A Role for PsbZ in the Core Complex of Photosystem II

Photosynthesis in oxygen-evolving organisms is driven by electron transport through the photochemical reaction centers photosystem I (PSI) and photosystem II (PSII), two large protein complexes located in the chloroplast thylakoid membrane. PSI and PSII each contain an array of light-harvesting antenna pigments that absorb light energy and transfer it to a reaction center core complex, where electron transport drives the conversion of energy into the stable chemical products NADPH and ATP. PSI absorbs predominantly red light and produces a strong oxidant that extracts electrons from water and releases oxygen (the water-splitting reaction of photosynthesis) and a reductant that transfers electrons on to PSI. PSI absorbs predominantly far-red light plus electrons from PSII to produce a strong reductant capable of reducing NADP⁺ to NADPH.

Light-driven ATP synthesis (photo-phosphorylation) is tightly coupled to electron transport through PSII and PSI and occurs within an ATP synthase complex embedded in the thylakoid membrane. Two other major complexes involved in photosynthetic energy conversion are cytochrome b₆f, which controls the shuttle of electrons from PSII to PSI via the mobile electron carriers plastoquinone and plastocyanin, and LHCII, a light-harvesting protein complex that functions as an auxiliary antenna for PSII and also appears to influence energy distribution between PSI and PSII.

Most of the proteins of these complexes, which together make up the photosynthetic apparatus, are encoded by the chloroplast genome. On the basis of the high degree of conservation of structure and composition of photosystems in all organisms examined to date, it is thought that all photosystems evolved from a single ancestor. In fact, there is a high degree of conservation among most of the ~120 genes encoded by numerous plastid genomes from plants and algae that have been sequenced completely (reviewed in Rochaix, 1997).

Suguira (1992) divided plastid genes into three major categories on the basis of their functions: plastome expression and maintenance (such as genes encoding rRNA, tRNA, and ribosomal protein subunits), photosynthesis, and unknown function. A total of 26 to 34 unidentified open reading frames were found in various chloroplast genomes, and at least 12 of these were highly conserved in size and sequence among the chloroplast genomes examined to date. Hallic and Bairoch (1994) introduced the term ycf (hypothetical chloroplast open reading frame) for chloroplast open reading frames of unknown function.

TARGETED INACTIVATION OF ycf9

Chloroplast transformation is possible via recombination between plastid genome sequences and homologous sequences that flank a gene expression cassette (which produces “transplastomic” plants), in contrast to nuclear transformation, in which an uncontrolled number of foreign gene copies are integrated into the genome at random sites. Thus far, most plastid transformations have been performed in Chlamydomonas reinhardtii and tobacco. Technical limitations have hindered plastid transformation in other systems, although progress in recent years holds promise for routine application in other higher plant species, and the technique has been used successfully in rice (Heifetz, 2000).

Targeted gene inactivation via chloroplast transformation has been used to characterize the function of several of the ycf genes in Chlamydomonas and tobacco. Thus, ycf6 (Hager et al., 1999) and ycf7 (Takahashi et al., 1996) were found to be essential components of the cytochrome b₆f complex in tobacco and Chlamydomonas, respectively. In this issue of The Plant Cell, Swiatek et al. (pages 1347–1367) describe the use of targeted gene inactivation of the chloroplast ycf9 gene in both Chlamydomonas and tobacco to provide strong evidence that the ycf9 product, named PsbZ, is a subunit of the PSII core complex.

Previous research on this gene has yielded ambiguous results. Because plant cells contain numerous chloroplasts, and thus numerous copies of the chloroplast genome, it can be difficult to obtain transplastomic plants that are homoplasmic for foreign transgenes. Mäenpää et al. (2000) were unable to obtain transplastomic tobacco plants homoplasmic for an inactivated ycf9 gene and concluded that the ycf9 gene product was likely essential for chloroplast function. Furthermore, the use of a polyclonal antibody raised against a portion of the ycf9 gene product on thylakoid fractions separated by standard techniques suggested that the ycf9 product was not associated with PSII or LHCII but possibly was associated with PSI. On the other hand, Ruf et al. (2000) produced homoplasmic ycf9 knockout tobacco plants that displayed no apparent phenotype under normal growth conditions. However, the ycf9 mutant plants showed impaired growth and oxygen-evolving capacity under low light, suggesting that they had reduced light-harvesting efficiency relative to wild-type plants. These ycf9 mutant plants...
also were found to have reduced levels of CP26, a minor antenna protein of LHCII, and in wild-type plants the ycf9 gene product was found to co-purify with LHCII fractions, suggesting that the Ycf9 protein formed part of the LHC complex (Ruf et al., 2000).

Swiatek et al. show quite convincingly that PsbZ is in fact a genuine subunit of PSII. Evidence is presented from both Chlamydomonas and tobacco, using specific antibodies against PsbZ on thylakoid fractions from wild-type psbZ-deficient mutant preparations as well as preparations from various other mutants lacking other components of the photosynthetic apparatus. Thus, PsbZ was found to comigrate precisely with PSII core subunits in wild-type Chlamydomonas and was present in mutants lacking PSI, ATP synthase, chlorophyll a/b antenna proteins, or the cytochrome b_{559} complex but was absent in other mutants lacking PSII cores. It was also found to be associated with PSII core fractions from wild-type tobacco plants, but it was missing from PSII core fractions from the psbZ-deficient mutant tobacco.

One of the great strengths of this study is the use of the two different systems, Chlamydomonas and tobacco, to investigate protein function. Somewhat different experiments were performed in each system, depending on the availability of mutants and techniques that were applicable to each, yet the results were in good agreement, making the conclusions far more robust than if only one of the systems had been used. This is perhaps a particularly important point in this case, because others had obtained somewhat different results concerning the function of ycf9 (Mäenpää et al., 2000; Ruf et al., 2000). This work also demonstrates the potential usefulness of Chlamydomonas as a model system for higher plant processes.

**PSII SUBUNIT ORGANIZATION**

PSII is made up of at least 17 subunits, most of which are embedded within the thylakoid membrane: the reaction center proteins D1 (PsbA) and D2 (PsbD); the chlorophyll-containing antennae CP47 (PsbB) and CP43 (PsbC); the cytochrome b_{559} α- and β-subunits (PsbE and PsbF); and a number of smaller subunits of largely unknown function (PsbH, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbN, and PsbX). Several other subunits, including cytochrome c_{550} (PsbV), PsbU, and PsbO, extend into the thylakoid lumen from PSII (Zouni et al., 2001).

PSII probably exists as a dimer in higher plants and algae (Santini et al., 1994; Shi et al., 2000; Zouni et al., 2001), and the dimer forms a PSII-LHCII supercomplex with LHCII subunits. There is evidence that some of the low molecular weight proteins, such as PsbH, PsbK, and PsbL, are involved in PSII dimer stabilization. Shi et al. (2000) showed that another low molecular weight protein named PsbW is involved in the stabilization of dimeric PSII complexes in Arabidopsis. In contrast to wild-type plants, the dimeric form of PSII could not be isolated from antisense psbW plants with more than a 96% reduction in PsbW protein content. Furthermore, the antisense plants exhibited a 50% reduction in oxygen evolution and a 40% loss of PSII reaction center proteins relative to wild-type plants, suggesting that the dimerization of PSII is important to maintain the stability of the photosynthetic apparatus. PsbW is a nucleus-encoded protein, leading to the interesting speculation that it represents a higher plant mechanism for providing a degree of nuclear control over photosynthetic activity in the partly autonomous chloroplast (Shi et al., 2000).

Swiatek et al. present evidence that the PsbZ protein is involved in maintaining the stability of PSII-LHCII supercomplexes. After membrane solubilization with an appropriate detergent, sucrose gradient sedimentation results in the separation of PSII core subunits into several different fractions corresponding to PSII-LHCII supercomplexes, PSII dimers, or PSII monomers. PSII-LHCII supercomplexes were readily identified from wild-type tobacco preparations but were completely absent in preparations from the psbZ-deficient mutant. Mutant preparations also failed to accumulate other PSII- and LHCII-associated proteins at the positions of PSII supercomplexes.

