

## 125

### Imprinted expression of the maize SET domain gene *Mez1*

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The Arabidopsis gene *Medea* is an important regulator of endosperm development. The expression of *Medea* displays an imprinted pattern of gene expression with only the maternal allele expressed in the endosperm. To date, no ortholog of *Medea* has been detected within any monocot species. A detailed analysis of the maize genome identified three Enhancer of *zeste*-like genes; *Mez1*, *Mez2* and *Mez3*. *Mez1* is most closely related to the Arabidopsis *Clf* while *Mez2* and *Mez3* are most closely related to *Eza1*. There is no *Medea*-like detected in the maize genome. Due to the critical role of *Medea* in regulating seed development in Arabidopsis, it is likely that another gene in maize is performing *Medea*-like functions. We performed allele-specific expression analysis of *Mez1* and *Mez2* in developing maize embryos and endosperms. *Mez2* was biallelically expressed in all tissues tested. *Mez1* displayed biallelic expression in the developing embryo. However, only the maternal allele of *Mez1* was detected in the endosperm. Maternal monoallelic expression of *Mez1* was documented for all alleles tested. The imprinting of *Mez1* suggests that *Mez1* may be providing *Medea*-like functions in maize. The promoter sequence and structure of *Mez1* is compared to that of other promoters for imprinted genes. This documents an example of the convergent evolution of imprinting within a closely related gene family in plants. The finding that at least one Enhancer of *zeste*-like gene is imprinted in both maize and Arabidopsis provides support for the requirement of imprinting of this gene product.

## 126

### A multi-locus analysis of variation in Arabidopsis: comparison between the outbreeders *Arabidopsis lyrata* and *A. halleri*, and the inbreeder *A. thaliana*

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Nucleotide variation in eight effectively unlinked genes was surveyed in species-wide samples of the closely-related outbreeding species *Arabidopsis halleri* and *A. lyrata* ssp *petraea*, and in three of these genes in *A. lyrata* ssp *lyrata* and inbreeding *A. thaliana*. Significant genetic differentiation was more frequently observed in the more continuously distributed *A. halleri*. Average estimates of nucleotide variation were highest in *A. l. petraea* and lowest in *A. l. lyrata*, reflecting differences among species in effective population size. In general, the data support theoretical predictions that outcrossing species have higher levels of genetic variability than inbreeding species, with the exception of *A. l. lyrata*. Additional aspects of the data suggest further that *A. l. lyrata* experienced a population bottleneck during its colonization of North America and that this bottleneck substantially reduced within-species variability.

## 127

### Genetic characterisation of rye samples with regard to leaf rust resistance

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The purpose of this research is identification of Lr-genes in 11 sources of rye resistance to leaf rust. The genetic control of leaf rust resistance has been investigated by test crosses, and the test-clone method in the rye samples Malysh 72-2, Chulpan 3, Immunnaya 4, Yaroslavna 3, Lovashpatonae 2, Gotor 2, Talovskaya 12-2, Braunrostresisten 2, Orlovskaya 9-2, 3098/18 and 4001/10. It has been discovered that the leaf rust resistance in the above samples is controlled by a set of nonidentical oligogenes. Dominant gene Lr6 controls leaf rust resistance in Chulpan 3 and Immunnaya 4; Lr5 does in Malysh 72-2; and Lr-c, in Yaroslavna 3. Test crosses have shown that gene Lr-a and Lr-b differ from the genes determining the resistance in Gotor 2 and Braunrostresisten 2. In German samples 3098/18 and 4001/10, the resistance to the Lviv population of the pathogen is controlled by nonallelic genes Lr-a and Lr-b. It has been found that gene Lr-b is not identical to the resistance gene of Lovashpatonae 2, and Lr-a is not identical to Lr-c. In all samples, resistance to the leaf rust population is controlled by dominant genes from the stage of seedlings

till maturity. Highly efficient Lr genes are present in the samples Malysh 72-2, Chulpan 3, Immunnaya 4, Yaroslavna 3, Lovshpatonae 2, Gotor 2, Talovskaya 12-2, 3098/18 and 4001/10. In addition to the oligogenes, most samples possess genes efficiently controlling particular clones of the pathogen.

