Meeting Report

The 46th Annual Maize Genetics Conference. Unlocking the Secrets of the Maize Genome

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For the first time in its history, the Annual Maize Genetics Conference was held in Mexico City, near the center of origin for many Zea species, including maize. Maize research has made many contributions to our understanding of plant physiology and development, the regulation of transposable elements and chromosome structure, and the epigenetic control of gene expression. In addition to the reported advances in these research fields, this year's meeting emphasized the tremendous genetic diversity present within maize races. Explorations of this variation in studies of domestication, population genetics, and crop improvement hinted at the tremendous potential that lies within the maize genome. Tapping into this potential will soon be made easier as highlighted in a workshop outlining advances in maize genomics. Remarkable progress has been made toward sequencing the genic regions of the maize genome, and strategies to anchor and finish the entire maize gene space by 2006 were presented and discussed. This report highlights the new developments made in these areas of maize biology.

SEQUENCING THE MAIZE GENOME: CONNECTING THE DOTS OF GENE ISLANDS

The maize genome is comparable in size to the human genome and is nearly twenty times the size of Arabidopsis and six times that of rice. Current estimates predict that the maize genome contains approximately 25,000 to 50,000 genes (Martienssen et al., 2004). These genes, which constitute less than 20% of the genome, are embedded within large arrays of highly conserved retrotransposons and other repetitive sequences. One of the highlights of this year's meeting was the tremendous progress on sequencing the ''gene islands'' within the maize genome. The Consortium for Maize Genomics (involving the Donald Danforth Plant Science Center, The Institute for Genomic Research, Orion Genomics, and University of Georgia) and collaborative teams from the University of Georgia, Purdue University, and the University of Arizona, and from Cold Spring Harbor Laboratory and Iowa State University, have employed two enrichment strategies to preferentially sequence the genic regions of maize as a rapid and cost-effective alternative to sequencing the entire genome (Palmer et al., 2003; Whitelaw et al., 2003). One gene-enrichment strategy, methylation filtration (MF), utilizes the fact that most maize genes are hypomethylated compared to the surrounding repetitive elements (Rabinowicz et al., 2003). Transformation of shotgun libraries into selected bacterial hosts results in degradation of methylated sequences and thus in enrichment of clones containing unmethylated sequences including genes. The high-CoT (HC) selection approach exploits differences in renaturation kinetics between denatured low copy sequences and repetitive elements to construct gene-enriched libraries (Yuan et al., 2003).

Together, these genomics consortia have generated more than approximately 900,000 paired-end sequence reads from MF and HC libraries, which have been compiled into assemblies covering approximately 250 Mb or more than 10% of the maize genome. Analysis of these assemblies revealed a 4-fold enrichment of gene sequences in the enriched libraries. More importantly, these assemblies cover approximately 70% of the nucleotide sequence of nearly 93% of known maize genes. Interestingly, only 50 Mb of sequence was redundant between the MF and HC assemblies, indicating that both enrichment strategies are complementary rather then redundant. MF resulted in a greater enrichment of upstream and first exon sequences whereas 3′ sequences were better represented in the HC libraries. Unfortunately, some genic sequences appear underrepresented in both gene-enriched libraries. As a result, less than half the genes are currently contained within a single assembly.

Major goals for the coming year(s) will be to complete the sequences of all genes and to anchor the sequenced gene islands onto the genetic and physical maps. Joachim Messing (Waksman Institute) presented progress on the assembly of a minimal BAC tiling path for maize, which is being constructed in collaboration with the University of Arizona, Munich Information Center for Protein Sequences, and the MIT Center for Genome Research. They have generated high-resolution restriction fragment fingerprint

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maps and BAC end sequences for more than 400,000 BACs, which is approximately 25 times the coverage of the maize genome. By integrating the physical mapping data previously generated by the Maize Mapping Project (University of Missouri, University of Georgia, and University of Arizona), a partial physical map comprising almost 3,000 contigs has been assembled and anchored to the genetic map. They will refine this physical map by using the BAC end sequences to identify maize BACs and contigs that map to the same syntenic regions of rice. In addition, because approximately 30,000 BAC end reads were found to include gene sequences, these BAC end sequences will be used to place the MF and HC assemblies onto the physical map. Several other strategies are currently being evaluated to complete and anchor the gene island sequences. Jeff Bennetzen and colleagues (University of Georgia, Purdue University, and the University of Arizona) developed two technologies that again exploit the difference in methylation pattern between unique and repetitive sequences. Hypermethylation-partial-restriction libraries are small insert libraries obtained after partial digestion with methylation sensitive enzymes that may allow the cloning of those genic regions underrepresented in the MF and HC libraries. In contrast, methylation-spanning-linker libraries contain very large insert clones obtained after complete digestion with methylation sensitive restriction enzymes, which are expected to span intergenic regions and to allow ordering of the gene islands. Pablo Rabinowicz (Cold Spring Harbor Labs), Brad Barbazuk (Donald Danforth Plant Science Center), and Agnes Chan (The Institute for Genomic Research) proposed that single or low pass sequencing of BACs will allow assembly and anchoring of over 90% of the maize genes.