It is known that interactions between the PSII core and the LHCII antenna are controlled by phosphorylation (Allen, 1992). Swiatek et al. found that, consistent with a role in PSII-LHCII interactions, the phosphorylation status of PSII cores and LHCII antennae was altered markedly in the psbZ-deficient mutants of both Chlamydomonas and tobacco.

Swiatek et al. present strong evidence that PsbZ is associated with the PSII core. In light of the previous results of Ruf et al. (2000) that suggest the protein is associated with LHCII and the evidence from Swiatek et al. that PsbZ is involved intimately with PSII-LHCII interactions, it seems likely that PsbZ occupies a position in the PSII core near the PSII-LHCII interface. Figure 1 portrays a model for the structure of PSII-LHCII supercomplexes showing the possible location of PsbZ subunits and adjacent LHCII subunits and minor antenna proteins.

**PsbZ AND NONPHOTOCHEMICAL QUENCHING**

Figure 1 shows PsbZ lying adjacent to the CP26 subunit, which is a minor antenna subunit of LHCII. The positioning is based on the location of unidentified PSII subunits from Zouni et al. (2001)
and the observation by Swiatek et al. that \textit{psbZ}-deficient mutants accumulated significantly less CP26 protein than did wild-type plants. CP26 and CP29 are violaxanthin binding proteins and appear to play a role in non-photochemical quenching (NPQ) of chlorophyll fluorescence, which involves interconversions between violaxanthin and zeaxanthin (the so-called xanthophyll cycle). Exposure of the photosynthetic apparatus to excess light energy induces a reversible decrease in the efficiency of photosynthetic energy conversion. The excess excitation energy can result in damage to the thylakoid membrane (photoinhibition) or it can be dissipated through a controlled mechanism (photoprotection) that is associated intimately with the xanthophyll cycle in many plant species (reviewed in Demmig-Adams, 1990; Horton, 1996).

In the xanthophyll cycle, zeaxanthin is formed from deepoxidation of violaxanthin via the intermediate antheraxanthin in a reaction catalyzed by a deepoxidase, and an epoxidase catalyzes the reconversion of zeaxanthin to antheraxanthin and violaxanthin. There is strong evidence that zeaxanthin functions in the dissipation of excess excitation energy, which protects the photosynthetic apparatus from the damaging effects of photoinhibition by preventing the accumulation of toxic reactive oxygen species. Demmig-Adams (1990) showed that there is a close correlation between zeaxanthin content and the capacity for quenching of chlorophyll \textit{a} fluorescence and photoprotective energy dissipation (NPQ) in many different species and under a wide range of conditions. However, the mechanism of energy dissipation involving zeaxanthin remains largely unknown.

Swiatek et al. found that \textit{psbZ}-deficient tobacco plants showed a greatly reduced capacity for NPQ under adverse growth conditions such as increased light intensity and/or
decreased temperature. The mutant plants also showed a dramatically altered xanthophyll cycle, as measured by HPLC analysis of various pigments, including violaxanthin, zeaxanthin, and antheraxanthin. When transferred from dim light to high light, wild-type plants showed an increase in the proportion of zeaxanthin from 4 to 33% of total xanthophylls, followed by a rapid decrease to 14% after a 10-min dark recovery period. In contrast, zeaxanthin content in the psbZ-deficient plants increased from 5 to 63% when transferred from dim light to high light and did not recover appreciably when subsequently placed in darkness for 10 min. The total xanthophyll pool also was significantly higher in the mutant plants relative to the wild type. Nonetheless, the mutant plants apparently were unable to use zeaxanthin as a photoprotectant as effectively as did the wild-type plants, as shown by a reduced capacity for NPQ and greater photoinhibition under stress conditions.

Horton (1996) discussed the possibility that zeaxanthin functions only indirectly in NPQ, perhaps by bringing about structural changes within LHCII. There is evidence for a significant role for minor LHCII components (i.e., the violaxanthin binding proteins CP26 and CP29) in NPQ. Swiatek et al. show that PsbZ plays a critical role in interactions between PSII and LHCII and in the formation of NPQ under conditions that give rise to photoinhibition. There are a number of organisms that lack a xanthophyll cycle and/or do not accumulate zeaxanthin yet maintain the capacity for NPQ and photoprotection (Demmig-Adams, 1990; Horton, 1996). In this regard, it is interesting that PsbZ appears to be highly conserved among all photosynthetic organisms, even in those that lack a xanthophyll cycle. Further investigation of the function of PsbZ may provide the key to the mechanism of NPQ and the photoprotective dissipation of excess light energy.

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REFERENCES
MEETING REPORT

Functional Evolutionary Genetics and Plant Adaptation: Linking Phenotype and Genotype

Conway Morris (2000) wrote that the only point of agreement among biologists discussing organic evolution seems to be "it happened." Until recently, those attempting to forge links between evolutionary and functional genetics might have responded that discussions of any kind would be nice. Thomas Mitchell-Olds (Max Planck Institute for Chemical Ecology, Jena, Germany) andMontserrat Aguadé (University of Barcelona, Spain) are two such scientists amon a growing group who are beginning to realize the benefits of discussions and collaboration between functional geneticists (such as developmental molecular biologists) and evolutionary geneticists (such as those studying population genetics and ecology). Mitchell-Olds and Aguadé organized a recent workshop on Functional Evolutionary Genetics and Plant Adaptation, held in Jena, Germany, on March 9 through 11, 2001, to promote interactions between these fields. The workshop was sponsored by the European Science Foundation Scientific Program on Plant Adaptation. Judging by the enthusiasm of the participants and the high quality of the presentations, the meeting was an unqualified success. This report presents a general overview of the meeting and summarizes the major themes that emerged. See http://www.ice.mpg.de/departments/Gen/conferences/esf_2001/ for a full listing of speakers and abstracts.

LINKAGE DISEQUILIBRIUM AND SINGLE NUCLEOTIDE POLYMORPHISMS

One of the most important concepts in studies of both functional and evolutionary genetics is linkage disequilibrium (LD). The extent and ramifications of LD in Arabidopsis thaliana was a topic of great interest at the workshop and was discussed in detail in talks given by Magnus Nordborg (University of Southern California, Los Angeles) and Karl Schmid (Max Planck Institute for Chemical Ecology). In a population with several polymorphic loci, LD occurs when alleles at two loci occur together more often than expected (Figure 1). LD is a statistical measure that quantifies the nonindependence of genotypes at several loci. When genotypes are correlated between loci, the information from a marker may predict genotypic function at another locus—hence, the importance of LD in functional genomics and studies of human disease. In some instances, the occurrence of LD suggests that selection favors chromosomes carrying particular multilocus genotypes. However, Nordborg pointed out that the extent of LD is a result of a complex historical process (M. Nordborg and S. Tavaré provide a comprehensive discussion of this topic in an unpublished review available at http://www-hto.usc.edu/papers/abstracts/tig.html). LD is expected to vary greatly because of the randomness of history, but the average rate of decay of LD (i.e., the genetic or physical distance over which LD can be measured) depends on the demographic history of the population and a number of other factors. In particular, in plant populations, the extent of selfing versus outcrossing can have a strong effect. Nordborg showed that LD is more extensive in selfing species, which is expected because populations of self-pollinating individuals tend to be largely homozygous (Nordborg, 2000).