## 128

### Specification of adaxial/abaxial polarity in maize

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The first steps in lateral organ formation include the recruitment of founder cells from the meristem and the establishment of new developmental axes relative to the main body axis. The semi-dominant *Rolled leaf1 (Rld1)* mutants of maize cause a partial inversion in adaxial/abaxial polarity in the leaf. We have recently cloned the *rld1* gene, which encodes an HD-ZIPIII protein with high homology to *REVOLUTA* in *Arabidopsis*. *rld1* is expressed in the shoot apical meristem, in pro-xylem, and on the adaxial side of young leaf primordia. In older leaf primordia, expression becomes restricted to less determined cells near the margins. Like the *Arabidopsis HD-ZIPIII* genes, *rld1* appears to be regulated post-transcriptionally by a miRNA-directed process. *rld1* transcripts contain a miRNA165/166 complementary site, which is mutated in all four *Rld1* alleles. As a result, *rld1* transcripts persist longer and accumulate on the abaxial side of *Rld1* mutant leaf primordia. Consistent with a role for miRNA166 in the regulation of *rld1*, one of the miRNA166 precursors (*MIR166c*) is expressed in a pattern complementary to *rld1*. *MIR166c* is expressed weakly on the abaxial side of young leaf primordia. During primordia development, strong *MIR166c* expression initiates near the midvein and expands towards the margins of the leaf. Moreover, *MIR166c* expression within the vasculature is limited to the phloem.

We previously proposed that *leafbladeless1 (lbl1)* is required for adaxial cell identity, as *lbl1* mutants form radially symmetric abaxialized leaves. Preliminary data suggests that *lbl1* may specify adaxial cell fate by restricting *MIR166c* expression to the abaxial side of developing leaves. We have isolated several maize homologs of the *Arabidopsis YABBY* genes. Unlike *Arabidopsis*, where *YABBY* expression is correlated with abaxial cell fate, *yabby* expression in maize is limited to the adaxial side of developing leaf primordia. The *yabby* expression patterns in *lbl1* and *Rld1* mutants suggest the *yabby* genes act downstream of *lbl1* and *rld1*. *yabby* expression is down-regulated in *lbl1* and up-regulated in *Rld1* mutants. Furthermore, our observations suggest the *yabby* genes may direct lateral organ outgrowth.

These data suggest that *lbl1*, *rld1*, *MIR166c* and the *yabby* genes act in the same pathway. *LBL1* may suppress *MIR166c* expression on the adaxial side thus allowing expression of *rld1* and the *yabby* genes, leading to adaxial/abaxial polarity and lateral organ outgrowth. Finally, our data suggest that aspects of adaxial/abaxial patterning have diverged between monocots and dicots.

## 129

### Tissue Specific Gene Silencing Mediated By Naturally Occurring Mutations Of A Chalcone Synthase Gene Cluster In Soybean

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Chalcone synthase is a key regulatory enzyme in the flavonoid pathway, leading to the production of multipurpose compounds such as flavonoids, isoflavonoids, proanthocyanidins and anthocyanins. In soybean, chalcone synthase is encoded by a multigene family composed of eight members. Previous studies on the structural organization of this gene family in soybean report the existence of a 10 KB region (*I* locus) comprising of tandemly linked *Chs1*, *Chs3* and *Chs4*. Mutations of a dominant allele (*I* or *i'*) to a recessive allele (*i*) delete promoter sequences, paradoxically resulting in increased total *Chs* transcript levels, manifested as pigmented (black) seed coats. A very high nucleotide similarity (95%) between most of the chalcone synthase gene family members has made it difficult to explore the unusual nature of the *I* locus.

