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Cytokinesis: The Art of Partitioning

In higher plants, cytokinesis partitions the cytoplasm of a dividing cell by forming a new cell wall between the two sets of daughter chromosomes. Although conceptually simple, this process involves a sequence of well-orchestrated events, starting with the determination of the division plane before the onset of mitosis (reviewed in Staehelin and Hepler, 1996; Heese et al., 1998; Smith, 1999). A transient preprophase band of microtubules and actin microfilaments marks a cortical division site which is in the same plane as the dividing nucleus. Following disintegration of the nuclear envelope and formation of the mitotic spindle, the chromosomes condense, align, and separate before the spindle moves the daughter chromosomes away from the division plane. Remnants of the spindle are incorporated into the phragmoplast, which appears in the interzone in late anaphase. The phragmoplast consists of two opposing sets of microtubules and actin microfilaments with their plus ends toward the plane of division. The phragmoplast mediates the translocation of Golgi-derived vesicles to the division plane where the vesicles fuse with one another to form the cell plate, a transient membrane compartment. The disc-shaped cell plate expands from the center of the division plane to the periphery and eventually fuses with the lateral cell wall at the cortical division site. During cell plate expansion, the phragmoplast microtubules depolymerize in the center and repolymerize along the edge such that additional Golgi-derived vesicles are targeted to the margin of the cell plate. While expanding, the cell plate undergoes a complex transformation from an initial network of thin fusion tubes to a solid plate, with polysaccharides secreted into its lumen (Samuels et al., 1995). In summary, somatic cytokinesis is a phragmoplast-assisted process of cell plate formation and expansion driven by targeted vesicle fusion.

The dynamics of the cell division process are not well understood, although a number of proteins have been localized either to the cytoskeletal arrays involved in cell division or to the forming cell plate. For example, the large GTPase phragmoplastin (also called Arabidopsis dynamin-like protein) accumulates in the cell plate (Gu and Verma, 1997; Lauber et al., 1997). However, it is not known whether this protein plays a role in the formation of thin fusion tubes from vesicles and/or is needed in the removal of excess membrane from the maturing cell plate. Another example is a MAP kinase that is activated in mitosis and localized to the cell plate (Bögre et al., 1999). Although the localization data suggest a role in cell division, the functional analysis of proteins requires some sort of bioassay. **On pages 979–990 of this issue of THE PLANT CELL**, Vos et al. report on use of microinjection into living stamen hair cells of Tradescantia to determine the role of the kinesin-like calmodulin binding protein (KCBP) in the process of cell division. KCBP is a minus-end directed microtubule motor protein (Song et al., 1997) that localizes to the cytoskeletal arrays associated with cell division, such as preprophase band, mitotic spindle and phragmoplast, but does not colocalize with cortical microtubules during interphase (Bowser and Reddy, 1997; Smirnova et al., 1998). KCBP interacts with microtubules in vitro, and this interaction is inhibited by calmodulin (CaM). The inhibition is abolished by an antibody raised against a peptide derived from the calmodulin binding domain of KCBP, suggesting that the antibody may keep KCBP in a constitutively active form (Narasimhulu and Reddy, 1998). To test this idea, the authors microinjected the anti-KCBP antibody into stamen hair cells at different stages of the cell cycle.

The authors report that injection into interphase cells does not block cytoplasmic streaming—in contrast to an anti-CaM antibody—thus ruling out nonspecific interference with physiologic processes. Injection into mitotic cells has differential effects. Late prophase cells are induced to break down the nuclear envelope precociously. Cells at prometaphase do not progress to anaphase. Their chromosomes condense as if arrested at metaphase, although the chromosomes do not subsequently stay aligned. By contrast, cells injected at late metaphase or early anaphase complete anaphase, and a considerable proportion displays telophase arrest without forming a phragmoplast or a cell plate. To test whether the microtubule cytoskeleton had been destroyed, the authors injected rhodamine-labeled tubulin prior to injection with the anti-KCBP antibody. Although the nuclear envelope break down precociously and the cells are subsequently arrested at prometaphase, rhodamine-labeled microtubules are still present. These results suggest that KCBP is involved in microtubule organization during M phase. The authors present a model in which they propose a role for KCBP in microtubule bundling and spindle assembly. Because KCBP shares sequence similarity with *Xenopus* XCTK2 and Drosophila Ncd, two proteins associated with the spindle poles and involved in the formation of convergent bipolar spindles, the authors propose that KCBP plays a similar role. Another interesting feature discussed by the authors relates to the