Sequencing gene-enriched libraries is thus a fast and cost-effective alternative to whole genome sequencing. However, the usefulness of this approach will depend on the successful assembly and annotation of the obtained sequence information. Analysis of the Arabidopsis and rice genomes revealed extensive gene duplications. Pat Schnable and colleagues (Iowa State University) carefully analyzed the MF and HC assemblies and found that the sequencing error rate is extremely low (approximately 4×10^{-3}). However, they also demonstrated that approximately 1% of maize genes have tandemly arranged nearly identical paralogs, which are likely to collapse into a single gene during the assembly process. Assembly and anchoring of the gene islands in maize may be further complicated by the fact that maize is an ancient tetraploid. To assess the level of conservation between homeologous chromosomal segments, the complete sequence of 5-Mb regions that are duplicated on chromosomes 1 and 9 are being determined. This information will also allow a more detailed analysis of the synteny between maize and rice.

The progress reported in the maize genomics effort this past year is particularly remarkable since the genomics workshop at last year's maize meeting still focused on discussion of sequencing strategies. This progress would not have been possible without the political support and lobbying efforts of the National Corn Growers Association. Gary Davis (National Corn Growers Association) articulated the importance of maize to the U.S. economy, and thus the National Corn Growers Association's strong interest in maize genome research. The current goal is to finish and map out the entire archipelago of gene islands within the maize genome by the end of 2006. This year's meeting will certainly also be remembered for the breaking announcement that several industrial parties are willing to share, through a licensing agreement, privately generated sequence information from more than two million expressed sequence tags (ESTs) and more than 25,000 full-length cDNAs with the public. This is a clear demonstration of the collaborative interactions between academia and the private sector that has been characteristic of maize research. The availability of genomic and cDNA sequence information will be an invaluable resource. It will greatly assist the ongoing publicly funded genome sequencing projects and facilitate the analysis of gene function through access to full-length cDNA sequences. Importantly, these sequence databases will facilitate comparative genomics among diverse races of maize and between related grass species such as rice, wheat, barley, and sorghum. The sequences have been made available to not-for-profit researchers. For information, visit http://www.maizeseq.org/.

EXPLORATIONS INTO MAIZE GENETIC DIVERSITY

In a fitting tribute to the diversity of maize present within Mexico, the first talk of the conference was given by Dr. Ed Buckler (USDA-ARS, Cornell University). Buckler presented evidence from his lab and colleagues that maize contains tremendous diversity; any two inbred lines of maize are about as related to each other as humans are to chimpanzees! This diversity can be attributed to a large effective population size, a high degree of outcrossing and nearly 0.5 million years of recombination in its progenitor teosinte lineage. Today, much of that diversity remains but is divided into three major subpopulations (Remington et al., 2001). To exploit this diversity in association genetics, Buckler and colleagues have developed statistical methods to control for this underlying population structure. Through a candidate gene approach, Buckler and colleagues have identified several loci where nucleotide variation can be linked to phenotypic variations in flowering time (Thornsberry et al., 2001) and starch content (Whitt et al., 2002). An important component of association studies is determining the levels of linkage disequilibrium (LD) in a population. Buckler and colleagues found that LD usually decayed after approximately 1,500 bp in several genes across a diverse germplasm collection (Remington et al., 2001). In a study of 12 U.S. inbreds,

Bi Irie Vroh (University of Missouri) also presented evidence that LD decayed rapidly (r2 = 0.4), within 500 bp to 1 kb, across 470 unigenes (EST assemblies) examined. These studies suggest that association studies can potentially be used to fine map the genetic variation underlying many traits to intragenic intervals.

