

Meeting report

## Plant and animal genomes achieve functionality

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A report on the conference 'Plant and Animal Genomes XIII', San Diego, USA, 15-19 January 2005.

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The early 1990s saw the birth of genomics, as high-throughput techniques coupled with early robotics and bioinformatics enabled large-scale data collection from genomic and expressed sequence tag clones. In agricultural research, the molecular maps being created for more and more species enabled researchers to consider marker-assisted selection for animal husbandry and plant breeding. Map-based cloning became a reality in several model species, such as *Arabidopsis* and tomato, and quantitative trait loci in crop plants and domesticated animals became more amenable to analysis by molecular genetics. Throughout this time, the annual Plant and Animal Genome (PAG) conferences, established nearly 15 years ago, have provided a much-needed meeting place and information exchange for those working on the technologies of plant and animal genomics. This year's conference, with more than 2,000 participants, represented a quantum leap forward, as the laborious tasks of sequence data collection, map and library construction, and development of analytical tools are bearing fruit in the form of functional analysis.

One such area of advance was presented by Masahiro Yano (National Institute of Agrobiological Studies, Tsukuba, Japan) who reported work on the network of genes that control the time of flowering (heading) in rice. A network of heading (*Hd*) genes has been cloned, and includes *Hd5a*, encoding a transcription factor that binds to the CAAAT sequences in DNA, and *Hd6*, encoding a protein kinase. The analysis reveals similarities to, and differences from, the control of flowering time in *Arabidopsis*, suggesting that the crucifer paradigm requires modification for other plant species. For example, rice *Hd1* is an ortholog of *Arabidopsis* *CONSTANS* (a key regulator of the long-day-dependent flowering pathway) and *Hd3a* is similar to the *Arabidopsis* *FLOWERING LOCUS T*, which promotes

flowering, but rice *Lhd4* (*CONSTANS*-like with characteristics of a retroviral gag-pol protein) and *Ehd* (which promotes flowering in both short and long days, and acts via a two-component signal transduction system) have no apparent orthologs in *Arabidopsis*.

Dick Flavell (Ceres Inc, Thousand Oaks, USA) described the application of high-throughput expression testing in *Arabidopsis* to discover genes that influence plant performance and architecture. He described how 30,000 cDNA clones were expressed from a strong promoter in *Arabidopsis* and multiple transformants for each clone were put through a gauntlet of 30-40 screens of different environmental nature, such as high and low nitrate, high and low phosphate, and salt stress. Between 5 and 10% of the transgenic plants showed phenotypic variation, but as the plants were grown on agar plates with unusually high levels of nutrients in Murashige and Skoog nutrient medium and were supplied with sucrose through the roots, one awaits phenotypic verification in soil-grown tests. From the results of these screens, Flavell concluded that "plant yield is controlled by architecture, nutrients and stress tolerance". I personally welcome the renewed recognition of the importance of plant architecture, as major agronomic advances over the last 6,000 years have come from its optimization towards human needs, for example, improvements in bread wheat, harvest index (the portion of the total plant mass used in the harvest), developments of the cabbage family, and many others.

On a historical note, William Haseltine (Boston, USA), who describes himself as a molecular anatomist, discussed the development of the Human Genome Project and projected the need to go from bench to bed - the medical equivalent of farm to table. He reported that the human haplotype map (HapMap) currently contains 10 million single-nucleotide polymorphisms (SNPs), most of them as yet unpublished, which will aid detection of disease susceptibility and guide drug treatments. Amazingly to those of us who work on other organisms, Haseltine concluded that the human

genome is not yet completely known, pointing out the recent publication in *Science* of 10,000 new poly(A)+ transcripts from liver.