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role of calcium in cell division. Specifically, CaM inhibits the interaction of KCBP with microtubules in vitro only in the presence of calcium. To explain the stage-specific effects of the anti-KCBP antibody, one could postulate that calcium is released from internal stores, such as from the ER, differentially and locally during M phase. In any event, the present report suggests a mechanistically plausible model of how calcium concentration could be involved in the reorganization of the microtubule cytoskeleton during cell division.

Although cytokinesis is usually tightly coupled to nuclear division, this is not always the case. One obvious exception occurs in the endosperm, which originates from the triploid fusion product of a sperm cell with the large central cell of the embryo sac. Initially, several rounds of synchronous nuclear divisions proceed without cytokinesis (Mansfield and Briarty, 1990; Olsen et al., 1995; Berger, 1999; Brown et al., 1999). The syncytial endosperm is then cellularized by the formation of anticlinal (radial) cell walls between the nuclei which have migrated toward the cell surface (Mansfield and Briarty, 1990; Olsen et al., 1995; Brown et al., 1999). How the cytoplasm of the large cell is partitioned to the nonmitotic nuclei has been controversial. According to the prevailing view, membrane furrows grow inward from the surface and separate neighboring nuclei. Such a process would be formally similar to cellularization of the Drosophila syncytial blastoderm embryo, during which membrane material is delivered to the base of inwardly growing furrows in a syntaxin-mediated manner (Burgess et al., 1997). In the Arabidopsis endosperm, microtubules and vesicles have been observed at the tips of inwardly growing membrane furrows (Mansfield and Briarty, 1990), suggesting that the anticlinal cell walls are formed by tip growth. Similar observations have been reported for other species (reviewed in Olsen et al., 1995); however, there is also evidence supporting the alternative view that anticlinal cell wall formation resembles cytokinesis of somatic cells. In the cellularizing wheat endosperm, membrane vesicles accumulate between adjacent nuclei and form a cell plate which then expands toward the surface of the central cell while its opposing "free" end grows toward the vacuole (Fineran et al., 1982). Compatible with either view is the observation that the newly forming cell membranes of the cellularizing Arabidopsis endosperm accumulate the cytokinesis-specific KNOLLE syntaxin (Lauber et al., 1997). This result also suggests that endosperm cellularization is mechanistically related to phragmoplast-assisted cytokinesis of somatic cells.

On pages 933–947 of this issue of THE PLANT CELL, Otegui and Staehelin take a fresh look at cell wall formation in the syncytial endosperm of Arabidopsis. The authors take advantage of the high-pressure freezing/freeze substitution technique, which better preserves membrane structure than does chemical fixation (Samuels et al., 1995). In addition, they focus on the micropylar zone of endosperm surrounding the developing embryo, where the single layer of nuclei is in a two-dimensional array parallel to the surface of the large central cell. In this region, cellularization results in a honeycomb-like organization of new anticlinal cell walls. At the time of cellularization, no mitotic spindles are present, but microtubules radiate from the surface of the nonmitotic nuclei. The process of cellularization is resolved in exquisite detail. Small groups of oppositely oriented microtubules, called mini-phragmoplasts, are assembled and assist in the formation of a special type of cell plate (syncytial-type) between sister and nonsister nuclei. Unlike the directional expansion and maturation of the cell plate in somatic cytokinesis, a patchwork of local cell plates is formed simultaneously by multiple mini-phragmoplasts. Initially, Golgi-derived vesicles fuse to form hourglass-shaped intermediates, which give rise to 45-nm wide tubules, unlike the narrow fusion tubes observed in somatic cells. The wide tubules coalesce into networks, and adjacent wide tubular networks merge into a coherent cell plate network that undergoes maturation in a patchy pattern. The authors also analyzed the composition of syncytial-type cell plates and endosperm cell walls using specific antibodies. Two distinctive features of this analysis are the lack of fucosyl residues on xyloglucans and the persistence of callose in the cell walls after the cell plate has fused with the parental plasma membrane, which may be related to the role of the endosperm in seed development.