A workshop on genetic diversity was led by Major Goodman (North Carolina State University). He presented a survey of the vast phenotypic diversity captured in maize germplasm and reminded the audience that the pressures now placed on germplasm banks will only increase in the years ahead. This sentiment was reiterated by Stephen Smith (Pioneer Hi-Bred) who warned that the increasing commercialization of maize in Latin America will displace the traditional role of farmers as conservators of germplasm collections, as more and more farmers utilize high yielding hybrids. Thus, there is an increasingly important need for public and private support of germplasm facilities. CIMMYT is the world's largest repository of tropical and semitropical maize germplasm, with more than 23,000 maize landrace accessions. Marilyn Warburton (CIMMYT) described recent programs at CIMMYT to incorporate more of this germplasm into breeding programs and to examine the genotypic variation found across these collections. Denise Costich (Boyce Thompson Institute) tapped into this diversity by utilizing Goodman's diverse germplasm collection to examine variations in light response. Costich and colleagues surveyed more than 100 semitropical, stiff stalk, and nonstiff stalk inbreds and found that North American corn belt stiff stalk and nonstiff stalk accessions were less responsive to light than tropical or semitropical inbreds. These results suggest that artificial selection has acted to attenuate light response pathways as varieties were developed for more temperate regions.

Commercial hybrids of maize are produced by crossing elite inbred lines belonging to different heterotic groups. The resulting F_1 hybrids are high yielding as a result of heterosis. Despite its obvious agronomic importance, the molecular mechanisms underlying heterosis remain obscure. Guo Mei (Pioneer Hi-Bred) and colleagues and Michelle Morgante (Universiti di Milano) exploited the nucleotide variation between inbred lines to examine allele specific expression of several nuclear genes. Importantly, the RT-PCR methods employed enabled them to quantify the levels of transcript encoded from each of the parental alleles in the F_1 hybrid. These studies revealed that 58% to 73% of the genes examined show allelic differences in expression levels which often vary depending on the tissue analyzed. Neither Mei nor Morgante found evidence for imprinting, suggesting it is unlikely that the differences in allele-specific expression patterns result from epigenetic variation. As discussed by Morgante, the allelic variation may be attributable to the high degree of noncoding sequence variation present throughout the maize genome (Fu and Dooner, 2002).

Maize's closest relative, teosinte, was also the subject of several explorations of genetic diversity. To investigate the effects of a domestication bottleneck on genetic diversity within maize, Maud Tenaillon (Station de Genetique Vegetale, Ferme du Moulon) examined nucleotide variation at 12 loci across chromosome 1 of maize's progenitor teosinte (*Zea mays* subsp. *Parviglumis*). She found strong evidence for selection at *teosinte branched1* and *zag1*, the maize homolog of *AGAMOUS*, and equivocal evidence for selection at *tasselseed2* and *dwarf8*. Variation among teosinte alleles was on average 38% higher than that found in maize. To explore the domestication process, several simulations were performed using the sequence data from maize and teosinte. Best-fitting models incorporated a bottleneck with population size to bottleneck duration of 4 to 5. That is, assuming a 1,000-year bottleneck, the effective population size was 4,000 to 5,000 individuals. Jerry Kermicle (University of Wisconsin) addressed the apparent paradox that despite the evolutionary relationship between maize and teosinte, both species are often found growing side by side in fields without cross pollination. Pollination barriers have evolved to prevent weedy teosintes from being cross pollinated by maize. Pollen carrying recessive alleles at *Gametophyte factor1* (*Ga1*) and the recently identified *Teosinte crossing barrier1* (*Tcb1*) are incompatible with pistils carrying dominant alleles at these loci. Weedy teosinte carries dominant alleles at both loci, while Mexican maize contains a recessive *tcb1* allele. This prevents pollination of teosinte by maize and protects weedy teosinte from hybrid extinction.

To exploit the genetic variation present in teosinte subsp. for crop improvement, Carlos Harjes (Cornell University) presented his work on generating advanced backcross (AB) populations between maize and *Zea diploperennis*. As expected many of the teosinte alleles had negative impacts on yield, but several quantitative trait loci (QTLs) were identified that had positive effects on grain quality. Although similar AB strategies (wild species × elite cultivar) have been successfully applied to increase yield in tomato and rice, gene flow from wild ancestors to maize is likely to have provided many opportunities for maize to acquire teosinte alleles that contribute to yield increases. Indeed, in a session held at the Museo National de Antropologia, Bruce Benz (Texas Wesleyan University) presented evidence from archaeological finds that some of the earliest selections over 6,000 years ago were for improved harvesting characteristics (e.g. no disarticulation and increasing rowing). Yield was clearly a primary target for early domestication just as it is today, and has transformed maize into the highest yield grain crop on the planet.

