

Meeting report

Communal weeding

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A report on the first annual Genomic *Arabidopsis* Resource Network (GARNet) meeting, York, UK, 2-3 October, 2000.

Over 200 researchers from the UK plant research community met to find out more about the functional genomics resources that will become available through the GARNet program [<http://garnet.arabidopsis.org.uk>], which is funded by a £4 million grant from the UK BBRSC Investigating Gene Function (IGF) initiative. Such resources will include transcriptome analysis, bioinformatics, metabolite profiling, proteome analysis and tagged genes. Presentations from international speakers on the progress being made with functional genomics tools put the project in context and provided insights into how useful such shared resources can be.

Transcriptome analysis

Pam Green (Michigan State University, USA) described successes from initial rounds of the NSF-funded Arabidopsis Functional Genomics Consortium (AFGC; [<http://afgc.stanford.edu>]) microarray service, which has developed microarrays containing 11,000 expressed sequence tagged (EST) clones representing about 8,000 unique genes. Applications are submitted via the web, and data from completed experiments are made public either immediately or within three months, depending on customer preference. Green used the *dst1* mutant, which results in defects in sequence-specific mRNA decay, as an example of the usefulness of microarrays for exploring previously recalcitrant regulatory pathways. Although *dst1* plants have no visible phenotype, a comparison of their transcript profiles to that of wild-type parental plants revealed new molecular phenotypes that can provide insights into the role of the mutant genes and the pathway they affect. Green also described progress toward monitoring the stability of thousands of mRNAs simultaneously.

These studies identified at least 50 genes that correspond to unstable mRNAs with a half-life of less than 60 minutes.

An important outcome of such profiling studies will be the use of 'spot histories' - the behavior of a particular gene through the range of experimental applications - for predictive modeling of gene networks. Such analyses will provide insights into signaling pathways involved in development and maintenance processes, with the ultimate goal of creating a virtual plant. The difficulties of comparative experimentation in plant biology, particularly having to deal with different accessions, variable growth conditions and the underlying diurnal and circadian rhythms, were highlighted in Green's talk and subsequent presentations.

Gene tags

The *Arabidopsis* Transposon Insertion Service (ATIS) is focused on the systematic production and sequencing of transposon tags. Jon Clarke (John Innes Centre, Norwich, UK) outlined ATIS's goal of providing sequence insertion sites (SINS) through a GARNet-funded initiative at the John Innes Centre [<http://www.jic.bbsrc.ac.uk/STAFF/michael-bevan/ATIS/index.htm>]. SINS are being generated from two existing populations - SLAT (48,000 insertions) and ACTIVATE (2,000) - and a third population, FGT (30,000) currently being produced as part of the Functional Genomics Technologies initiative. The populations encompass gene disruption (loss-of-function), activation tag (gain-of-function) and gene trap (gene expression pattern) lines, respectively. SINS will be integrated with the *Arabidopsis* Genome Initiative gene models within the ATIS database (expected launch date is December 2000). This database allows query-based searches via a web interface, including searches by chromosome, genetic or molecular marker, BAC/P1 location, protein class, transposon class or by BLAST. A controlled vocabulary for defining gene

expression patterns is also being developed in collaboration with Sue Rhee at the *Arabidopsis* Information Resource [<http://www.arabidopsis.org/>]. This will provide a basis for text search features within an extended *Arabidopsis* Transposon Insertion (ATI) database holding gene expression patterns generated by the FGT gene trap lines. By the completion of the project in 2003, it is anticipated that 20,000-30,000 SINS will be available to the research community, with seed, or seed pools, associated with each SIN distributed by the Nottingham *Arabidopsis* Stock Centre (NASC).