So what is the take-home message? In a simplified view, partitioning of the cytoplasm appears to be comparable in endosperm cellularization and somatic cytokinesis, although the details differ. In both processes, Golgi-derived vesicles are translocated to the plane of partitioning by phragmoplast microtubules, and vesicle fusion, mediated by the cytokinesis-specific KNOLLE syntaxin, results in cell plate formation. In this way, endosperm cellularization can be viewed as a variant of somatic cytokinesis. It may thus be worthwhile to reexamine, with the high-pressure freezing/freeze substitution technique, other modes of cytokinesis that have been described for meiotic and gametophytic cells, to elucidate their similarities and dissimilarities to somatic cytokinesis.

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Maize Genetics 2000—And Beyond

Representing the interests of maize genetics, grass genomics, and plant biology, over 400 scientists attended the 42nd annual maize genetics conference this year in Coeur d'Alene, Idaho (March 16–19, 2000). During the meeting, data from all areas of maize biological research, and discussions of innovative research tools, were presented in a forum that included plenary and satellite talks, workshops, panel discussions, and extended poster sessions. Maize has a long and rich history as a model organism for genetic research. Recently, new avenues of research have been fostered by the synthesis and coordination of genomic research on maize, rice, sorghum, and other closely related grasses. Exciting advances in grass genomics were showcased at the conference, and numerous reports attested to the creative use of maize transposons as a cornerstone in analyses of new and interesting genes and gene functions. In this report, we present some highlights from the annual meeting of the minds in maize.

CYTOGENETICS

The large chromosomes of maize have long been a favorite subject of cytological investigation. Peter Carlton (University of California, Berkeley) described the use of fluorescently labeled centromeric and telomeric sequences to visualize meiotic chromosome pairing in three dimensions. Chromosome pairing is a central tenet in eukaryotic genetics and is known to be associated with stage-specific clustering of telomeres (i.e., the bouquet formation) and centromere–telomere polarization. Carlton employed telocentric maize chromosomes, created through centric fission of chromosome 3, to demonstrate that telomere clustering determines centromere–telomere polarization. These results indicate that the telomere bouquet at zygotene plays a major role in chromosome pairing.

Graham Moore (John Innes Centre, Norwich, UK) presented progress toward cloning *Ph1*, a gene involved in pairing and recombination between

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homeologous chromosomes in hexaploid wheat. The *ph1* mutation proves critical for breeding purposes, because it allows wheat chromosomes to recombine with homeologs from related species (Dubcovsky et al., 1995; Foote et al., 1997). Moore and coworkers generated a series of overlapping deletions of the *Ph1* locus in wheat, and identified a syntenous region in rice to within 200 kb. This 200-kb interval was sequenced to identify four candidate genes, one of which belongs to a class of genes also involved in plant development. These exciting results are a testament to the increasing use of comparative genomics and genome synteny in plant biological research.

In a cytogenetic analysis of selfish DNA, Evelyn Hiatt (University of Georgia, Athens) compared the maize meiotic drive system *abnormal 10-1* (*ab10-1*) and *abnormal 10-2* (*ab10-2*), which is derived from the wild maize progenitor teosinte (Dawe and Cande, 1996; Buckler et al., 1999). Both meiotic drive systems are associated with meiotic neocentromere activity, in which heterochromatic knobbed regions of chromosomes migrate prematurely to the spindle pole. Hiatt demonstrated that in *ab10-1,* neocentromere activity involves the tandemly repeated (TR-1) knob-specific sequence. However, in *ab10*-2 meiotic drive, neocentromeres are associated with a different knob sequence, the 180-bp repeat. These results may reveal a potential mechanism for the amplification of different chromosomal knob-related repeated sequences during genome evolution.