However, yield was not the only target for selection. Maize has been central to many native cultures throughout the Americas, which led to the selection of numerous alleles at loci involved in the accumulation of anthocyanin pigments. Genetic analysis of these diverse alleles has led to the discovery of several

epigenetic phenomena, including imprinting, paramutation, and transposon-induced gene silencing. Chris Della Vedova (University of Missouri) reported that the semidominant *inhibitor diffuse* allele of chalcone synthase, *C2-idf*, suppresses the normal *C2* gene through yet another epigenetic mechanism. He showed that *C2-idf* posttranscriptionally silences *C2* via an RNA interference or RNAi-like mechanism. As is characteristic for RNAi induced gene silencing, *C2-idf* mediated suppression was found to be correlated with the presence of small interfering RNAs (siRNAs) derived from *C2* as well as *C2-idf* itself. RNAi is usually triggered by the presence of double stranded or aberrant transcripts, and consistent with this, the *C2-idf* locus was found to contain partially duplicated copies of the *C2* gene. Many plant RNA viruses encode proteins that interfere with the RNAi machinery. Infection of *C2/C2-idf* plants with maize dwarf mosaic virus or with maize necrotic streak virus, which encode such inhibitors of RNAi, resulted in increased *C2* transcript levels and more intense anthocyanin pigmentation.

In addition to RNAi, translational controls mediated through short microRNAs may also play an important role in plant development. In *Caenorhabditus elegans*, short 21 nt RNAs can mediate translational repression of genes through complementary annealing across the 3′UTR. Importantly, translational repression is mediated through imprecise annealing whereas full complementarity triggers the RNA degradation (RNAi) pathway. To examine the role of miRNA is plant development, Jean Philippe Vielle-Calzada (CINVESTAV-IPN) used a bioinformatics approach to search the Arabidopsis genome for potential miRNA targets. Jean Philippe and colleagues queried the Arabidopsis genome for sequences that matched several criteria for known miRNAs from *C. elegans* and identified 10 potential miRNA-regulated genes. Detailed characterizations of two, AtmiR-2 and AtmiR-9, indicate that both play a role in regulating the splicing machinery of the plant. These candidate miRNAs are conserved across animal and plant genomes, suggesting that the miRNA pathway may also be conserved between plants and animals.

DISSECTING THE MAIZE LIFE CYCLE

The maize seed has clear agronomic value and, not surprisingly, has been the subject of detailed developmental studies for decades. However, the genes controlling the differentiation process have remained elusive. By exploiting genomics/transcriptomics resources, Thomas Dresselhaus (University of Hamburg) and colleagues reported the characterization of three genes encoding small, secreted peptides important for gametophyte development. The female gametophyte develops after three rounds of mitosis from the functional megaspore and comprises four specialized cell types: synergids, antipodal cells, central cell, and the egg cell. The coordinated development of the female gametophyte likely depends on signaling between these different cell types. The three genes were identified in a differential screen of cDNA libraries prepared from microdissected embryo sacs and zygotic tissues. Two of the peptides, including a defensin-like molecule, are specifically expressed in the unfertilized embryo sac. Mutational analyses suggest they play a role in cell-cell communication during development of the egg apparatus and pollen tube guidance. The third, a CWAK-box peptide, is more widely expressed including in the male gametophyte. Antisense suppression caused pollen abortion, sterility, and dwarfism, while overexpression in Arabidopsis suppressed the differentiation of several structures, including cotyledons, root hairs, and chloroplasts. These results suggest this peptide may suppress differentiation of the developing gametophytes.

Jose Gutierrez-Marcos and colleagues (University of Oxford) also used a differential screening approach to identify genes that show a parent-of-origin pattern of expression during seed development. Most imprinted genes identified (>30) are expressed from the maternal allele only, but a single gene with a paternal-specific expression pattern was also identified. Detailed analysis of one of the maternally expressed genes, *meg1*, showed that it is imprinted only during the first 10 d of endosperm development. Expression of *meg1* depends on *ZmMRP1*, a Myb transcription factor, which induces transcription specifically in the basal endosperm transfer layer. This tissue is specialized for the uptake of nutrients from maternal tissue into the endosperm. Localization of MEG1 to the basal endosperm transfer layer thus suggests that this process may initially be under maternal control.

