Meeting report

Frontiers in plant genetics

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A report of the American Society for Plant Biologists' meeting 'Plant Genetics 2003: Mechanisms of Genetic Variation', Snowbird, Utah, USA, 22-24 October 2003.

This very successful meeting ranged widely, covering topics such as natural variation, speciation, epigenetics, chromosome structure and developmental genetics. The common theme of using genetics to address biological questions served to bring together scientists who do not often attend the same meetings.

The analysis of natural variation is particularly interesting in regard to the spread of plants into new areas and the domestication of crops. Barbara Schall (Washington University, St Louis, USA) studies tamarisk, a plant that is on the 'most wanted list' of invasive species in the United States. Tamarisk grows naturally in Iran, where it is controlled easily by natural predators, including camels, but in the western USA, tamarisk has dislodged the natural desert flora by draining the limiting supply of water with its long tap-root. In Iran, two genetic varieties of tamarisk are found in separate regions, and Schall described how these two varieties overlap geographically in the US, resulting in hybrids. The invasiveness of this pest species may result from hybrid vigor.

Cris Kuhlemeier (University of Bern, Switzerland) has been studying the evolution of different pollination mechanisms in petunia species. *Petunia axillaris parodii* has white flowers with a long corolla tube and is pollinated by moths. *P. integrifolia inflata* has pink flowers, a short corolla and lacks the odor and abundant nectar found in the white species; it is pollinated by bees. Although the two petunia species do not cross in nature, they do in the laboratory, allowing Kuhlemeier to make recombinant inbred (RI) lines that vary in flower color, odor and shape. He reported that, interestingly, hawkmoths preferentially pollinated, and bees avoided, an RI line that was white, even though it had the shape of the *P. integrifolia inflata* flower. As petunias are genetically transformable, Kuhlmeier tested this preference by adding a color gene to the white RI line. Hawkmoths were able to distinguish this line from the otherwise identical white line and avoid it.

Hybridization appears to have played a part in the ecological divergence of wild sunflowers. From two parental species, three ancient hybrids are thought to have evolved, each of which now inhabits a different environment, and the hybridization is thought to be of adaptive significance. Loren Rieseberg (Indiana University, Bloomington, USA) described a comparison of the natural hybrid sunflowers with synthetic hybrids made by the researchers, at the genome level and for their ability to survive in salt-marsh conditions. Both the ancient and synthetic hybrids that were able to survive well in the salt marsh contained the same combinations of parental chromosome segments, which conferred on them the remarkable ability to survive in this harsh environment.

Chromosomes and enigmatic mutations

Two speakers highlighted the unique DNA sequences found at the ends and centers of chromosomes. Dorothy Shippen (Texas A&M University, College Station, USA) discussed her work on *Arabidopsis* mutants lacking telomerase. The enzyme telomerase is required to maintain the length of the protective telomeres at the ends of the chromosomes; in the mutant, the telomeres get shorter and shorter in each successive generation produced by self-pollination. After six selfings, Shippen and her colleagues found that the loss of telomere function caused chromosomal DNA bridges to form at anaphase of mitosis, producing cycles of chromosomal breakage and fusion. They amplified the DNA across the fusion junctions and found that the sequence at this repair point is not found anywhere else in the genome. Kelly Dawe (University of Georgia, Atlanta) provided a look at the centromeres of maize chromosomes. He has found retrotransposons at the centromere that are not found elsewhere in the maize genome but that are conserved in other cereals. The variant histone CENH3 is found at the centromere and can

be immunoprecipitated with the transposon DNA, suggesting that it is bound to it. Although the centromere is considered to be inactive chromatin, the centromere retrotransposon is transcribed.

A question that has interested biologists for years is the interaction of loci during interphase. Are the interacting alleles from homologous chromosomes found near each other? Antonius Matzke (Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg, Austria) described a technique to visualize transgenes in interphase nuclei, with which he observed that alleles are not found near each other.

Robert Pruitt (Purdue University, West Lafayette, USA) provided some enigmatic data that captivated the audience. His *Arabidopsis* mutant *hothead*, which was induced by ethane methyl sulfonate mutagenesis, reverts at a frequency of about 1-15%, whereas reversions normally never occur. The reversion is a single-nucleotide change back to the correct nucleotide. Speculation went fairly wild in trying to explain this finding, but included cytoplasmic RNA templates that preserve a copy of the wild-type sequence and allow reversion at such high frequency.