REGULATION OF CELL DIVISION

The regulated coordination of cell division is a fundamental component of plant development. Two mutations that alter planes of cell division were described at the meeting. Laurie Smith (University of California, San Diego) described the cloning and characterization of the *tangled1* (*tan1*) gene. In the *tan1* mutation, normal longitudinal divisions are replaced by oblique divisions (Smith et al., 1996). This defect is primarily due to the failure of cell plate guidance to the preprophase band location (Cleary and Smith, 1998). Smith showed that the *tan1* gene encodes a protein related to the basic region of the human *apc*-encoded (for adenomatous polyposis coli) protein. This region of this protein has been shown to mediate interactions with microtubules, suggesting that TAN1 might interact with microtubules. In support of this model, Smith reported that monoclonal antibodies raised against the TAN protein interact with all microtubular structures in dividing cells.

In the *extra cell layer1* (*xcl1*) mutation, normal protodermal anticlinal divisions are replaced by periclinal divisions. Sharon Kessler (University of California, Davis) reported that the extra cell layers produced by abnormal periclinal divisions differentiate according to their lineage and not to their position. Kessler showed that the shoot apical meristems in *xcl1* individuals are shorter than wild-type siblings, and may form two tunica layers instead of one. These data indicate that the defective cell division phenotype of the *xcl1* mutant may be manifested quite early.

REGULATION OF GENE EXPPRESSION IN THE SHOOT APICAL MERISTEM

Plant meristems are the sites of an intricate and dynamic equilibrium during organogenesis, which is characterized by the loss of cells and cell replenishment from central tissues. The shoot apical meristem (SAM) consists of a peripheral (organogenic) zone and a central (cell-replenishing) zone. Coordination of the number of cells that enter and exit the peripheral zone is important for regulation of meristem size. Elliot Meyerowitz (California Institute of Technology, Pasadena) gave an overview of what is known about the *CLAVATA* (*CLV*) signaling pathway. Mutations in any of the three *CLV* loci of Arabidopsis result in larger shoot and floral meristems. CLV3 is a small putatively secreted protein that may act as the ligand to CLV1, a leucine-rich repeat (LRR) receptor serine kinase. The central zone and rib zone, marked by *CLV1* and *CLV3* expression, respectively, are much larger in both *clv1* and *clv3* mutants. These findings suggest not only a feedback mechanism, with the *CLV* signaling pathway acting from *CLV3*-expressing central zone cells to limit cell proliferation of the rib zone cells, but also an unknown signaling mechanism from the rib zone to promote central zone cell proliferation (Meyerowitz, 1997; Fletcher et al., 1999). Consistent with this interpretation, ectopic expression of *CLV3* effectively shuts down meristem proliferation after the first few vegetative leaves are formed. Meyerowitz estimated that 75 to 100 LRR receptor kinases exist in the Arabidopsis genome, which exceeds the number of identifiable cell types in the plant. He went on to suggest that if each cell can be identified by a unique set of receptors and ligands, then by identifying these products we may be able to piece together the networks of interactions that account for plant growth, development, and environmental responses.

At least six loci that function to regulate meristem size in maize are identified by the *fasciated ear* (*fae*) mutations. David Jackson (Cold Spring Harbor Laboratory, NY) discussed these mutations that alter meristem size in maize. Jackson cloned the *fae2* allele and showed that the gene encodes an LRR protein that might act as a receptor involved in meristem organization. The gene maps to the vicinity of a quantitative trait locus (QTL) for kernel row number in maize ears. These data are

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especially intriguing, because the maize ear is derived from the differentiated lateral meristem of maize.

The importance of regulated *knotted1*-like homeobox (*knox*) gene expression during development of plant lateral organs was first demonstrated in maize (Smith et al., 1992; Jackson et al., 1994). Jane Langdale (University of Oxford, UK) reviewed recent progress toward understanding the role of ROUGH SHEATH2 in the control of *knox* expression in maize. The recessive *rough sheath2 (rs2*) mutation causes a rough leaf sheath and a displaced ligule phenotype, mimicking the phenotype of dominant mutations in several *knox* genes, including *Knotted1*, *Rough sheath1* (*rs1*), and *Liguleless3* (*lg3*). Indeed, *rs2*, which encodes a Myb-like putative transcription factor homologous to the Antirhinnum gene *PHANTASTICA* (*PHAN*), seems to be necessary for excluding homeobox gene expression from the leaf (Timmermans et al., 1999; Tsiantis et al., 1999a). A reduction in apical dominance is seen in *rs2* mutants, which may indicate a defect in auxin transport. Interestingly, maize plants grown on auxin transport inhibitors exhibit many of the phenotypes of *rs2* mutant plants, although ectopic *knox* gene expression is not detected in these plants (Tsiantis et al., 1999b). A model is thus suggested in which RS2 acts upstream to repress *knox* gene expression, which itself acts to affect cytokinin:auxin ratios in the plant. Thus, an intricate interaction between *knox* genes and growth hormones might regulate shoot meristem and lateral organ identities.