Towards attributing the increase in seed coat pigmentation in the genotypes with the recessive *I* allele to one or more chalcone synthase genes, we have employed a powerful and sensitive technique, fluorescent real time RT-PCR. Gene-specific TaqMan® primer-probe sets were designed for the eight members using Primer Express. Purified total RNA was retro-transcribed and the cDNA amplified using the ABI Sequence Detection System. The relative expression levels of the *Chs* gene family members in the isolines carrying the *I* → *i* and *i'* → *i* mutations were quantitated. Our results indicate that at least five of the eight members were expressed at variable levels in both the seed coats and cotyledons of the different isogenic pairs. A significant increase in *Chs* expression of at least three members was observed in the recessive *i* genotype (pigmented seed coats) of all the mutant pairs, unlike the cotyledons where the mutation did not seem to affect *Chs* expression levels. This suppressive effect of the *I* locus in soybean is one of the very few known endogenous gene silencing mechanisms that is tissue specific.

## 130

### Using RAPD for estimating genetic polymorphism in and phylogenetic relationships among species of the genus *Lycopersicon* (Tourn.) Mill.

Volodymyr Ugorchak, Scientific Research Inst Of Plant Protection

RAPD genome analysis of 53 species and cultivars of the genus *Lycopersicon* (Tourn.) Mill. revealed their

high genetic polymorphism (Tourn.) Mill., based on which their phylogenetic relationships were inferred. In total, 248 polymorphic DNA fragments were amplified. Intraspecific polymorphism was maximum (79%) in *L. peruvianum* and minimum (9%) in *L. parviflorum*. In general, genome divergence among cross-pollinating tomato species was substantially higher than in self-pollinating species. An UPGMA dendrogram constructed from the RAPD patterns was consistent with the *Lycopersicon* phylogeny inferred from the molecular data of RFLP, ISSR, and microsatellite analyses and with classification based on morphological characters. The relationships of taxa within the genus *Lycopersicon* are discussed.

## 131

### Chloroplast-targeted variants of Anthranilate Synthase lead to accumulation of tryptophan in soybean

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Anthranilate Synthase (AS) controls the first step in tryptophan (trp) biosynthetic pathway, the conversion of chorismate to anthranilate. The plant enzyme is a heterotetramer composed of two functionally distinct alpha and beta subunits. The alpha subunit, whose activity is feed-back inhibited by trp, catalyzes the amination of chorismate and elimination of enolpyruvyl side chain. The beta-subunit belongs to the family of triad glutamine-dependent amidotransferases which transfer nascent ammonia via an intramolecular channel to the synthase active site. One strategy to increase trp levels in plants relies on overexpression of feedback resistant alleles of AS alpha subunit. When a previously identified feedback resistant maize AS alpha protein was targeted to the soy chloroplast, up to 4,000 ppm trp accumulated in transgenic seeds. In addition to the maize AS allele, the *Agrobacterium tumefaciens* AS gene, expressing both alpha and beta subunits as one contiguous polypeptide, was cloned by sequence homology and expressed in *E. coli*, *A. thaliana* and *Glycine max*. Studies done with purified enzyme show that Agro AS is sensitive to feedback inhibition by trp. The chloroplast-targeted Agro AS enzyme is active and exhibits a specific activity hundred folds higher than that seen in wild-type soybeans. As a consequence, levels of up to 8,000 ppm trp accumulate in transgenic soy seeds. Agro AS mutant alleles showing various degrees of feedback inhibition were rationally designed by narrowing the trp-binding pocket, and stably transformed into soy. This strategy was successful as well and seeds generated by such lines accumulated more than 10,000 ppm trp.