Two groups reported on the regulation of meristem function and leaf initiation by plant hormones. Recent studies have shown that members of the *knotted1 homeobox* (*knox*) gene family in dicots inhibit transcription of gibberellic acid (GA) biosynthesis genes (Sakamoto et al., 2001; Hay et al., 2002). In fact the repression of GA production was found to be essential for meristem maintenance. Michael Muszynski (Pioneer Hi-Bred International) presented data indicating that in maize, GA biosynthesis is controlled independent of *knotted1*. However, he showed that the role of *knox* genes in regulating cytokinin production is conserved between dicots and maize. Cytokinin induces cell division and functions in leaf initiation and phyllotaxy. The *abphyl1* mutation in maize forms a larger than normal meristem early during embryogenesis. As a result, *abphyl1* mutants establish a decussate/opposite phyllotaxy rather than the normal distichous/alternating phyllotactic pattern. David Jackson (Cold Spring Harbor Laboratory) reported that *abphyl1* encodes a cytokinin inducible response regulator homolog. These are transcription factors that are activated in response to cytokinin but, because one of the targets is a repressor of the response regulator, actually block cytokinin signaling at high cytokinin levels. *abphyl1* is

expressed in a wedge of cells above and partially overlapping with the incipient leaf primordium. This suggests that in response to high cytokinin levels near the incipient primordium, ABPHYL1 may locally reduce cell division thus delineating zones with distinct growth rates associated with the separation of the new leaf from the remainder of the meristem. Increased cytokinin signaling in the *abphyl1* mutant is thought to lead to more cell divisions in the meristem. The resulting enlargement of the meristem may permit the initiation of two rather than one leaf per node.

Classical surgical experiments have shown that the meristem produces a signal that is required for adaxial/abaxial patterning of the leaf (Sussex, 1951). Michelle Juarez (Cold Spring Harbor Laboratory) reported that *rolled leaf1* (*rld1*) encodes a class III homeodomain Leu zipper (HD-ZIPIII) protein that is required for the specification of adaxial/upper cell fate. Expression of *rld1* in the leaf is induced by *leafbladeless1*, and becomes limited to the adaxial side due to miRNA166-mediated cleavage of *rld1* transcripts on the abaxial side. She showed that miRNA166 initially accumulates immediately below the incipient leaf but subsequently in a progressively expanding pattern via the abaxial side throughout the developing primordium. RLD1 and other HD-ZIPIII family members contain a START lipid-sterol binding domain and are thought to specify adaxial identity in response to the meristem-derived signal. The proposed model suggests that RLD1 specifies adaxial/abaxial polarity by incorporating positional information established by two opposing signals that originate outside the incipient primordium: the adaxializing signal from the apex of the meristem and the miRNA166 signal from below the incipient leaf.

The formation and activity of axillary meristems is a major determinant of plant architecture and was an important selected trait during the domestication of modern maize. Currently, lateral meristem activity is still an important developmental trait because of its potential to improve crop yield. The *barren stalk* (*ba*) mutants in maize are defective in lateral meristem formation and therefore lack ears and develop tassels devoid of branches and spikelets. Andrea Gallavotti (University of California, San Diego) and colleagues showed that *ba1* encodes a bHLH domain protein orthologous to the *lax1* gene from rice. The expression pattern of *ba1* on the adaxial side of lateral meristems and at the boundary between spikelet and floral meristems suggests a role for BA1 in meristem formation in response to a signal from the main shoot apical meristem. This signal could be polar flow of auxin, as the *ba1* tassel resembles the *pinoid* mutant inflorescence in Arabidopsis (Christensen et al., 2000). Consistent with the possibility that selection at *ba1* contributed to the domestication of maize, only two haplotypes passed through from teosinte to maize. Moreover, *ba1* maps to one of the five major QTLs controlling most of the morphological differences between maize and teosinte.

Elizabeth Kellogg (University of Missouri) provided further evolutionary perspective on grass flower development in her analysis of *Sepallata*-like genes. The *Leafy hull sterile* (*Lhs1*) mutant in rice is semidominant and affects flower development due to overexpression of a MADS box gene related to the *SEPALLATA* genes of Arabidopsis (Pelaz et al., 2000). *LHS* is expressed in the spikelet meristem of all grasses examined but later expression patterns varied. In species, including maize and rice, with a basipetal pattern of flower development and maturation within a spikelet, *LHS* was expressed only in the terminal flower of the spikelet. In species such as oats, with an acropetal pattern of flower development, expression was observed in all flowers of the spikelet. It was hypothesized that the evolution of *LHS* function contributed to the morphological diversity of grass flowers.