Interactions among the multitude of *knox* genes were explored by Mark Lubkowitz (University of California, Berkeley). Lubkowitz reported that the ectopic expression of any *knox* gene is accompanied by ectopic expression of the *lg3 knox* gene, suggesting that LG3 functions downstream of other KNOX gene products. Lubkowitz also used the reverse yeast one-hybrid screen to look for downstream targets of LG3. He identified a 13-nucleotide binding site seen in introns of *lg3*, *lg4,* and *lg4a* genes, suggesting an autoregulatory function for these genes.

Whereas the *phan* mutation leads to loss of adaxial-abaxial (dorsal-ventral) polarity in Antirrhinum leaves, such phenotypes in maize do not appear to be associated with mutations in *phan*-like or *knox* genes. Jen Nelson (University of California, Berkeley), however, showed results from a genetic mosaic analysis of *Rolled1*, a mutation that does alter abaxial-adaxial polarity in leaves. Mutant leaves show ligule flaps (normally an adaxial feature) on the abaxial leaf surface, and the presence of the mutant gene in abaxial epidermis is necessary and sufficient for production of the mutant phenotype.

Luzie Wingen (Max Planck Institute, Cologne, Germany) described the cloning of the *Tunicate1* (*tu1*) gene, a classical, dominant gain-of-function mutation that causes the outgrowth of glumes in ears, leading to a pod maize type phenotype. Wingen reported that a cloned MADS box gene (*zmm19*), mapping close to the *tu1* locus, is normally expressed only in leaf sheaths, and detects an RFLP between *Tu* and *tu* individuals. The promoter region has a *MuDR* insertion, and other alleles all show alterations in the promoter region, thereby leading to overexpression of *zmm19* in ears and tassels causing glumes to grow and take on a leaf sheath type phenotype.

REPRODUCTIVE DEVELOPMENT

Distinct developmental phases in plants include embryonic, juvenile, adult, and reproductive stages. Phase change mutations have been studied in maize and also described in Arabidopsis (Lawson and Poethig, 1995; Telfer et al., 1997). Matt Sauer (University of Pennsylvania, Philadelphia) showed that the maize mutation *early phase change1* (*epc1*) is tightly linked to the *Zea mays* ortholog of *hasty1 (zmhasty)*, which encodes an importin β involved in phase change in Arabidopsis. Juvenile leaves in *epc* mutants display adult leaf features, and transcript levels of the *zmhasty* gene are altered in two independently isolated *epc* mutants, confirming that mutations in *zmhasty* lead to *epc* phenotypes. Mike Muszynski (Pioneer HiBred, IA) reported on attempts to modulate floral transition. Whereas early maize inbreds make an average of 14.5 leaves prior to flowering, inbreds at mid and full maturity average 15 to 18.5 and >19 leaves, respectively. The *indeterminate1* (*id1*) gene encodes a zinc-finger transcription factor that promotes floral transition, possibly by regulating a transmissible signal generated in leaves (Colasanti et al., 1998). Upregulation of *id1* expression in transgenic plants leads to early floral transition. In plants homozygous for the *id1* transgene, this early transition represents three to four fewer nodes, and the effects are reproducible in widely different growth locations.