What was of great interest to those at the meeting was that Nordborg also showed that although LD is extensive in A. thaliana, it is far from genome wide (as had been considered by many to be the case). He presented evidence that LD in A. thaliana decays over a distance of 100 to 200 kb. This has significance for those interested in using single nucleotide polymorphisms (SNPs) in association studies (i.e., linking phenotype with a SNP marker). A genome-wide "SNP chip" for conducting association studies with A. thaliana could be constructed using 2000 SNPs spaced at 50- to 100-kb intervals, a prospect that is possible with currently available resources and technologies. Nordborg favored the idea of sequencing 500- to 1000-bp segments every 50 to 100 kb (1% of the genome) in 100 individuals, which could be used to produce a SNP chip with 10 or so tightly linked SNPs every 50 to 100 kb. Schmid discussed efforts of the Max Planck SNP Consortium to develop a database of 1300 SNPs, evenly spaced at 100 kb, by using a selection of 12 A. thaliana accessions that are among the most highly diverged (based on previous amplified fragment length polymorphism studies) plus data from the closely related species Arabidopsis lyrata and Arabis drummondi.

GENETIC VARIATION AND THE MATING GAME

One of the major topics of discussion was the idea that the type of mating system (i.e., selfing versus outcrossing) can have a significant effect on the evolution of plant genes. A high degree of selfing produces highly homozygous populations, which effectively reduces
recombination (as discussed above) and complicates efforts to determine the adaptive significance of a particular trait or gene. Because *A. thaliana* is a highly selfing species, a number of evolutionary geneticists have turned to the closely related outcrossing species *A. lyrata* as a model for investigating adaptive variation (North American and European subspecies formerly known as *Arabis lyrata*, *Arabis petraea*, and *Cardaminopsis petraea* are now considered subspecies of *Arabidopsis lyrata* [O’Kane and Al-Shehbaz, 1997]). Work with *A. lyrata* comes with the disadvantage that very little genomic sequence is available. However, its close relationship with *A. thaliana* makes it particularly amenable to studies of “candidate genes,” in which genes that are suspected of being associated with important phenotypic traits (e.g., based on the biochemical function of the protein) are selected as possible candidates influencing adaptively significant variation. Candidate genes can be identified (mapped, cloned, and sequenced) in *A. lyrata* quite easily because of the high degree of homology and colinearity between the two species. Associations between the candidate gene and the phenotypic trait may sometimes be easier to demonstrate in the outcrossing *A. lyrata*; thus, the adaptive role of the specific candidate could be confirmed. Because of the wealth of genetic and genomic information available regarding *A. thaliana*, comparisons between *A. lyrata* and *A. thaliana* can be expected to provide critical information not only about the importance of mating system in shaping evolutionary outcomes but also about the possible adaptive and functional significance of various genetic loci.

The effect of mating system on DNA variation was discussed in a number of presentations. Because selfing produces populations of homozygotes and reduces the effective recombination rate, it is commonly thought that selfing reduces DNA variation. However, selfing actually has little effect on the overall amount of DNA variation within a species. Rather, it affects how variation is structured. DNA variation in a selfing species tends to be structured into distinct haplotypes coupled with extensive LD (a haplotype is a specific multilocus combination of alleles that occurs in an individual, such as *A1B1*). Conversely, recombination among heterozygotes in an outcrossing species reduces LD and breaks up haplotypes.

Outi Savolainen (University of Oulu, Finland) compared sequence variation in *A. lyrata* and *A. thaliana* for alcohol dehydrogenase (*Adh*), the model gene of sequence variation in plants, and the candidate genes *CONSTANS* (*CO*) and *FRIGIDA* (*FRI*), which have been associated with flowering time in a number of species. The overall (within-species) level of variation was about the same in both species; however, a higher degree of within-population polymorphism was found in *A. lyrata* compared with that of *A. thaliana* populations. Selfing is expected to decrease the effective population size, and thus within-population variation, by twofold relative to outcrossing species, but within-population variation was decreased more than twofold in *A. thaliana* relative to *A. lyrata*. In addition to the effect of selfing, the recent and rapid expansion of *A. thaliana* from Asia to other parts of the world, leading to founder effects or so-called bottlenecks, is seen as an important factor leading to low within-population variation in *A. thaliana* (Savolainen et al., 2000). This idea was

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**Figure 1.** Two Populations Are Depicted with Polymorphisms at the A Locus and the B Locus.

In the first population, allelic state at the A locus is independent of alleles at the B locus. Genotypes at the two loci are independent, hence the population is in linkage equilibrium. In the second population, A alleles are paired with B alleles (and a alleles occur with b alleles) more often than would be expected if they were independent. Such correlation (or nonindependence) among loci is called linkage disequilibrium. Linkage disequilibrium is important in genomics because marker loci may be correlated with functional variants (such as disease genes). This permits identification of disease genes and the prediction of disease risk.
echoed by a number of other speakers. Interestingly, CO and FRI showed more sequence divergence between the two species than did Adh, suggesting that different constraints may be acting on these genes.

The difficulty of inferring the action of natural selection in selfing species was raised in several presentations. For example, Aguadé presented data on sequence variation in genes encoding enzymes of the phenylpropanoid pathway in A. thaliana. Two patterns of variation emerged: in some genes (typified by FAH1), variation was structured into two highly divergent haplotypes that exhibited little within-haplotype diversity, whereas in other genes (typified by CHI), there was no clear evidence for two haplotypes. In outcrossing species, the presence of two highly divergent haplotypes in regions with normal levels of recombination can be a strong indication of balancing selection in that region. However, in a selfing species, this pattern could equally be attributable to genetic drift, given that recombination is effectively reduced (Aguadé, 2001).

Information about the potential adaptive significance of genes can come from examining patterns of DNA variation in the context of gene function and phenotype. Michael Purugganan (North Carolina State University, Raleigh) presented data on DNA variation among genes involved in flower development, which again suggested that two basic patterns of variation exist in A. thaliana: genes that are somehow organized into distinct haplotypes versus those that are not. Thus, one group of genes, including TFL, showed a low amount of variation in the coding sequence, but high levels of variation organized into distinct haplotypes in the promoter region; other genes showed either low (LFY) or normal (AP3, PI) levels of variation throughout coding sequence and promoter regions. The low level of variation in the LFY floral meristem identity gene is consistent with the idea that natural selection has driven an advantageous LFY mutation to fixation in all A. thaliana populations (directional selection). But selection on the LFY coding region also could be attributable to genetic “hitchhiking” (e.g., directional selection on a nearby locus, resulting in reduced nucleotide variation for a chromosomal region surrounding the selected locus). The size of the affected region depends on LD; thus, it will be larger in a selfing population.

TFL1, which appears to function by repressing LFY, is perhaps even more complex. Although the low amount of variation in the coding sequence may suggest directional (purifying) selection (as for LFY), two distinct promoter haplotypes suggest balancing (diversifying) selection acting on the promoter region. Association studies with 21 A. thaliana accessions suggest that the TFL promoter types are associated with different numbers of inflorescence meristems. Moreover, Purugganan presented results from field experiments conducted by his collaborators, the Arabidopsis ecologists Johanna Schmitt and Cynthia Weinig (Brown University, Providence, RI), which suggest that the number of inflorescence branches has an impact on overall plant fitness.

Wolfgang Stephan (Ludwig-Maximilians-Universität, Munich, Germany) presented data on the effects of recombination and mating system on DNA variation in the tomato genus Lycopersicon. His group has studied patterns of nucleotide diversity in the selfing species L. chmielewski and L. pimpinellifolium and the outcrossers L. peruvianum, L. chilense, and L. hirsutum. Although the amount of within-population polymorphism was much lower in the selfing relative to the outcrossing species, the level of nucleotide diversity was not highly correlated with recombination rate, as it is in Drosophila. Thus, there is a strong effect of mating system on the structure of nucleotide diversity, but it is by no means the only factor. Stephan suggested that demographic factors, such as population subdivision and population size, also have dramatic effects on the structure of nucleotide diversity (Stephan and Langley, 1998).

DOES EVOLUTION PLAY FAVORITES?

A number of speakers presented evidence that certain loci have been “recruited” for a particular function independently multiple times, either in different species or within a single species. This pattern has been observed before. For example, Paterson et al. (1995) reported on independent mutations at corresponding (e.g., homeologous) genetic loci among different cereal crop species. Mark Rausher (Duke University, Durham, NC) stated that the reasons for apparently nonrandom patterns of substitution are unclear. Has it occurred simply by chance? Do some genes have excessively high mutation rates (for unknown reasons)? Or is the answer that mutations in many genes impinging on a particular pathway are detrimental or lethal, whereas mutation in one of the genes is allowed?