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### Development of a system to score C to T or G to A transitions in a CpG or CpNpG context

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In 2000, Kovalchuk *et al.* (EMBO J. 19: 4431-4438) described a method to monitor mutation frequencies in plants. This was achieved by introducing inactivating missense and nonsense mutations into an active  $\beta$ -glucuronidase (*gus*) gene in *Arabidopsis thaliana*, followed by histochemical staining of these plants. A blue spot appears wherever the mutant nucleotide is restored to the wild-type, and from the number of blue spots observed the mutation frequency can be deduced.

We want to use a similar system to monitor transitions of methylated cytosine to thymidine residues, or from guanine to adenine on the complementary strand. Unfortunately, none of the *gus* mutants described by Kovalchuk *et al.* (2000) allow to check this kind of mutation, and consequently we had to develop them ourselves. As cytosine methylations more frequently occur in a CpG or CpNpG (symmetric) motive, as compared to non-symmetric cytosines (Gruenbaum *et al.* 1981, Nature 292: 860-862), all point mutations result in a symmetric cytosine residue.

Using site directed mutagenesis, we currently generated several *gus* genes that carry an inactivating point mutation, as such that a C to T transition restores the wild-type *gus* sequence. All these *gus* sequences are cloned in an *E. coli* expression vector, and transformed in a strain that does not show any background GUS activity.

In the future, these mutant *gus* genes will be used to generate *Arabidopsis* plants containing a single mutant *gus* locus. Such plants can be used to monitor C to T or G to A transitions in different experimental setups, if necessary after crossing the mutant *gus* locus into the lines to be studied.

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### FLEXIBLE MULTIPLEX SNP GENOTYPING IN ARABIDOPSIS USING SNPWave™

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Large scale genotyping of genetic markers such as single nucleotide polymorphisms (SNPs) is important to map mutants and identify quantitative trait loci (QTL) in *Arabidopsis* and other model organisms. In addition, SNP genotyping is essential for marker-assisted selection in plant and animal breeding programs. Since a large diversity of cost-effective platforms for SNP detection exist, the main cost component is preparation of the detection mixture. In order to reduce the cost per datapoint, efficient multiplex amplification technologies and miniaturization of reaction volumes are needed.

We are developing a 100-plex SNPWave™ assay to facilitate whole genome screens and fine-mapping based on SNP genotyping in *Arabidopsis*. For this purpose, 100 polymorphisms between the Landsberg and Colombia ecotypes were selected from the TAIR database. With 20 polymorphisms per chromosome, the average inter-marker distance ranges between 3 and 6 cM per chromosome.

SNPWave is based on highly multiplexed allele-discrimination, followed by selective amplification of 10 SNP loci in a single polymerase chain reaction (PCR) using the amplified fragment length polymorphism (AFLP) technology. The use of AFLP allows the amplification of various subsets of SNPs, including 10 subsets for whole genome screening which each contain 1 SNP of every chromosome arm, or 10 subsets for fine mapping which each contain 10 SNPs derived from a single chromosome arm. Detection of SNPWave reaction products is performed on a (capillary) sequencer, such as the MegaBACE™, using multiple fluorescent labels and short running times. SNPWave allows co-dominant scoring of over 23,000 SNP loci on one MegaBACE 1000 in a 6 hour period, using 96 capillaries, 4 labels, 10 SNP loci per reaction and 6 consecutive runs.

We expect this SNPWave assay to become a valuable and flexible tool for gene mapping in *Arabidopsis*.

AFLP is a registered trademark of Keygene N.V.

The AFLP and SNPWave technologies are covered by patents and patent applications owned by Keygene N.V.

Application for trademark registration for SNPWave has been filed by Keygene N.V.

MegaBACE is a trademark of Amersham BioSciences.

## 134

### Functional analysis of Elongator in plant development

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During the development of the plant, pattern formation, growth and differentiation are overlapping rather than consecutive events. These processes are reiterated throughout the life cycle upon the formation of every new organ. Using the model plant *Arabidopsis thaliana*, the genetic control of organ growth is studied using the leaf as the experimental system.