To examine the developmental mechanism regulating maize anther development, David Skibbe (Iowa State University) utilized microarrays to probe gene expression differences in anthers dissected from upper and lower florets. Skibbe compared RNA expression profiles using an array containing more than 12,000 cDNA clones. By scanning the slides at multiple laser power settings, Skibbe was able to increase his resolution by 30% to 40% and found that nearly 10% of the genes expressed in anthers were differentially expressed between upper and lower florets. These studies strongly suggest that the upper and lower floret anthers are physiologically distinct.

MAIZE CYTOGENETICS: STUDYING CHROMOSOME BEHAVIOR

Maize has been a longstanding model for meiotic studies, and continues to show its outstanding characteristics as a model for plant cytology. Olivier Hamant (University of California, Berkeley) reported progress in understanding sister chromatid cohesion, a critical step in nuclear division. In the meiotic mutant *absence of first division1* (*afd1*), chromosome pairing and synapsis are disrupted resulting in 20 univalents at metaphase I, instead of the normal 10 bivalents. The gene encodes a REC8 homolog that localizes to the synaptonemal complex. AFD1 is required for the establishment of Rad51 foci, involved in recombination. In other systems, proteins protect REC8 to maintain centromeric cohesion (Rabitsch et al., 2004). A reverse genetic screen for mutants in such proteins identified a Mutator insertion, *mtm99*, which displayed precocious loss of centromeric cohesion in prophase II of meiosis, suggesting a conserved function between maize, fungi, and Drosophila.

A technical limitation to maize cytogenetics has been the inability to identify chromosomes during mitosis. Jim Birchler (University of Missouri) presented the results of his group's efforts to develop a chromosome painting procedure that allows the identification of all ten chromosomes in mitotic meta-

phase. A fluorescent in situ hybridization (FISH) cocktail was composed of probes to various repeat elements including telomeres, subtelomeres, 5S RNA, centromeres, and knobsequences. Each probe was labeled with one of four different colored fluorescent dyes. The cocktail uniquely identified each chromosome by their hybridization pattern in either mitosis or meiosis. Interestingly, there was variation in the number and arrangements of the various repeats in different inbred lines. This user-friendly technique should provide a rapid means to survey genome structure in maize germplasm and perhaps related grasses such as teosinte and tripsacum.

BIOCHEMICAL GENETICS

Several groups presented work on understanding the molecular genetic basis of physiological responses to stress. Phosphorous is the most limiting nutrient in the alkaline soils that predominate in many developing countries, because phosphate is rapidly bound by soil particles and made inaccessible to plants. A plenary speaker from Mexico, Luis Herrerra-Estrella (Centro de Investigacion y Estudios Avanzados del IPN), presented his lab's work on dissecting phosphorous stress responses in Arabidopsis. Arabidopsis, like other nonmycorrhizal plants, adapts to low Pi availability by modifying root architecture to maximize Pi uptake efficiency. Cell division in the root apical meristems permanently arrests, and lateral roots initiate, resulting in a short, highly branched root system that allows the plants to explore the upper soil strata, which tend to be more phosphorous rich. In addition, the arrested meristems express high levels of acid phosphatase, and all epidermal cells differentiate as trichoblasts, allowing them to function as specialized Pi absorption structures. Genetic screens identified the *lpi* (*low phosphate insensitive*) mutant that does not show the typical root architecture response to low phosphate levels, but has a normal root hair response. This mutant was defective in external Pi sensing but showed normal systemic responses to internal Pi levels.

Patricia Leon (Universidad Autónoma de Mexico) gave a plenary talk on Glc signaling in plants. Arabidopsis seedlings usually arrest development on 7% Glc but *Glc-insensitive* (*gin*) mutants do not show this inhibition. Surprisingly, the molecular characterization of *gin* genes revealed that they were known components of ABA synthesis or signaling pathways, including *aba3*, and *abi4*. Other known *aba* mutants were tested and also found to be Glc insensitive. ABA application to Glc-treated ABA synthetic mutants restored the developmental arrest. Of the *abi* mutants, only *abi4* and *abi5* proved to be Glc insensitive. Glc caused an ABA-dependent induction of *ABI4::GUS* expression, as well as the expression of other *ABA* biosynthetic genes, suggesting a positive feedback loop. It will be interesting to discover the physiological basis of how Glc sensing relates to ABA signaling.