Morning glory (Ipomoea) has a tremendous amount of variation in flower color. Rausher’s group examined the phylogeny of 40 Ipomoea spp and found that white flower color has evolved independently many times. Furthermore, transitions in flower color were found to be highly asymmetric; for example, there are many cases of purple to white transitions but not of the opposite transition of white to purple. The data collected so far suggest that this pattern may be the result of “knockout” mutations. For example, I. purpurea (normally purple flower) produces some variants at the W locus that produce white flowers in homozygous individuals and white-purple flowers in heterozygotes. W was cloned and found to be homologous with the myb transcription factor An2 in petunia, which is
known to be involved in flower color. The white-flowered \textit{I. purpurea} variants seem to have frameshift deletions and an introduced stop codon in this gene. Rausher’s group will test the knockout hypothesis by introducing a wild-type copy of \textit{W}, presumably the functional gene, into white-flowered plants.

There are at least eight genetic routes that can produce white-flowered plants in \textit{Ipomoea}. Rausher’s group is collecting data to determine the genetic changes associated with flower color in 15 independently evolved white-flowered species. They also will test various hypotheses that the pattern of knockout mutations is not random. For example, there may be fewer deleterious pleiotropic effects associated with regulatory genes that are highly specifically expressed only in certain tissues (such as \textit{An2}, which is expressed specifically in flower petals in petunia) relative to structural genes or other regulatory genes that are more widely expressed throughout the plant. Another hypothesis is that there are fewer deleterious pleiotropic effects associated with mutations in downstream compared with upstream structural genes. The data collected so far suggest that downstream structural genes may be more subject to mutations that persist than are upstream structural genes.

Caroline Dean (John Innes Centre, Norwich, UK) gave another example of nonrandom occurrence of mutations at the \textit{FRI} locus in \textit{A. thaliana}, which is a major determinant of flowering time (Johanson et al., 2000). The majority of \textit{A. thaliana} accessions are winter annuals that are late-flowering plants that require vernalization, or a prolonged cold period, to flower. Winter annualism in \textit{A. thaliana} is chiefly a monogenic trait that maps to the \textit{FRI} locus. \textit{FRI} encodes a novel protein with no strong homology with any other known proteins. It is a single gene that appears to confer a vernalization requirement by overriding flowering promotion pathways. The rapid-cycling accessions

Columbia and Landsberg erecta carry loss-of-function \textit{FRI} alleles. Interestingly, early flowering appears to have evolved multiple times in \textit{A. thaliana} through loss of \textit{FRI} function. This is quite surprising, because \textit{fri} mutations account for relatively few vernalization and flowering time mutants obtained through mutagenesis experiments. Further analysis of \textit{FRI} over a wide range of populations in \textit{A. lyrata} could prove to be extremely interesting as well.

THE HOLY GRAIL: LINKING PHENOTYPE AND GENOTYPE

Significant progress has been made toward linking phenotype and genotype for a number of plant traits and genes. In addition to the research on genes involved in flower development and flowering time discussed above, numerous presentations were giving linking genes to a wide variety of phenotypic characteristics, such as trichome development, hypocotyl length and seedling development, seed storage lipids, insect and pathogen resistance, and salt tolerance. A few of these presentations are summarized here.

Flower Shape

Enrico Coen (John Innes Centre) spoke on the evolution of floral characters and what determines whether flowers are asymmetrical, as in Antirrhinum (snapdragon), or symmetrical, as in the Asteraceae (daisies). The wild-type Antirrhinum flower has two dorsal, one ventral, and two lateral petals and emerges from an indeterminate meristem. A peloric mutant of Antirrhinum, which carries a mutation in a gene named \textit{CYCLOIDIA}, has radially symmetrical flowers comprising five ventral petals produced from a determinate meristem. In the early stages of flower development, when the bud appears radially symmetrical, \textit{CYCLOIDIA} is expressed quite specifically only in the dorsal petal regions.

Daisies and other members of the Asteraceae (composites) have what appear to be radially symmetrical flowers. However, the composite flower consists of inner disc florets and outer ray florets, and although the disc florets are symmetrical, each ray floret is asymmetrical. Coen reported on research on \textit{Senecio} spp conducted in collaboration with Richard Abbott and Amanda Gillies (University of St. Andrews, UK) and Pilar Cubas (Universidad Autónoma de Madrid, Spain). \textit{Senecio vulgaris}, a composite native to the United Kingdom, has yellow disc florets but no ray florets. The Italian species \textit{S. squalidus}, which has yellow disc and ray florets, escaped from the Oxford botanical gardens 300 years ago and is now widespread throughout the United Kingdom. The two species interbred, and a rayed variant of \textit{S. vulgaris} appeared. Restriction fragment length polymorphism analysis has shown polymorphism cosegregating with the rayed and nonrayed forms of \textit{Senecio} that is tightly linked to a \textit{CYCLOIDIA}-like gene, which is expressed only in floral meristem tissue that forms into ray florets. Future work will focus on providing definitive evidence that this gene is responsible for the rayed versus the nonrayed phenotype. In another example, Coen showed a striking mutant of \textit{Cosmos}, another composite with ray and disc florets, in which the ray florets formed tubes instead of flattened petals. Floral symmetry is thought to have evolved independently many times. It will be very interesting to determine if the \textit{Cosmos} mutation is associated with a \textit{CYCLOIDIA}-like gene and if a similar mutant can be created in \textit{Senecio}.

Insect Resistance

Juergen Kroymann (Max Planck Institute for Chemical Ecology) provided ev-
idence for almost complete association of phenotype and genotype for genes involved with insect resistance in A. thaliana, from work done in collaboration with others in the Mitchell-Olins group (Kliebenstein et al., 2001a, 2001b; Kliebenstein and Mitchell-Olins, 2001). They identified candidate genes through subtractive hybridization and gene expression analysis from plants damaged by insect herbivores compared with mechanically wounded control plants and analyses of a number of mutants and different accessions of A. thaliana showing variation in insect resistance. They chose to focus attention on genes involved in glucosinolate biosynthesis, because glucosinolates are known to be associated with insect interactions (the breakdown of various glucosinolates leads to the accumulation of toxic products) and glucosinolate composition and accumulation is highly variable among A. thaliana accessions. Quantitative trait loci for insect resistance and aliphatic glucosinolate composition were mapped, and a complete association was found between glucosinolate profiles and the expression of two genes, AOP2 and AOP3. Accessions such as Col-0 that appear to have both genes knocked out, based on sequence analysis and expression studies, accumulate a methylsulfinylalkyl glucosinolate precursor. Accessions found to express a functional AOP2 accumulated an alkynyl glucosinolate and did not express AOP3, and AOP3 was expressed only in those ecotypes that produce hydroxalkyl glucosinolates. None of the accessions examined was found to express both genes. Work is in progress to determine the extent to which these genes influence insect resistance.

**Pathogen Resistance**

Pathogens exert tremendous selection pressure on plants. It is well known that pathogens produce recognition (avirulence) factors that trigger rapid defense signaling cascades in plants. Recognition by the plant is conferred by so-called R (resistance) genes, a large group of genes that share sequence motifs including leucine-rich repeat (LRR), Toll/Interleukin-1 receptor, nucleotide binding (NB), and serine/threonine kinase domains. There is evidence for tremendous sequence exchange that produces a diversity of NB-LRR-type R proteins, which may provide plants with a mechanism for maintaining resistance against rapidly evolving pathogen populations. Although R loci are highly polymorphic, resistance-signaling genes appear to be rather more conserved. Jane Parker (Sainsbury Laboratory, Norwich, UK) presented data on two genes, PAD4 and EDS1, that are required for R gene-mediated resistance to a range of microbial pathogens. These two genes function upstream of defenses mediated by the phenolic signaling molecule salicylic acid and appear to be necessary for the induction of systemic immunity. Both genes encode lipase-like proteins and thus may function by hydrolyzing a lipid-based substrate (Parker et al., 2000).