The value of a publicly accessible functional resource was echoed by Rob Martienssen (Cold Spring Harbor Laboratory, New York, USA), who has been instrumental in developing the [*Arabidopsis* Genetrap database] [http://spot.cshl.org/genetrap_database/mainframe.html], which describes a collection of transposon insertion lines generated in the Martienssen lab. Analysis of the insertion sites revealed a nonrandom distribution of transposons. Transposon insertions in both the nucleolar organizer region and heterochromatic knob were under-represented, whereas significant insertion hotspots were found in chromosomes V and II. In addition to the utility of being able to examine cell-specific expression patterns of heterozygotes, the insertion lines allow the question of genetic redundancy to be addressed. The analysis of *Arabidopsis* genome sequence has revealed significant (80%) segmental duplication, resulting in genes with common function (for example, *AGL1* and *AGL5*). Consequently, many disrupted genes give no phenotype. Individual members of a gene family can be knocked out and the resultant homozygous lines analyzed individually or in combination with other family members. The power of this technique was illustrated through the analysis of double and triple mutants of the homeotic genes *ap1/cal/ful*, which revealed a function for *ful* in flower development previously obscured by redundancy with *ap1* and *cal*.

Proteomics

In plants, proteomic analyses are less well developed than genomic analyses but are an essential step towards our understanding of coordinated gene function. Traditional two-dimensional (2D) electrophoresis continues to provide the basic technology for comparative proteomics. Paul Dupree (University of Cambridge, UK) described advances using dual Cy3/Cy5 labeling that enable the comparison of two proteomes on a single 2D gel. Justin Roberts (University of California, Riverside, USA), described the elegant use of affinity chromatography to selectively purify and concentrate classes of proteins that are not accessible using conventional subcellular fractionation techniques. The choice of resins with specific immobilized nucleotides or broad-spectrum metabolite analogs (dyes), coupled with sequential elution, enabled the resolution of classes of proteins with specific biochemical properties. In addition to achieving significant enrichment, affinity purification also facilitated the

application of mass spectrometry (MS) and 2D electrophoresis to study associated multisubunit protein complexes. The selective use of ligands also has applications in the use of combinatorial chemistry in drug and herbicide discovery. Roberts tempered the discussion with a cautionary tale of technical bottlenecks, particularly the difficulty in achieving *in vivo* labeling reproducibly.

Bottlenecks in sample preparation were also addressed by Johan Gobom (Max-Planck-Institute for Molecular Genetics, Berlin, Germany). Gobom discussed improvements in high-throughput screening through the application of advanced materials technology. These included arraying multiple samples using 200 µm colloidal gold particles as hydrophilic 'anchors' on hydrophobic Teflon-coated matrix-assisted laser desorption ionization (MALDI) probes, and the rapid purification of 2D spots for MALDI-MS. The latter technique used pre-structured sample supports that efficiently bind peptides within 10 seconds of contact, enabling rapid and quantitative removal of salt, thus removing a major restriction to high-throughput MALDI screening. This clearly highlighted the value of on-site integration of plant functional genomics programs with related projects. Although only briefly touched on, systematic large-scale yeast two-hybrid screens, protein crystallization and associated structural genomics undertaken at 'The Protein Structure Factory' [<http://userpage.chemie.fu-berlin.de/~psf/>] clearly bear this out.

Metabolomics

A highlight of the meeting was the demonstration of the power of 'metabolomics' by Oliver Fiehn (Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany). Using a combination of gas chromatography (GC)-MS and liquid chromatography (LC)-MS, 1,000 distinct compounds can be distinguished in *Arabidopsis* leaf extracts, although less than 20% have so far been identified. New compounds being discovered include citramalate, which suggests the existence of a tricarboxylic acid cycle bypass previously found only in bacteria. In a refreshing approach, Fiehn described how data mining is accomplished in a nonbiased way by the application of cluster analyses on large data sets to generate testable hypotheses. Metabolic phenotypes derived by principal components analysis are assigned to 'cliques' of metabolic profiles. By interpreting linear correlations between metabolic ratios as enzymatic links, metabolic networks can be constructed, and then tested through a combination of biochemical and genetic approaches. The use of metabolic profiling to complement existing functional genomics techniques is elegantly illustrated in Figure 1, where the metabolic phenotypes of Columbia-0 wild type, C24 wild type, and the corresponding F1 progeny are visualized. Fiehn also showed how a phenotypic dwarf mutant *dgd1*, which is altered in the biosynthesis of a thylakoid membrane lipid,