Once floral transition has occurred, the inflorescence meristem is programmed to produce a series of meristem types. Numerous branch meristems are produced by the inflorescence meristem; these give rise to two spikelet meristems, which in turn produce two flower meristems, which make the floral organs. Thus, all meristems except for the inflorescence meristem produce determinate structures. Debbie Laudencia-Chingcuanco (University of California, Berkeley) described the *indeterminate floral apex1* (*ifa1*) mutation. In this mutation, all determinate meristems continue to proliferate, suggesting that the *ifa1* gene is required to maintain determinacy in these structures. In double mutations of *ifa1* and *zag1* (Schmidt et al., 1993), floral meristems revert to inflorescence meristems. Similarly, in double mutations of *ifa1* and *ids1* (Chuck et al., 1998), spikelet meristems are converted to inflorescence meristems, and in *id1* single mutants (Colasanti and

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Sundaresan, 1996), spikelet meristems do not terminate but give rise to a shoot apical meristem. These results suggest that all determinate meristems have the potential to be converted into an indeterminate inflorescence meristem.

EMBRYONIC DEVELOPMENT—THE EMBRYO AND THE ENDOSPERM

The process of double fertilization in flowering plants gives rise to a diploid embryo and a triploid endosperm. The endosperm develops without a meristem, and only drastic defects in endosperm development alter plant survival. The food reserves in the endosperm are mobilized via the aleurone layer, which forms the outermost layer of the endosperm. Phil Becraft (Iowa State University) examined the cell lineage relationship of the endosperm and aleurone. Using clonal mosaics, Becraft showed that aleurone and endosperm cell lineages are initially identical, differentiating rather late in development. Aleurone cells contribute daughter cells internally within the seed up until the last two rounds of cell division in the endopserm, and differentiation of endosperm and aleurone tissue is dependent upon positional cues and not on differences in cell lineage origins per se. Becraft also described the *defective kernel1* (*dek1*) mutation, in which development of aleurone is diverted into the development of starchy endosperm. However, revertant wild-type sectors take on aleurone fate, suggesting that cues to direct aleurone development are present until the end of cell divisions in the endosperm. Similarly, late loss of DEK1 function can cause peripheral cells to take on starchy endosperm fate, suggesting that DEK1 is needed to perceive cues that both specify and maintain aleurone cell fate.

Odd-Arne Olsen (Agricultural University of Norway) described endosperm development from the central cell of the megagametophyte. After a period of free nuclear division, cellularization is initiated. Adventitious phragmoplasts direct centripetal cell wall formation until cellularization is complete at four days after pollination (DAP). The expression of molecular markers demarcates the four major cell types of the endosperm. While still in the syncytial stage, the *end1* transcript is localized to the region of cytoplasm that will become the basal endosperm transfer layer. Shortly after cellularization, the *embryo surrounding region* (*esr*) genes are expressed in cells of the region of endosperm surrounding the embryo. The *lipid transfer protein1* (*ltp1*) gene is apparently expressed in the aleurone layer at about 8 DAP. The starchy endosperm cells comprise the remaining bulk of the endosperm. Olsen also reported on mutants that disrupt the development of the basal transfer cells and aleurone.

TRANSPOSON TAGGING AND GENE IDENTIFICATION

There is a great variety of mutant phenotypes of the maize endosperm, most of which are poorly understood. Mark Settles (University of Florida, Gainesville) described an efficient method for identifying new *Mutator* (*Mu*) transposon-tagged mutations affecting aleurone development in maize endosperm. Settles devised a thermal asymmetric interlaced polymerase chain reaction protocol (Liu and Whittier, 1995) for use in concert with representational difference analysis subtraction in progenitor and new mutant progeny to identify novel *Mu* transpositions. The procedure will permit the identification of candidate loci in maize populations prior to aleurone mutant screening.

Laurel Mezitt (University of California, Davis) described the identification of the *sucrose export defective1* (*sxd1*) gene. Mutations in *sxd1* dramatically lower the level of sugar export from the leaves, and result in the distortion of minor veins in the leaf blade and plasmolysis of phloem parenchyma cells (Russin et al., 1996). Strikingly, plasmodesmata linking cells between the bundle sheath and phloem parenchyma are blocked by an improper deposition of cell walls. The gene encodes a protein with no identifiable functional domains, and no homologs have been found in animals (although it is present as a single copy in Arabidopsis). The *sxd1* gene is expressed in bundle sheath cells and is upregulated in maturing leaf blades. Mezitt cloned the *sxd1* allele using a protocol for differential display of *Mu* transposons by amplification of insertion-mutagenized sites (Frey et al., 1998). *Mu* transposon-tagging is a popular strategy of mutagenesis in maize, and the continued development of new *Mu*-cloning procedures promises to augment the existing protocols for gene discovery.