**Starch Biosynthesis**

Barbara Schaal (Washington University, St. Louis, MO) spoke about the search for genes that have played a role in the evolution of carbohydrate metabolism and starch synthesis in cassava (Manihot esculenta). Very little research has been conducted on cassava, although it is an essential staple crop for 600 million people, particularly in Africa. Schaal’s group has pinpointed the probable progenitor of the cultivated crop as the wild species M. flabelifolia, the natural range of which is in the Amazon basin (Olsen and Schaal, 2001). Luis Carvalho, a longtime collaborator of Schaal’s, has made some striking discoveries about cassava by examining how people in this area grow and use the crop. Unlike most other parts of the world, where cassava is grown as a monoculture and used mainly for its starchy tuber, in the Amazon it is intercropped and there are many uses for the plant. Numerous cultivars are grown; some are grown for the leaves as well as the tubers, and others are used to make juice, alcohol, or flour for bread. Analysis of carbohydrate content in the different varieties showed that some of the Amazon cultivars contain very little starch but high levels of free sugars, compared with the levels in world germbank varieties. This was an important discovery because people in other parts of the world are trying to engineer cassava for higher sugar content, thus far with little success. Schaal hypothesized that a mutation in the starch biosynthesis pathway led to a new biochemistry, and she plans to look more carefully at M. esculenta and M. flabelifolia in the Amazon basin to understand the evolution of these genes.

In summary, the renewed interest in collaborations among functional and evolutionary geneticists is highly promising for plant genetics research. Functional geneticists can provide information and ideas about genes and mechanisms that may be ecologically significant targets of natural selection. Evolutionary geneticists can provide information on the degree and structure of DNA variation, which can form the basis for the development of new tools, such as mapped recombinant inbred lines and marker densities for LD mapping, and the isolation of new genes involved in development and physiology. A number of plant geneticists began studying the outcrossing species A. lyrata with the idea that it might provide a plant equivalent of Drosophila, which has large random mating populations in which levels of within-population DNA variation are correlated with recombination rate in
local chromosomal regions. The structure of variation in *A. lyrata* may resemble that of *Drosophila* more than that of *A. thaliana* does. However, Nordborg pointed out that the main reason *Drosophila* is a “good model” for population genetics is that it can fly, so there is random mating on a global scale, and that population structure, perhaps more than the type of mating system, has a critical influence on patterns of DNA variation. Thus, it should not be surprising to find that the pattern of DNA variation in *A. lyrata* does not resemble that of *Drosophila* very closely. Nonetheless, evolutionary geneticists should benefit greatly from more extensive analyses and comparisons of *A. lyrata* and *A. thaliana*. Perhaps the joint efforts of plant functional and evolutionary geneticists will redefine what it means to be a “good model” for population genetics.

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**REFERENCES**


MEETING REPORT

An Evolutionist Visits the 43rd Annual Maize Genetics Conference

From modest roots of a few dozen maize geneticists sitting around a small room in the Allerton House in Illinois in the 1950s, the Maize Genetics Conference has grown this year to a less modest but still comfortable size of nearly 500 attendees, the largest maize meeting to date. It was held at the Grand Geneva Resort in southern Wisconsin, easily accessible but secluded enough to allow the participants to focus on maize. It featured four plenary talks and two additional invited talks, 25 contributed talks, and more than 170 posters. Packaged into just four days (March 15 to 18), the meeting had an intensity that kept the participants engaged, if not overwhelmed, leaving at least one of them ready to track back home to download and process the flood of new ideas, contacts, and information. In this report, I offer no pretense of providing a comprehensive abstract of all or even most of the talks and posters. Rather, this report provides the admittedly biased perspective of an evolutionary geneticist. Still, I hope to convey the excitement and energy of the meeting, and I offer my apology to the many fine speakers and poster presenters whose work I do not mention. Abstracts for all of the talks and posters can be found at http://www.agron.missouri.edu/Coop/Conf/2001.html.

PLENARY TALKS

Kelly Dawe (University of Georgia, Athens) kicked off the meeting with his talk on maize Abnormal chromosome 10 (Ab10) and its influence on chromosomal segregation. The Ab10 chromosome contains factor(s) that produces “meiotic drive” such that Ab10 is transmitted to progeny at frequencies in excess of Mendelian expectations. It was demonstrated by Marcus Rhoades in the 1940s that this system for meiotic drive involves heterochromatic regions (knobs) that can act as neocentromeres in the presence of Ab10. This allows chromosomes with knobs (Ab10 itself has a very large knob) to be pulled to the poles more rapidly than chromosomes lacking knobs and thus to be represented at greater than Mendelian proportions in the distal megaspore that forms the embryo sac. Dawe presented work from his laboratory demonstrating that the mode of neocentromere movement is distinct from that of the true centromeres, involving the action of neither MAD2 (a regulatory protein of the spindle) nor CENPC (a structural protein of the centromere). Dawe’s laboratory also tested whether polar flux of the spindle microtubules might be involved by using taxol to stabilize the microtubules. Because taxol did not stop neocentromere movement, it does not appear that the neocentromeres are simply hitching a ride—via polar flux—on the microtubules. Dawe and colleagues are investigating the possibility that members of the kinesin superfamily of proteins, which are involved in chromosome and organellar movement in animals, might control the movement of the neocentromeres in maize.

June Nasrallah (Cornell University, Ithaca, NY) gave an overview of her exceptional work on the genetics of self-incompatibility (SI) in Brassica. SI is controlled by the S locus, a compound locus consisting of two separate genes: SRK, which encodes a receptor kinase, is the stigmatic element; SCR, which encodes a cysteine-rich peptide, is the pollen element and is predicted to be a ligand for SRK. A third gene, SLG, which encodes a glycoprotein localized to the cell wall of the stigma epidermis, is found in the majority of S haplotypes and appears to be required for stabilization of SRK. Having worked out the control of SI in Brassica, Nasrallah and her group have been investigating the evolution of SI and the S locus in Brassica and other genera. In Brassica, they have shown that SRK (the female element) and SCR (the male element) have undergone a form of coevolution such that the allele phylogenies of these two genes are nearly identical. This is what one would expect if each change in the male component was matched by a change in the female component (or vice versa), as required for SI to be maintained over time. Allelic polymorphism at the S locus has been shown to date from 20 to 40 million years ago, a result expected under frequency-dependent selection. If one allele becomes too abundant in the population, plants carrying this allele have a disadvantage in finding a mate, whereas plants with rare alleles cross successfully with most others. Under frequency-dependent selection, genetic drift does not lead to the loss of alleles at the normal rate; therefore, ancient variants are maintained much longer. The Nasrallah group has found that in Arabidopsis the S locus is non-functional and contains pseudogenes for SRK and SCR, explaining the self-compatible mating of this colonizing weed.

Laurie Smith (University of California, San Diego) presented an elegant talk about her work on the cell biology of...
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leaf development. She briefly mentioned her published work on tangled (tan1), a mutant with abnormal cell divisions in all tissue layers of the leaf and elsewhere in the plant. Tan1 encodes a highly basic protein that can bind microtubules. She then discussed three classes of mutants (brick, discordea, and pangloss) that affect the proper orientation of asymmetric divisions in the leaf epidermis. Smith used most of her 40 minutes to discuss the brick mutants, in which the pavement cells of the leaf blade epidermis lack their characteristic lobed margins so that they superficially resemble the cells of the sheath epidermis (or bricks). The brick mutants enabled Smith and her student Mary Frank to test two models for the formation of the lobes during cell differentiation. One model is that the lobes are produced during cell expansion because of thick and thin bands of cellulose along the cell wall. When the cell expands, the regions of thinner cellulose deposition would bulge out to form the lobes. An alternate possibility is that the lobes are formed by tip growth, the same form of growth seen in pollen tubes or trichomes. Smith argued in favor of the tip growth model for lobe formation in part because the brick1 mutant also has short trichomes (reduced tip growth) and because actin is deposited in the lobes of epidermal cells, as expected for tip growth. Smith’s group has cloned Brick1 and found that it encodes a small protein of only 86 amino acids that also is found in humans, Drosophila spp, and Caenorhabditis elegans.