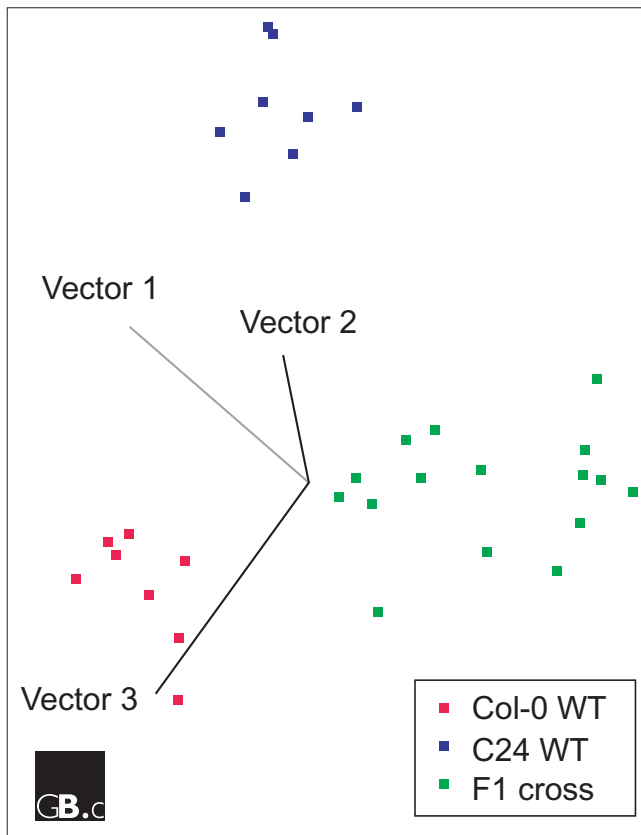


Figure 1

Principal component analysis visualization of the 'metabolic phenotypes' of Columbia-0 wild type, C-24 wild type, and the corresponding cross (F1 generation). Plants grown under controlled environmental conditions were harvested at the same developmental stage. Eight pools, each containing eight plants, were extracted and analyzed by 'metabolite profiling' (for protocols, see <http://www.mpimp-golm.mpg.de/fiehn/index-e.html>). GC/MS was used to automatically quantify 500 metabolites. Principal components analysis (which uses an n -dimensional vector approach) was undertaken to summarize metabolic profiles covering 500 metabolites. After application of PCA algorithms, vectors 1, 2 and 3 were chosen to best represent the distinct metabolic profiles associated with each genotype. Each point in the parental lines represents a linear combination of all the metabolites from an individual sample. Courtesy of Oliver Fiehn, with acknowledgements to Thomas Altmann and Bernd Essigmann for contributions to the experimental design.

clustered with its parental accessions despite having a significant percentage of its metabolites altered, thus reinforcing the theme of distinct genotypic metabolic profiles. Discussion centered on the way to refine such analyses, in particular the possibility of profiling subcellular organelles rather than heterogeneous tissue samples.

The meeting succeeded in bringing together the major players in Europe and the USA, and should stimulate greater collaboration, for example in the provision of gene-specific tags (GSTs) and informatics resources. The meeting also

provided the platform to launch the IGF Brassica Functional Genomics initiative, which will interact closely with GARNet. Ottoline Leyser (University of York, UK; a GARNet principal investigator) hopes results arising directly from this initiative will be discussed in future meetings, which will be crucial to assess the progress of the different technologies and to shape research policy for future initiatives.