Our study on the function of the DEFORMED ROOTS AND LEAVES (DRL1) gene adds to the accumulating evidence that organ growth is controlled genetically or epigenetically by the regulation of transcription of sets of genes (Mizukami and Fischer, 2000; Autran *et al.*, 2002). The DRL1 protein is homologous to a yeast protein that associates with Elongator, a histone acetyl transferase complex that binds the RNA polymerase II transcription elongation complex. Knockout *dr11* mutants in *Arabidopsis* display specific phenotypes that relate to defective plant meristem activity and organ growth (Nelissen *et al.*, 2003). The DRL1 gene appears to function early in leaf development and specifies a pathway for lateral growth in parallel with the leaf polarity genes that establish dorsal-ventral asymmetry early in leaf blade formation. DRL1 acts upstream of ANGUSTIFOLIA (AN), a transcriptional co-repressor with a function in the expansion stage during leaf growth (Kim *et al.*, 2002). AN is the first gene for which altered transcription was demonstrated in *dr11* knockout mutants. *Arabidopsis* homologs for the six Elongator components are present in the genome and these are functionally analysed (knock-out, double mutants). The targets of the Elongator-mediated regulation during leaf organ formation are being determined using cDNA-AFLP and Affymetrix micro-array genome-wide transcription profiling of Elongator mutants.

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### 135

#### **Anthocyanin Intensity in Rice Grain is Regulated by Splicing Efficiency of Dihydroflavonol-4-reductase and is Temperature Sensitive**

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Temperature during grain ripening stages affected grain anthocyanin intensity in purple rice. In winter, anthocyanin content was significantly higher than in summer. Dihydroflavonol 4-reductase (DFR) is the key enzyme in anthocyanin synthesis. RT-PCR analysis from RNA isolated during grain development in summer revealed an additional unexpected DFR transcript containing the intron 1 in purple rice. In white rice, only unspliced transcripts were detected during grain filling period. In silico translation revealed that the 112 bp unspliced intron 1 translated in-frame with the flanking exons created premature stop codon. This putative unspliced transcript was not a genomic DNA contamination. The inefficient splicing was not originated from mutation at its splice junctions. Further comparison between white and purple rice for the C1, OsB1 and OsB2, the known transcription regulators of DFR, identified interesting SNPs. In the C1 of a white rice, a 3 bp deletion causing an exclusion of glutamic acid from a domain similar to the Ribosomal DNA Enhancer Binding Protein 1 (REB1) was found. This REB1 in mammalian systems functions as a pre-mRNA splicing controller. It is possible that C1 regulate alternate splicing and gene expression in DFR in response to temperature. It become evidences that post-transcriptional control regulates genes underlying a QTL in plants.

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#### **High-Resolution Fine-Mapping of *sun*, a Locus Controlling Tomato Fruit Shape.**

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The locus *sun* on the short arm of tomato chromosome 7 controls morphology of the fruit. Alleles from wild relatives impart a round shape, while alleles from certain cultivated varieties impart an oval shape typical of roma-type tomatoes. Large genomic insert clones were identified and mapping of the end-fragments in the high-resolution recombinant population showed that these clones fell into two non-contiguous contigs flanking the fruit shape locus *sun*. Progeny testing of recombinant plants revealed that *sun* was located in the region between the two contigs comprising 0.2 cM. Fiber-FISH analysis with clones from each of the two contigs showed that the physical gap was less than 66 kb, corresponding to a region with a physical to genetic distance ratio of 287 kb/cM, which is lower than the genome average of 750 kb/cM. Our results indicated even lower physical to genetic distance ratios, from 71 to 160 kb/cM, in the regions flanking *sun*. The recombinant screens revealed a ten-fold difference in recombination rate between two different populations used to fine-map *sun*. In addition, we observed significant differences between F3 families derived from the same cross in crossover rates and distribution of recombination events. Lastly, our results indicated the presence of a previously unknown paracentric inversion of the top 16 cM of chromosome 7 in *L. pennellii* LA716.