In has long been known that nuclear genomes tightly regulate the expression of chloroplast-encoded genes, but how this is accomplished is not understood. In this context, Alice Barkan (University of Oregon, Eugene) presented work from her laboratory on the control of chloroplast gene expression. Barkan’s group has used mutant screens to identify and clone nuclear genes that regulate the expression of genes encoded in the chloroplast genome. They initially screened Mutator populations for phenotypes suggestive of defects in photosynthesis (e.g., pale green seedlings) and performed secondary screens to identify mutants that failed to splice group II introns in the chloroplast. By this means, they identified and cloned the chloroplast RNA splicing (crs1 and crs2) genes. crs1 specifically functions in the splicing of a single group II intron, whereas crs2 assists in the splicing of multiple group II introns. crs2 is a homolog of peptidyl-tRNA hydrolase enzymes, but it has acquired several unique features that may account for its new function in group II intron splicing. crs1 is a member of a gene family of unknown function that is represented by 16 genes in Arabidopsis. Members of the crs1 family contain one or more copies of a 10-kD domain of ancient origin with features suggestive of a novel RNA binding domain. Evidence was presented that different members of the crs1 family in plants may function in the splicing of different group II introns, with one member of the family functioning in concert with crs2. The genetic approach being used by Barkan’s group is revealing at a fine molecular level how eukaryotic genomes have taken over the control of gene expression in the chloroplast, which is a relic of a once free-living bacterium.

FUNCTIONAL GENOMICS MEETS POPULATION GENETICS

A decade ago, one of the leading lights of maize genetics declared at a conference that he did not want to hear any talk about “allele frequencies.” Indeed, many academic departments were cleansing themselves of population genetics, so the discipline is now restricted largely to departments of evolutionary biology. The genomics era, with its mountains of sequence data and the puzzle of so many genes and so few known functions, has opened a crack through which population genetics is again slipping back into the mainstream of biology, although sometimes under the cloak of bioinformatics. The trend was visible at this year’s maize meeting, with three separate groups presenting experimental results on the association of phenotypes with sequence polymorphisms in populations, so-called association analyses (Cardon and Bell, 2001). The logic behind association analyses is simple, even if the statistics are not. First, one measures a phenotype (or hundreds of phenotypes) on individuals in a population. Second, one determines the sequence of a gene (or hundreds of genes) on the same individuals. Finally, one looks for statistical associations between the phenotypes and allelic variants at the genes.

Ed Buckler and his group (United Agricultural Research Service/North Carolina State University, Raleigh) presented three posters and a talk at the conference related to association analysis. Artfully leaving out the details of his statistical analyses, Buckler presented compelling evidence that a tryptophan-to-arginine substitution in sugary (su1), which encodes an isoamylase, gave rise to sweet maize (a conclusion presented independently at the meeting by Jason Dinges from Martha James’s group [Iowa State University, Ames] on the basis of biochemical evidence). Buckler went further and suggested that variation at su1 also is involved in making maize flour more palatable than was that of its ancestor, teosinte, by modifying the balance of different forms of starch in the endosperm. Jeffry Thornsberry of Buckler’s group presented equally compelling results that Dwarf8 (a transcriptional regulator of the SCARECROW class) controls natural variation in silking date in maize. Finally, Bradley Rauh, also of Buckler’s group, presented evidence that the prolamin binding box factor (Pbf) shows a strong signature of selec-
tion during maize domestication and is associated with natural variation in the abundance of protein in the endosperm.

As Buckler's group has shown, part of the promise of association analyses is that one can study dozens (or hundreds) of phenotypes and genes simultaneously without the need to develop specialized stocks or populations for each phenotype and gene. In principle, any variable phenotype in maize can be tested for association with any gene for which there is allelic variation. Part of the power and appeal of association analyses is that they can provide far greater resolution than quantitative trait loci (QTL) mapping. In theory, if there has been sufficient recombination, then association analyses will have the power to identify specific nucleotide or amino acid substitutions that control phenotypic differences among individuals.

Nevertheless, there is a clear risk that association analyses could give false-positive results under some circumstances, such as when the controlling polymorphism is of recent origin or is located in a region of suppressed recombination. For this reason, it is essential to investigate some cases in which the link between gene and phenotype is well established so that any misleading associations with neighboring genes can be exposed. To explore the utility of association analysis for U.S. breeding lines, Kelly Palaisa of Antoni Rafalski's group (DuPont Agricultural Genomics, Newark, DE) is examining polymorphism for yellow (y1), a gene with a well-understood phenotypic effect. y1 encodes phytoene synthase and controls yellow versus white kernel color. The Rafalski group was able to show that an INS2 insertion in the promoter of y1 is associated with the yellow kernel phenotype, but because there was extensive linkage disequilibrium at y1, the phenotype is associated with other polymorphisms as well. The Rafalski group will be extending their study to surrounding genes to learn if the yellow kernel phenotype is associated with polymorphisms in neighboring genes. This type of study will make clear whether there has been sufficient historical recombination in U.S. maize to break up the linkages between the polymorphisms in genes that control phenotypes of interest and polymorphisms in neighboring genes.

THE RICE GENOME PROJECT: A WAKE-UP CALL FOR THE MAIZE COMMUNITY

The meeting included a workshop on the rice genome project that featured eight talks organized by Sue Wessler (University of Georgia, Athens). Takuji Sasaki (National Institute of Agrobiological Resources, Tsukuba, Japan) provided a progress report on the International Rice Genome Sequencing Project (IRGSP), which is proceeding ahead of schedule (see http://rgp.dna.affrc.go.jp). A draft version of the rice genome sequence is expected in mid 2002, with a full, high-quality version due in 2004. In the meantime, anxious users can BLAST or download the unfolding public rice sequence via GenBank. Rod Wing (Clemson University, Clemson, SC) and Robin Buell (Institute for Genome Research, Rockville, MD) reported IRGSP's progress on rice chromosomes 3 and 10, Gerard Barry provided an overview of Monsanto's draft sequence of the rice genome, and Steve Briggs discussed Syngenta's draft sequence of the rice genome. Other talks in the workshop centered on developing new tools for rice genetics or applying old ones more effectively in a world in which the full rice genome sequence is on line. David McElroy of Maxygen spoke about Maxygen's "Delete-a-Gene" technology, which combines fast neutron mutagenesis and polymerase chain reaction screening of DNA pools to identify deletions in genes of known sequence. Masahiro Yano (National Institute of Agrobiological Resources) beautifully outlined how QTL for phenotypes such as heading date can be readily cloned once the full genome sequence is on line (Yano et al., 2000). Importantly, his research revealed that to do this, one needs complete access to the full genomic sequence.

One announcement of interest in the rice workshop came from Steve Briggs, who estimated that the rice genome contains 50,000 genes, fully two-thirds more than are estimated to be nested in the nucleotides of our own species. Given that maize, an ancient polyploid, could easily contain 50% more genes than rice, a "true" diploid, one might speculate that maize contains 75,000 genes. Is it conceivable that maize has twice the gene content of humans, with enough left over to encode the C. elegans genome? Venter et al. (2001), in their discussion of the surprisingly low gene content of humans, discuss an outdated population genetics theory that the maximum number of possible genes for humans is ~30,000 (see Muller, 1967). This argument is based on a maximum amount of genetic load given a certain mutation rate and fitness effects of the mutations. This theory implies that the unexpectedly low gene number in humans may reflect a limit imposed by genetic load. The theory was incomplete, however, because it did not consider the possibility of epistasis between deleterious genes (see Eyre-Walker and Keightley, 1999). Therefore, there is no theoretical reason that maize should not have twofold or more the number of genes as humans or that the human number should not be greater than 30,000.