As a further testament to the power of *Mu*-transposon mutagenesis in maize genetic analysis, Chuck Dietrich (Iowa State University) presented a poster describing 80 mutations of the *gl8* locus derived from *Mu*-tagged populations. Astonishingly, 75 of 80 *gl8* mutations were found to contain a *Mu* transposon in the target gene. Although these results indicate that *gl8* may represent a “hotspot” for *Mu* insertions, Dietrich’s work provides promise that most mutations derived from *Mu* stocks may lead to the cloning of distinct genes.

Virginia Walbot (Stanford University) summarized ongoing progress in the maize gene discovery project, which utilizes two main approaches toward the identification of expressed genes in maize. One approach is a large expressed sequence tag (EST) sequencing project, which has thus far identified 53,000 ESTs (representing 17,000 unique genes) from various tissue-specific libraries. Another strategy strives to generate insertion mutations at every maize gene utilizing *RescueMu*, a *Mu1*

element engineered to contain a pBluescript plasmid. *RescueMu* permits simple plasmid rescue of genomic DNA flanking *Mu* insertions, greatly facilitating gene cloning in maize

Mu transposons are also useful in maize targeted mutagenesis, as described by Bruce May (Cold Spring Harbor). This "reverse genetic" strategy relies on PCR-based screens to identify *Mu* insertions in known genic sequences. Once putative transposon insertions are identified in *Mu*-bearing progenitor plants, the corresponding progeny can be genetically analyzed for mutant phenotypes.

EPIGENETIC REGULATION OF TRANSPOSONS AND GENE EXPRESSION

Damon Lisch (University of California, Berkeley) discussed a putative mechanistic connection between two fascinating yet perplexing epigenetic phenomena—*Mu* transposon suppression and paramutation. Silencing of *Mu* transposon activity and genetic paramutation both involve heritable changes in gene activity without changes in DNA sequence. Lisch reported that *modifier of paramutation1* (*mop1*), a mutation which prevents paramutation at several loci in maize, also prevents the methylation and concomitant inactivation of *Mu* elements. This evidence suggests that paramutation and transposon silencing may share common components of an epigenetic regulatory system.

The global control of plant gene expression is the focus of a genomics initiative described by Vicki Chandler (University of Arizona). The goal of this project, funded largely by NSF and led by Rich Jorgensen (University of Arizona), is the identification and characterization of genes affecting chromatin structure. Characterization of function involves reverse genetics approaches, including screening T-DNA insertion lines and RNA interference techniques that should silence all related genes, eliminating the problem of gene redundancy in viewing phenotypes.

DEVELOPMENT OF TOOLS FOR MAIZE AS A BIOLOGICAL SYSTEM

The need to enhance techniques for maize transformation was the subject of a panel discussion chaired by Kelly Dawe (University of Georgia, Athens). Although the generation of transgenic maize plants is no longer as problematic as in the recent past, panel members Steve Moose (University of Illinois, Urbana-Champaign), Jeff Bennetzen (Purdue University, West Lafayette), Wayne Parrot (University of Georgia, Athens), Michael Spencer (Monsanto), Vicki Chandler (University of Arizona, Tucson), and Pat Schnable and Lyuda Sidorenko (Iowa State University, Ames) discussed strategies aimed at reducing the costs and complications encountered with existing transformation protocols. In a separate but related presentation, Kelly Dawe introduced a genome project sponsored by NSF that is designed to construct maize artificial chromosomes. Crucial to the project is the identification and cloning of a functional maize centromere, and the construction of novel transformation vectors. If successful, this project promises to greatly advance the field of maize transformation.