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#### **Feedback resistant corn anthranilate synthase increases trp levels in transgenic Arabidopsis and soy**

Marguerite Varagona, Stephanie Back, Wayne Brown, Diane Buhr, Paul Chomet, Tanya Fagaly, Steve Kan, Yuki Lewis, Michael Luethy, Kathy Malloy, Siva Manjunath, Tim Oulmassov, Brad Reznick, Gabriela Vaduva, Dale Voyles, Joan Wang, Lisa Weaver, Ken Gruys (Monsanto Co.) Lyle Crossland, Al Kriz, Jihong Liang (Revensen LLC)

Soy meal is a dietary source of protein for animals and humans. However, plant protein sources are deficient in aspartate and shikimate derived amino acids. Currently, amino acids are supplemented using higher cost food additives or fermentation products. Our work focuses on elevating the levels of tryptophan (trp) in soy seed to generate a low cost substitute for commodity soy meal in animal diets. The poster describes research to discover a gene involved in trp biosynthesis that can be used to elevate trp in transgenic plants. The first committed step in trp biosynthesis is the conversion of chorismate to anthranilate by anthranilate synthetase (AS). In plants, this complex has an alpha2/beta2 structure. The alpha subunit is responsible for the amination of chorismate to form anthranilate using an amine group from glutamine and provided by the beta subunit. AS is negatively feedback regulated by trp binding to the alpha subunit. By selecting maize cultured cells for resistance to the trp analog 4-methyl-trp, a line was found to have a feedback insensitive allele of AS alpha. Corn plants derived from the culture showed elevated trp in several tissues. The gene causing the phenotype was isolated and had a single point mutation changing an amino acid to render the protein trp insensitive. This allele was expressed in corn using an embryo promoter, and seed from the resulting transgenic plants showed elevated trp. To use this AS alpha gene in soy, it must be able to interact with the endogenous soy beta subunit. This interaction was tested by expressing the maize gene in Arabidopsis and soy seed. In both plants, the gene was shown to elevate trp levels in the seed. Enzyme assays confirmed the presence and function of the AS enzyme in the transgenic seed.

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#### **Programmed cell death, meristem fasciation and cell cycle arrest caused by dysfunction of Arabidopsis ribonucleotide reductase**

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Cell division plays an important role in plant pattern formation and in the establishment of the final body plan. How cell proliferation is regulated within the context of plant development remains largely unknown. Mutations in *Arabidopsis TSO2* gene cause the fasciation of the shoot meristem, abnormal leaf and flower morphology, partial homeotic transformation in floral organs, aborted embryos and hypersensitive to UV-C treatment. Using a map-based approach, we showed that *TSO2* encodes a small subunit of ribonucleotide reductase (RNR). RNR catalyzes the rate-limiting step in dNTPs production and thus plays an essential role in DNA synthesis and repair. There are two additional RNR small subunit genes, *R2A* and *R2B*, in the *Arabidopsis* genome. We isolated *r2a* and *r2b* single and double mutants that exhibited no visible phenotype. However, *r2a* or *r2b* dramatically enhances *tso2* phenotypes either in homozygous or heterozygous forms. RNA expression studies indicated that *TSO2* is cell cycle-regulated and play a leading role in *Arabidopsis* RNR function during cell proliferation and DNA damage repair. In situ and GUS reporter gene, used to monitor the expression of cell cycle markers, showed that cell cycle arrests at S and G2-M phases in *tso2* mutants. Further, programmed cell death (PCD) is observed in *tso2 r2a* double mutants. RT-PCR analysis showed that many genes induced in hypersensitive (HR) cell death are also upregulated in *tso2 r2a* double mutants. Hence, *tso2*-mediated PCD shares common components with HR-mediated PCD. Our data provided the first detailed genetic and in vivo functional analyses of RNR gene family in higher eukaryotes. We proposed that impaired RNR activities in *tso2* mutants led to altered dNTPs homeostasis and led to DNA damage and genome-instability which may trigger PCD to eliminate damaged cells.