If the rice workshop made anything clear to me, it was that the future of maize as a model organism is in jeopardy without the complete genome sequence. The elegant work of Yano et al. (2000) on the cloning of QTL and similar work in Arabidopsis (Alonso-Blanco and
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Koornneef, 2000) underscore the ease with which such work can be accomplished when the genome is fully sequenced; they also show the futility of working in systems that lack this advantage. Although the colinearity among the grass genomes will allow maize geneticists to play off the rice genome to some degree, it is no substitute for having the maize genome sequence itself.

A WORKSHOP ON MAIZE GENOMICS AND FUTURE DIRECTIONS

Torbert Rocheford (University of Illinois, Champaign-Urbana) moderated a workshop on maize genomics that featured a series of 12 five-minute updates on a selection of maize genome projects. Here are a few of the topics: Donal O’Sullivan (University of Bristol, UK) reported on his group’s analysis of the rp1 disease resistance locus; Michele Morgante provided an overview of DuPont’s bacterial artificial chromosome and expressed sequence tag libraries for maize; Pablo Rabinowicz from Rob Martienssen’s group (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) presented their strategy for sequencing hypomethylated portions of the maize genome; Tom Brutnell (Boyce Thompson Institute, Ithaca, NY) and Paul Chomet (Mystic Research, Mystic, CT) discussed improvements in technologies for forward and reverse genetics using Ac and Mu; Kan Wang (Iowa State University) discussed protocols for maize transformation using Agrobacterium tumefaciens; and Antoni Rafalski (Dupont Agricultural Genomics) discussed the effort to assemble a single nucleotide polymorphism database for maize.

After this whirlwind tour of maize genomics, Jeff Bennetzen (Purdue University, West Lafayette, IN) and the other members of the Maize Executive Committee stepped onto the stage to discuss future directions for maize. Bennetzen outlined the committee’s priorities for maize, and topping the list was sequencing of the maize genome. The announcement was followed by remarkably little discussion, reflecting the consensus among maize geneticists that the genome must be sequenced. The plan would involve sequencing ~350 Mb of the 2500-Mb maize genome, restricting the effort to hypomethylated or gene-rich regions. The combination of the sequence of the gene-rich regions for maize and the complete sequence for rice would keep maize genetics competitive with other model organisms. The greatest challenge may be to avoid the problems that have arisen with other genome projects when commercial and public efforts were either uncoordinated or antagonistic. With this in mind, the Executive Committee has proposed to organize a workshop on maize genome sequencing involving the key public laboratories and companies such as DuPont, Syngenta, and Monsanto.

POSTERS AND SHORT TALKS

It would be hopeless for me to try to summarize the 200 posters and short talks and worthless for me to present a single sentence on each of a dozen or so. So with my apology to those not mentioned, let me discuss three of my favorites at slightly greater length.

Quantitative genetics (the inheritance of quantitative traits) operates on the assumption that variation is under genetic control with some environmental noise. Epigenetics has not been factored into the linear models usually used in quantitative genetics, yet epigenetic mechanisms may contribute to quantitative variation. Ken McWhirter, Julie Zinnert, and Bill Eggleston (Virginia Commonwealth University, Richmond) presented a poster on the interface of quantitative genetics and epigenetics. Their work is on the R locus, which is a regulatory gene controlling anthocyanin deposition in the aleurone of developing maize kernels. R is one of several loci in maize that have served as models for the investigation of paramutation. It is known that the level of R gene expression for the paramutable alleles is inversely correlated with the level of methylation. McWhirter and colleagues isolated a series of R alleles that no longer participated in paramutation but that exhibited a range of phenotypes from pale to dark purple kernels. They further reported that this quantitative variation was correlated with the level of methylation at the 3’ end of the gene. No structural differences were found among these derived alleles, suggesting that differences in their epigenetic state (i.e., methylation) underlie the observed quantitative variation. Because the variation was stable, heritable, and likely epigenetic, it raises the possibility that quantitative variation in populations may be controlled in part in an epigenetic manner. McWhirter and colleagues speculate that this phenomenon may occur at genes throughout the genome. This mechanism, if it is found to be general, will require the extension of quantitative genetic models to accurately describe the inheritance of quantitative variation.

There remains much to learn about the fine structure of the maize genome and the extent to which the structure varies among regions. Pioneering work from Jeff Bennetzen’s laboratory has indicated that the maize genome has its genes scattered between long runs of retrotransposons, the latter making up 60% or more of the genome (San Miguel et al., 1996). Bennetzen’s work confirmed data from DNA reassociation kinetics that also indicated that in maize, the genes are like islands in a sea of repetitive DNA (Hake and Walbot, 1980). Although this model certainly still holds, Huihua Fu of Hugo Dooner’s group (Rutgers University, Piscataway,
NJ) found a striking exception to the rule. In a 32-kb stretch surrounding the bronze locus on chromosome 9, there are 10 genes without any intervening retrotransposons. The average distance between transcripts was a mere 1 kb. Dooner’s group speculates that there may be a connection between the high gene content of this region and the fact that bronze has the highest known recombination rate for a maize gene. Their observation also raises a number of questions about the structure and history of the maize genome. How frequent are such regions of high gene density? During the expansion of the retrotransposon families, why did the bronze region escape being infiltrated by retrotransposons, or were they once there and subsequently lost?

An interesting question for a large genome plant such as maize is: how far upstream of a coding sequence can regulatory elements be found? Generally, in plants, all elements necessary for proper tissue-specific expression are located within a few kilobases of the coding sequence, and because Arabidopsis has one gene approximately every 5 kb, it would seem that regulatory elements are usually nearby in plants. In this context, Maike Stam from Vicki Chandler’s laboratory (University of Arizona, Tucson) reported the mapping of both an enhancer and sequences for paramutation of the b1 locus between 90 and 103 kb upstream of the coding sequence. This observation suggests that there is long-range communication between these distant regulatory elements and the proximal promoter. If such distant regulatory elements are a common feature in maize and other large genome plant species, they will pose a special challenge to molecular quantitative geneticists, because it is a clear possibility that such distant enhancers may figure prominently in the subtle quantitative effects that contribute to natural variation and evolutionary change.

SEE YOU NEXT YEAR

Next year, the Maize Genetics Conference moves south to Orlando, Florida, for a warmer venue. The Maize Steering Committee has reserved extra rooms, anticipating that many will bring their families along for a premeeting or post-meeting trip to Disney World. With the anticipated record-breaking attendance, it would be best to register early.

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Secretory proteins are first inserted cotranslationally into the endoplasmic reticulum (ER) and then transported through the secretory pathway to the cell surface or to inner hydrolytic compartments in a traffic process that is mediated mostly by the Golgi complex. Addition of the oligosaccharide glycan Glc₃Man₉GlcNAc₂ to Asn residues (N-glycosylation) is a common cotranslation modification of secretory proteins (reviewed in Helenius and Aebi, 2001). Although individual glycoproteins exist that are not affected markedly when synthesized without glycans, no eukaryotes have been identified that can survive without N-glycosylation, indicating that some proteins need N-linked glycans to function properly and/or to avoid rapid degradation.