GENOME STRUCTURE AND EVOLUTION

A dominant theme of all plant genetic research is the need to understand genome structure, function, and evolution. Mei Guo (Pioneer Hi-Bred, IA) and Anjali Dogra (University of Missouri, Columbia) presented two different approaches to understanding the classical phenomenon of heterosis. Hypotheses to explain the phenomenon are the dominance theory, which postulates that heterosis results from masking of recessive, deleterious traits, and the heterozygotic advantage theory, which states that heterozygosity is inherently advantageous (Xiao et al., 1995; Monforte and Tanksley, 2000). No direct evidence exists for either theory. Dogra attempted to distinguish between these hypotheses by constructing triploid inbred and hybrid strains in various combinations, and found that triploid inbreds are outperformed by diploid inbreds, whereas triploid hybrids perform better than diploid hybrids. Thus, triploidy itself is not advantageous, and these results argue against the dominance theory and for the heterozygotic advantage theory. Guo used expression profiles to determine whether changes in gene expression could be correlated with heterosis. Guo showed in maize hybrids that most genes are expressed within the range manifested by parental plants. Unexpectedly, a bias toward gene expression from male parental chromosomes was discovered in all hybrids examined, and this bias correlates with lower yield.

Molly Jahn (Cornell University, Ithaca) discussed ongoing work in her lab to analyze genome similarities among three species of *Solanaceae*, namely, tomatoes, potatoes, and peppers (Livingstone et al., 1999). General aspects of karyotype evolution have been elucidated from the analysis of these systems, and the focus now is on the evolution of resistance gene (*r*) function. One common theme is that *r* genes are found by chance in clusters more than expected in all three lineages, and clusters contain either all dominant or all recessive genes. Additionally, *r* genes are conserved in position in syntenous regions among the different genera, but the specificity of particular *r* genes has changed among lineages, reflecting rapid evolutionary change in functions of *r* genes.

Jeff Bennetzen (Purdue University,

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West Lafayette) discussed strategies for comparing sequences from orthologous regions among maize, barley, rice, sorghum, and wheat. Such comparisons should improve our understanding of grass evolution and gene colinearity, and will reveal the (apparently limited) extent of colinearity with the dicot Arabidopsis. Andy Kleinhofs (Washington State University) proposed strategies for using synteny in positional cloning. In attempts to clone the barley *Rpg1* rust resistance locus, the gene was found to be missing in the syntenous region in rice, thereby offering a caveat with respect to the use of genomic synteny in cloning disease resistance genes.

THE EVOLUTIONARY ORIGINS OF MAIZE

Nick Lauter (University of Minnesota) described an interesting approach to uncover genetic variation in apparently invariant traits in teosinte, the wild maize progenitor. This issue is important because evolution must have variability upon which selection can act, and many lines of teosinte are equivalent in traits that changed during the domestication of maize. By crossing two varieties of teosinte, then crossing the F_1 to a maize inbred line, Lauter was able to uncover variation among the different teosinte lines and perform QTLs mapping of traits involved in maize evolution. These results have certain implications for how maize evolution occurred, perhaps pointing to the presence of stabilizing mechanisms that mask otherwise invariant traits.

John Doebley (University of Wisconsin, Madison) gave an overview of work in his laboratory during the last few years in identifying the five major QTLs involved in the evolution of teosinte to modern maize (Doebley and Stec, 1993; Doebley et al., 1995). The QTLs on 3L and 5S have modest effects on many traits, but no candidate genes have been identified. The main QTL for one trait, affecting inflorescence phyllotaxy, is on chromosome 2S and maps close to a maize *leafy1* homolog. Doebley is generating knockouts of *leafy1* in maize to see if these mutants affect phyllotaxy. The *teosinte glume architecture 1* (*tga1*) mutation maps close to a QTL on chromosome 4 involved in development of the cupule fruit case, and cloning of *tga1* is underway. A major plant architecture QTL maps to chromosome 1L and corresponds to the *teosinte branched 1* (*Tb1*) gene, which is responsible for suppressing vegetative lateral branches in the axils of leaves (Doebley et al., 1997). The *tb1* gene encodes a potential basic helix-loop-helix transcription factor that is expressed in axillary buds, where it probably represses growth, thereby converting the highly branched teosinte shoot into the largely unbranched maize shoot.

CONCLUSION

One overall theme of the conference was the melding of traditional and new approaches to understanding maize biology. Progress in plant genomics and accompanying technologies has greatly energized the field, yielding high-throughput techniques for studying plant processes. Nevertheless, many genomic approaches also make use of transposon tools, for instance in gene identification and dissection of gene function. With the continued advances in tools and resources available to the scientific community, the next decade will see unprecedented progress in the understanding of maize biology.

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