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#### **Comparative and evolutionary analysis of the ovate-containing region in tomato and its orthologous region in eggplant**

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The *ovate* locus was mapped on the long arm of chromosome 2 in the tomato genome, while the syntenic region was mapped on the chromosome 2 in the eggplant

genome. A 105-kilobase bacterial artificial chromosome (BAC) clone containing the *ovate* gene, BAC19, was isolated from a genomic library. Eighteen genes were predicted in BAC19, with 11 confirmed by exact EST matches. An orthologous 106-kilobase eggplant BAC clone, SM77N19, was identified and annotated. Twelve genes were predicted in SM77N19 and six genes had *Arabidopsis* homologous matches. Nine genes were conserved and colinear between the 81 kb eggplant sequence and the 71 kb tomato sequence, which indicated that this fragment was descended from the close common ancestor. The speciation time of tomato and eggplant will be evaluated using the average extent of the amino acid substitution of these conserved genes. Comparisons of  $K_a$  and  $K_s$  for each gene will be reported. The investigation of these two regions will elucidate the evolutionary forces that act in this gene rich region in Solanaceae species, enhancing our understanding of the fine scale genomic structure change in the close related

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### A WD40-repeat containing protein is required for efficient H3K9 dimethylation and normal development in *Arabidopsis*

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Epigenetic control of (trans)gene expression can occur at either post-transcriptional or transcriptional levels. In higher plants, mammals, and filamentous fungi, transcriptional gene silencing (TGS) is frequently associated with DNA methylation. By contrast, in the green alga *Chlamydomonas reinhardtii*, single copy transgenes can become transcriptionally silenced without discernible cytosine methylation of the introduced DNA. A WD40-repeat containing protein, Mut11p, similar to the fungal transcriptional co-repressor Tup1p, is essential for this process and appears to have a global role in gene regulation in *Chlamydomonas* (Zhang et al., 2002, Plant J. 31: 25). The *Arabidopsis* genome encodes two putative homologs of *Chlamydomonas* Mut11 (At3g49660 and At4g02730). We have examined the role of the closest homolog (At3g49660, AtMut11) in the development of a multicellular plant. Northern blot and RT-PCR analyses indicated that AtMut11 is expressed in both vegetative and reproductive organs. These results are also consistent with the expression of  $\beta$ -glucuronidase driven by the AtMut11 promoter in transgenic *Arabidopsis* lines. A T-DNA insertional mutant, disrupted in AtMut11, revealed that this gene is essential for embryogenesis. Homozygous mutant embryos are arrested very early in development (before the heart stage) and only phenotypically wild-type progeny can be recovered by self-fertilization of the heterozygous mutant. This defect in embryogenesis can be complemented by transforming the mutant line with either a genomic copy of AtMut11 or a cDNA copy driven by the native promoter. Transgenic lines, where expression of AtMut11 was down-regulated at different stages of development by inducible RNA interference, also showed defects in leaf, stem and flower development. These results suggested that AtMut11 is required throughout plant development. The transgenic lines showing down-regulation of expression of AtMut11 also had global defects in the dimethylation of histone H3 at lysine 9 and in the formation of heterochromatin. Further, consistent with a role in transcriptional control, an AtMut11:GUS:gf fusion protein localized in the nucleus. Our results suggest that AtMut11 is a component of a molecular machinery involved in the modulation of chromatin structure and transcriptional repression in *Arabidopsis*.

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### Functional and Molecular Characterization of Genes and Mutant Alleles of the Soybean Flavonoid Pathway.