Depending on the conformation of the glycoprotein and the cell type, the oligosaccharide can be modified after transfer to the polypeptide, mainly by glycosidases and glycosyltransferases present in the Golgi complex (reviewed in Lerouge et al., 1998). Golgi-mediated modifications are critical in vertebrates, in which they determine the life of many serum proteins and specify cell identity, but they are dispensable for plant life (von Schaewen et al., 1993) and are very much simplified in yeast. The first modifications of N-linked glycans, however, occur before glycoproteins leave the ER. In a process that seems to be common to all eukaryotes, the oligosaccharide is modified within minutes after the completion of protein synthesis by the removal of the three terminal Glc residues and the transient readaddition of one Glc residue through the action of ER-located enzymes. This appeared for years a puzzling process, until it was discovered that two lectins that reside in the ER, calnexin and calreticulin, exclusively bind glycans with the structure Glc₁Man₀GlcNAc₂ and that the readaddition of one Glc residue occurs only on glycoproteins that have not yet reached the correct final folding or assembly (reviewed in Helenius and Aebi, 2001). Thus, the structure recognized by the lectins is present only in folding and assembly intermediates or in structurally defective (misfolded) proteins. The cycle of removal and addition of Glc, therefore, is part of the quality control mechanism that operates in the ER to ensure that only structurally correct proteins proceed along the secretory pathway: newly synthesized glycoproteins are retained in the ER by interactions with the lectins and, during the cycles of binding and release, have the opportunity to fold properly. Defective glycoproteins eventually are targeted for degradation (Helenius and Aebi, 2001).

Two glucosidases operate in the ER: glucosidase I removes the outermost, α1,2-linked Glc residue; subsequently, glucosidase II removes the two remaining α1,3-linked residues and the single residue that is readded by the ER-located glucosyltransferase. Two recent articles report the phenotypes of plants in which the synthesis of these ER-located glucosidases has been inhibited genetically (Taylor et al., 2000; Boisson et al., 2001).

An Arabidopsis mutant impaired in the synthesis of glucosidase I was isolated during the screening of a T-DNA insertion collection in a search for mutants affected in seed development (Boisson et al., 2001). The mutation is lethal in the homozygous state: the seeds do not germinate, and the embryo is blocked at the heart stage. The mutant seeds also lack typical storage vacuoles, have abnormally enlarged cells, and have altered cell walls. Glycan modifications occurring in the Golgi complex begin with the removal of part of the Man residues by Golgi mannosidase I, followed by the addition of one terminal GlcNAc residue by Golgi GlcNAc-transferase I. The structure that is formed, GlcNAc₁Man₀GlcNAc₂, is then subjected to further Golgi modifications by other glucosidases and by glycosyltransferases that mediate the addition of GlcNAc, Fuc, Xyl, and Gal residues to produce the so-called complex glycans (Lerouge et al., 1998). The actions of mannosidase I and GlcNAc-transferase I, which require the previous removal of the three Glc residues, are committed steps in the production of complex glycans. Consistently, the glycoproteins of the Arabidopsis mutant seeds lack the typical complex structures and contain exclusively Glc₃Man₇₋₈GlcNAc₂ glycans (Boisson et al., 2001).

However, it is unlikely that the absence of complex glycans on glycoproteins is the causal agent of the lethal phenotype, because it has been shown that an Arabidopsis mutant lacking GlcNAc-transferase I does not have growth or reproduction defects (von Schaewen et al., 1993). This strongly suggests that the severe phenotype of the glucosidase I mutant is attributable to the fact that ligands for calnexin and calreticulin are not generated, impairing...
ER quality control (Boisson et al., 2001).

The synthesis of ER glucosidase II was inhibited in potato by antisense downregulation (Taylor et al., 2000). These transgenic plants have no apparent phenotype when grown in the greenhouse, but they have severely reduced growth and tuber production when grown in the field. They also show signs of plasmolysis and have reduced cell wall lignification and pectin content. This is accompanied by an increase in transcripts for BiP, the HSP70 chaperone of the ER. BiP also is part of the ER quality control system and has a more general role than the mechanism based on the terminal Glc residues, because it recognizes the polypeptide backbone of nascent or defective secretory proteins (Vitale and Denecke, 1999). Most likely, BiP transiently binds hydrophobic sequences exposed in folding and assembly intermediates, inhibiting irreversible aggregation in the crowded ER environment. When proteins are correctly folded and assembled, these sequences are buried inside the protein, effectively concealing BiP binding sites.

An increase in BiP synthesis is diagnostic of ER stress and is induced by conditions that negatively affect protein folding in the ER (Vitale and Denecke, 1999). This induction, termed “unfolded protein response,” is common to the other helpers of folding in the ER, such as protein disulfide isomerase and calreticulin (Shorrosh and Dixon, 1991; Denecke et al., 1995). Thus, transgenic potato plants appear to undergo ER stress when grown in an open field. Taylor et al. (2000) hypothesize that the lack of glucosidase II activity impairs the calnexin/calreticulin quality control mechanism, negatively affecting the folding of newly synthesized glycoproteins, and that the increase of BiP synthesis is a partial compensation for this defect.

Why is the phenotype manifested only in the field? This is not easy to explain, but possibly the stress is favored by the more severe environmental conditions experienced in an open field than in the greenhouse (Taylor et al., 2000). It also should be noted that the antisense strategy greatly reduces but does not completely block glucosidase II activity. Residual glucosidase II activity may be sufficient to create substrates for calreticulin and calnexin, provided that growth conditions are optimal.

Pulse chase experiments using ER glucosidase inhibitors in developing cotyledons or plant cell cultures have failed to show adverse in vivo effects on protein intracellular transport (Chrispeels and Vitale, 1985; Lerouge et al., 1996). Plant calnexin (Huang et al., 1993) and calreticulin (Denecke et al., 1995) have been cloned, and interactions have been shown between calnexin and the newly synthesized subunits of vacuolar H\(^+\)-ATPase (Li et al., 1998) and between calreticulin and BiP (Crofts et al., 1998). However, glycan-mediated binding of glycoproteins to calnexin or calreticulin in plants has not been demonstrated directly. The involvement of Glc residue removal in regulating glycoprotein assembly in the plant ER has been shown using an in vitro translation and translocation system combined with ER glucosidase inhibitors (Lupattelli et al., 1997). The studies by Taylor et al. (2000) and Boisson et al. (2001) extend this observation, showing that impairment in Glc removal has adverse effects on plant growth and therefore that ER quality control probably is fundamental for the synthesis of secretory proteins required for plant viability. Indeed, several plant embryogenesis mutants are affected in other aspects of the secretory pathway, providing support for the notion that its full integrity is critical for plant life (reviewed in Sanderfoot et al., 2000; see Lukowitz et al., 2001).

The in vivo pulse chase experiments performed previously may have been too limited in both the length of treatment with glucosidase inhibitors and the plant material used to allow the detection of detrimental effects (Chrispeels and Vitale, 1985; Lerouge et al., 1996). It will be interesting to determine if only a few key proteins are affected negatively by the absence of Glc removal or if this is a more general phenomenon.

The common detrimental effect on cell wall structure observed in the two studies is particularly intriguing. In yeast, ER glucosidase I and II are not essential for growth, but mutated strains have defects in cell wall synthesis and reduced levels of 1,6-β-glucan (Simons et al., 1998). One of the possibilities raised at the time of this discovery involved the glycosidases as processing enzymes of a hypothetical transient primer for the synthesis of 1,6-β-glucan. However, it has been shown that this is not the case; instead, one Golgi protein involved in the synthesis of 1,6-β-glucan is selectively unstable in strains defective in glucosidase I (Abeijon and Chen, 1998). In this respect, it is interesting that potential N-glycosylation sites are present in RSW1, a catalytic component of cellulose synthase (Arioli et al., 1998), and in two plant glycosyltransferases for which a role in the synthesis of matrix polysaccharides of the cell wall has been confirmed (Edwards et al., 1999; Faik et al., 2000). Moreover, the lethal cyt1 mutation of Arabidopsis, which results in N-glycosylation deficiency (because it affects the enzyme that produces GDP-Man, a precursor for the synthesis of N-linked glycans), also causes a marked reduction in cellulose content (Lukowitz et al., 2001). This may be attributable to the synthesis of unglycosylated RSW1 or the unglycosylated form of other unknown subunits of cellulose synthase (Lukowitz et al., 2001). Therefore, plant glycoproteins necessary to synthesize the cell wall may require perfectly functional quality control in the ER, acting through the cycle of Glc removal and addition.
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Uncovering Secretory Secrets: Inhibition of Endoplasmic Reticulum (ER) Glucosidases Suggests a Critical Role for ER Quality Control in Plant Growth and Development

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