Rab17, a LEA protein that accumulates in response to ABA and osmotic stress, is the most highly phosphorylated protein in the embryo. Montserrat Pages (Departament de Genetica Molecular, IBMB) reported that casein kinase 2 (CK2) is required for Rab17 phosphorylation and that phosphorylation regulates the subcellular localization of Rab17. Wild type Rab17 is localized in the cytoplasm and nucleus, but is not found in the nucleolus. However, a mutant, nonphosphorylatable form of Rab17 was located in the nucleolus. Furthermore, when expressed in Arabidopsis, wild type Rab17 mediated seedling germination arrest under NaCl stress conditions whereas the nonphosphorylatable form did not arrest. Thus, stress regulation of Rab17 function appears to be mediated through phosphorylation by CK2.

Jorge Nieto-Sotelo (Institute of Biotechnology, UNAM) presented an analysis of the Hsp100 proteins required for induced thermotolerance in maize. The protein contains two ATP-binding domains separated by a middle region predicted to form a coiled-coil. Wild type yeast Hsp104 formed hexamers in the presence of ATP but mutations in the coiled-coil caused the protein to remain monomeric. Therefore, this middle region is required for multimerization of Hsp100 proteins.

Cereal grains are limited in the content of several essential amino acids, including Lys and Thr. The manipulation of amino acid content has been a major target for crop improvement and Chun-Hsiang Chang (Pioneer Hi-Bred) reported on efforts to improve Lys content through metabolic engineering. Lys and Thr biosynthesis are feedback inhibited by Lys inhibition of the Asp kinase and dihydrodipicolinate synthase (DHDPS) enzymes. A bacterial *dapA* gene codes for a Lys insensitive DHDPS and introduction of this gene into maize increased Lys content in the grain. Site-directed mutagenesis was performed to generate a Lys-insensitive Asp kinase that shunted flux to the Thr pathway, increasing Thr content in the seed. Combining these two Lys feedback insensitive enzymes resulted in a significant increase in both amino acids in the grain.

MAIZE PLASTID BIOLOGY

Plastid function is central to virtually all aspects of plant biology and work ranging from chloroplast biogenesis to pigment degradation was featured. Nigel Walker (University of Oregon) presented an update on the activities of a collaborative functional genomics project targeting chloroplast biogenesis. Researchers at the University of Oregon and Boyce Thompson Institute have generated a collection of approximately 2,200 mutants defective in photosynthetic pigment accumulation. Mutants were phenotypically characterized, including visible traits as well as the analysis of various chloroplast protein and RNA accumulation. The data, in addition to stock ordering

information and protocols for performing forward or reverse genetics for mutant gene identification are accessible at http://chloroplast.uoregon.edu/. The Web site also has photosynthesis and genetics tutorials for students in grades 7 to 12.

Carotenoids are necessary for photosynthesis, are precursors to abscisic acid, and pigment several nonphotosynthetic tissues such as flowers and maize kernels. Dietary β-carotene is also a precursor for vitamin A. The dominant *White cap* (*Wc*) mutant confers a dose-dependent carotenoid deficiency in maize kernels. Molecular analysis by Bao-Cai Tan (University of Florida) showed that the *wc* gene encodes a carotenoid cleavage dioxygenase (CCD). Expression in bacteria that were engineered to produce β-carotene showed that WC catalyzes β-carotene cleavage to produce β-ionone. In the endosperm of yellow kernels, expression of *wc* is low, while in homozygous *Wc* mutant endosperm, expression is dramatically increased. Thus, Wc appears to catalyze the degradation of carotene. Interestingly, the rice ortholog of *Wc* (*OsCCD1*) is expressed in rice endosperm and thus could limit carotenoid accumulation in transgenic Golden Rice. Engineering reduced *Wc* activity in rice kernels might, therefore, increase β-carotene content and the nutritional value of Golden Rice.