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An extensive collection of ESTs and the 9,280-cDNA clone arrays, have allowed the identification and cloning of genes of the soybean flavonoid pathway. Three independent loci (*I*, *R* and *T*) control pigmentation of seed coats in soybeans and are distinct from those controlling flower color (*W1*, *W3*, *W4*, *Wm* and *Wp*). The *T* locus also controls pubescent hair color and alters a leaf wavy phenotype. An epistatic effect of the *t* allele results in cracked seed coats. We found the *T* locus to encode a flavonoid 3-hydroxylase gene (F3.H) that was highly expressed in early stages of seed coat development and very low or not at all in other tissues. F3.H genomic and cDNA sequence analysis of color mutant lines with varying *t* alleles revealed a frame shift mutation in one of the alleles. In another line derived from a mutable genetic stock, the abundance of the mRNAs for F3.H was dramatically reduced. Analysis of this allele's cDNA and its genomic sequence, including 800 bp of its promoter region, has not revealed the molecular defect. The lack of F3.H expression in this latter mutation does not affect the expression of other flavonoid pathway genes when a global transcription analysis was done via microarrays. Expression data for this and other genes in the pathway suggest that the F3.H gene is programmed to be turned on in the seed coat specifically. However, as part of the defense response of the soybean plant to a bacterial infection, this F3.H gene is induced in leaf tissues. This induction is not as rapid as the one observed for the isoflavone synthase 1, the enzyme leading to the synthesis of isoflavones and phytoalexins. We will present data that led to the identification and cloning of the *Wp* gene via microarrays and partial characterization of a *wp* mutable allele.

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### LHP1 subnuclear localisation patterns during *Arabidopsis* development

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Epigenetic processes are involved in the control of developmental processes in higher eukaryotes. This control is mediated by various chromatin modifications and key chromatin-associated proteins such as proteins involved in the formation of silent chromatin which belongs to the HETEROCHROMATIN PROTEIN 1 (HP1) family. HP1-like proteins are highly conserved chromosomal proteins, present in most eukaryotes ranging from *S. pombe* to humans and plants, with chromatin packaging and gene silencing functions and a particular structure, which comprises a chromo domain and a related chromo shadow domain, connected by a hinge region. The *Arabidopsis thaliana* LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) is involved in developmental control as some of its animal counterparts. Mis-expression of LHP1 leads to defects in plant architecture, leaf morphology and to precocious floral transition. In order to get a comprehensive understanding of the dynamics of silent chromatin in *Arabidopsis* during development and gain insight into LHP1 function, transgenic plants carrying GFP-LHP1 fusions have been obtained. Their analyses and the corresponding subnuclear localisation of the GFP-LHP1 fusion protein will be presented. Comparisons with animal HP1 localisation patterns will be discussed.

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### Genetic variation of AA genome *Oryza* species measured by MITE-AFLP

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MITEs (miniature inverted-repeat transposable elements) are the major transposable elements in *Oryza* species. We have applied the MITE-AFLP technique to study genetic variation and species relationship in the AA-genome *Oryza* species. High polymorphism was detected within and between species. The genetic variation in the cultivated species, *O. sativa* and *O. glaberrima*, was comparatively lower than in their ancestral wild species. In comparison between geographical lineages of the AA genome species, African taxa, *O. glaberrima* and *O. barthii*, showed lower variation than the Asian taxa, *O. sativa*, *O. rufipogon*, and *O. nivara*, and Australian taxon *O. meridionalis*. However, another African taxon, *O. longistaminata*, showed highest genetic variation. Species relationships were analyzed by the pattern of presence of absence of homologous fragments, because nucleotide sequences of the detected MITE-AFLP fragments revealed that the same fragments in different species shared very high sequence homology. The clustering pattern of the AA-genome species matched well with the geographical origins (Asian, African and Australian), and with the Australian taxon being distant to the others. Therefore, this study demonstrated that the MITE-AFLP technique is amenable for studying genetic variation and species relationship in rice.