Plant proteins on the move

David Jackson (Cold Spring Harbor Laboratory, New York, USA) reported studies on the direct cell-to-cell movement of the homeobox protein Knotted1 (Kn1) during leaf development. He has shown previously that the direction of protein transport is differentially regulated in the leaf and meristem. Kn1 moves from the mesophyll (inner layer) to the epidermis in the leaf, but not *vice versa*, whereas in the meristem, Kn1 moves in both directions. He has also devised a movement assay using Kn1 and the protein Glabrous1 (Gl1), which is required in the epidermis to produce trichomes. In a *gl1* mutant plant, which normally makes no trichomes, expression of wild-type Gl1 in internal mesophyll tissues under the direction of the ribulose biphosphate carboxylase promoter did not rescue trichome formation, but expression of a fusion of Gl1 and Kn1 did. This indicated that the fusion protein had moved into the epidermis from the mesophyll, where it was made, under the influence of the Kn1 moiety. This assay revealed that movement is selective. For example, the *Arabidopsis* KNOX homeobox proteins Stm and Knat1 could both substitute for Kn1, but Knat2, 3, 4, 6 or an interacting Bel1-related protein did not.

Jackson also confirmed that Kn1 can specifically cause the movement of its own mRNA into the cells where it is normally expressed. A stop codon was introduced into the *GL1-KN1* construct after the *GL1* coding region, so that only the Gl1 protein was translated. When this protein fusion was expressed from the mesophyll-specific promoter, no trichomes formed. When Jackson expressed this construct in plants constitutively expressing Kn1, however, trichome formation was rescued, indicating that Kn1 can transport the *GL1-KN1* fusion mRNA from the inner leaf to the epidermis. This system is now being used to dissect the trafficking signals and to screen for mutants in cell-to-cell trafficking.

Chromatin and RNA

Genes encoding the Polycomb group of proteins are involved in chromatin and required for proper fertilization and embryo development. Ueli Grossniklaus (University of Zurich, Switzerland) explained that known proteins in this group in *Arabidopsis* include Fis, Fie and Mea, and gel-filtration experiments show that Mea is part of a protein complex of 650 kDa. A newly discovered protein, Msi-1, also a member of the Polycomb group, is part of this 650-kDa protein complex, and when *MSI-1* is mutated, it produces a similar fertilization phenotype to mutations in *MEA*. Grossniklaus reported the results of gene-expression studies in recently pollinated *mea* and *fie* mutants. Two genes consistently upregulated in the *mea* and *fie* mutant background were the MADS-box gene *PHERES* and the gene encoding S-phase kinase-associated protein 1 (SKP1), a core component of the SCF ubiquitin-ligase complex that marks cell-cycle regulators and transcription factors for degradation. *PHERES* is normally expressed in embryos and endosperm at early stages of development only. In *mea* mutants, however, expression is high and stays high. Using chromatin immunoprecipitation, Grossniklaus's group was able to show that the *PHERES* promoter was specifically immunoprecipitated by antibodies to Mea or Fis. Thus, the *PHERES* gene is likely to be a direct target of the Mea and Fis proteins. Grossniklaus also reported that lowering the level of *PHERES* transcripts by antisense techniques rescued the *mea* phenotype. Thus, *PHERES* is a direct target of Mea and its lack of repression is responsible for a large part of the *mea* mutant phenotype.

Regulation by RNA was a topic that threaded through a number of talks. Bonnie Bartel (Rice University, Houston, USA) gave an historical overview of the recent discovery of microRNAs in plants. John Bowman (University of California Davis, USA) described work showing that the genes of the HD-ZIP family, which encode transcription factors that regulate leaf dorso-ventral development, are likely to be controlled by microRNAs. Bowman has found HD-ZIP genes in all the plant species he has examined, including non-seed plants such as lycopods and ferns. Interestingly, the microRNA binding site is conserved at the nucleotide level throughout plants, suggesting that regulation by microRNAs may be an ancient mechanism.

The meeting was a great success and is likely to be repeated in two years' time. Plants are special in that they provide a glimpse into evolution as it happens. Given that pollinations can occur between distantly related species, geneticists can cross different species to ask how varieties arise and what

genes are selected for in different environments; Rieseberg's talk on sunflower hybrids is a good example of this. As more genomes are sequenced and scientists move from model organisms to plants in the wild and crops, the stories can only get better.