David Stern (Boyce Thompson Institute) presented results of his studies on transcriptional regulation in plastids. Plastids contain two RNA polymerases; the plastid-encoded RNA polymerase (PEP) resembles bacterial polymerases, while the nuclear encoded polymerase (NEP) resembles bacteriophage T7 polymerase and is dual targeted to plastids and mitochondria. Bacterial RNA polymerases gain specificity through association with sigma factors and the maize nuclear genome encodes six sigma factor-like proteins. Five of these are targeted to the plastid but one, Sig2B, is dual targeted to plastids and mitochondria. This was surprising because mitochondria are only known to contain the T7-like NEP, thought not to utilize sigma factors. Within a given cell, Sig2B is targeted to either plastids or mitochondria, not both. This appears to occur through developmentally regulated differential promoter selection leading to alternate translation start sites. Sig2B is only able to confer DNA sequence specificity to the PEP in vitro and a *Mu* transposon mutant has reduced levels of PEP, but not NEP, regulated transcripts. The function within mitochondria remains enigmatic.

Manli Yang (University of Toledo) showed that the *lethal leaf spot1* (*lls1*) encodes pheophorbide A oxygenase, an enzyme conserved between maize and Arabidopsis and required for the autumnal degradation of chlorophyll. The protein is localized to the inner membrane of the chloroplast. The light-dependent cell death phenotype also requires the presence of chlorophyll as indicated by suppression of the *lls1* phenotype by the chlorophyll deficient mutant *Oil yellow* or in albino stripes of *iojap*. Thus, it is hypothesized that LLS1 is required for the elimination of chlorophyll degradation products that are photoreactive and highly toxic.

TRANSPOSONS AND TRANSPOSITION

Although a plethora of maize transposons have been staples of past maize meetings, this year Nancy Craig (Johns Hopkins University School of Medicine) provided the maize community with a fascinating story of nonplant hAT element transposition. The hAT superfamily of transposons was defined by founding members *hobo* (Drosophila), *Ac* (maize), and *Tam3* (*Antirrhinum majus*) that encode transposases with similar structural characteristics, have similar terminal inverted repeats (TIR) and create 8-bp target site duplications upon integration into the host genome. Craig and colleagues established an in vitro system to study excision and integration of the hAT family member *hermes* transposon and has shown that *hermes* excision occurs using a cut-and-paste mechanism that creates DNA hairpins flanking donor site DNA. In her studies of the bacterial transposon Tn7, Craig showed that the Tn7-encoded protein TnsC plays a central role in regulating the transposition process. This ATP-dependent regulator serves as a checkpoint to ensure that DNA breakage only occurs when the Tn7 target site is engaged by the interacting protein TnsD.

In addition to transposon-encoded proteins, host proteins also play an important role in the transposition process. Cagla Altun (Purdue University), showed that *mre11* null mutants of Arabidopsis are stunted and sensitive to DNA damage. The MRE11 ortholog in yeast forms a complex with Rad50 and is necessary for double strand break repair. Using a PCR assay to monitor *Ac* excision, Altun was unable to detect any products in Arabidopsis *mre11* mutants. These results suggest that MRE11 is necessary for either *Ac* transposition or double strand break repair associated with *Ac* excision events.

Akemi Ono (Stanford University) explored the mechanisms of host-mediated control of *Mutator* transposition through DNA methylation. Using transgenic plants expressing a *mudrB* protein driven by the 35S promoter, Ono observed silencing of the transgene that paralleled silencing endogenous *Mu* elements. However, 35S:mudrB lines were reactivated in the subsequent generation whereas endogenous elements remained inactive. Conversely, fusions of the terminal inverted repeats of *Mutator* to a LUC cassette failed to result in LUC silencing in multicopy MuDR lines. Together, these results indicate that coding regions of the *Mu* transposon are necessary and sufficient to induce gene inactivation and that sequences in the TIR are necessary to maintain the repression in subsequent generations.

The maize genome is large, the generation time long and no variety has yet been developed that grows well in petri dishes. Nevertheless, the rapid advances in maize genomics, including genome/cDNA se-

quence, transposon collections, and powerful statistical methods to analyze gene associations and genome structure, are providing geneticists, developmental biologists, and plant breeders with exciting new opportunities to pursue basic and applied studies. In particular, the availability of complete gene sequence from multiple grass genomes including maize will ultimately provide the genetic blueprints that molecular biologists and plant breeders will use to develop crops that are better adapted to marginal soils and environments, are endowed with improved nutritional qualities, and that display enhanced disease and pest resistance. Indeed, the possibilities are as exciting for maize genetics today as they were for the first plant breeders in Mexico nearly 6,000 years ago.

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