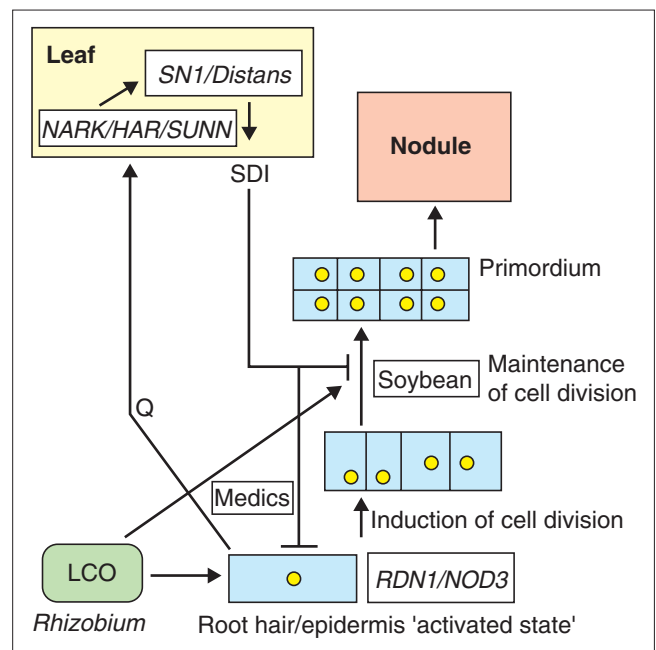
Gene discovery and the recognition of relevant expression patterns by the use of promoter-reporter gene fusions, or by transcriptome, proteome or metabolome profiling, continue to be a major part of the conference. As techniques for evaluating the functions of genes discovered in this way, RNA interference (RNAi) and targeted identification of local lesions in genomes (TILLING) were highlighted in the workshop 'Functional Genomics with TILLING and Eco-TILLING Technology'; 13 years ago, we would never have thought that a PAG conference would be addressing ecology. In Eco-TILLING, variation among organisms is detected by PCR and mismatches are recognized from their cleavage by the nuclease *CelI*. A similar approach is used when looking at experimentally induced genetic variation, for example in an EMS-M2 population, the second generation after seed mutagenesis. On the technical front, working with pea and soybean, Abdel Bendahmane (Unité de Recherche en Génomique Végétale, Evry, France) reported that a newly discovered enzyme (*endoI*) is highly specific at recognizing and cleaving mismatches in DNA, perhaps surpassing the specificity of the *CelI* enzyme traditionally used in the TILLING technique. Examples of the isolation of mutations via TILLING were presented by Bendahmane and others from the model legume *Lotus japonicus*, pea, *Glycine max* (soybean) and *Medicago*. As many as nine SYMRK mutants (affecting the symbiosis receptor kinase needed for early mycorrhizal development in legumes) were isolated from 2,304 M2 plants.

The nodulation of legumes by nitrogen-fixing bacteria featured in several presentations, with emphasis on the autoregulation of nodulation (AON) mechanism. It is already known that supernodulation, characterized by an increased nodule number and extended intervals between nodules along the root, results from the loss of a systemic regulation system that works in legumes predominantly through the shoot - most probably through the leaves. Julia Frugoli (Clemson University, Clemson, USA) reported the discovery, using induced mutagenesis, of three loci controlling supernodulation in the model legume *Medicago truncatula*. One of these loci (*Mtsunn*) is equivalent to the *GmNARK/LjHAR1* locus cloned previously from *G. max* and *L. japonicus*, respectively. These genes encode a receptor kinase with an extracellular leucine-rich repeat region (LRR) with similar structure to the *Arabidopsis CLAVATA1* (*CLV1*) receptor, which is known to control apical meristem proliferation and cell fate. It is tempting to assume that certain components of the *CLAVATA* system also participate in the SUNN/NARK/HAR1 complex. In connection with this, Akira Miyahara (University of Queensland, Brisbane, Australia) presented a poster showing the isolation and characterization of two kinase-associated protein phosphatase (*KAPP*)

genes from soybean and one from *Lotus*. *KAPP* in *Arabidopsis* acts as a negative regulator of *CLV1*. The investigation of these systems by RNAi and TILLING will presumably be one of the next steps.

The second *M. truncatula* gene reported by Frugoli is *Mtsn1*, which like *Mtsunn* acts in the shoot. This mutation may be equivalent to the as yet unpublished *Lotus Distans* mutant described at the International Congress of Nitrogen Fixation in Beijing last year by Masayoshi Kawaguchi's group at the University of Tokyo, Japan. Publication of both findings is eagerly awaited. The third locus reported by Frugoli (*MtRDN*) is root-controlled and may be equivalent to the pea supernodulation locus (*Psnod3*) found 20 years ago (but still not cloned - positional cloning in pea is very difficult because of its large genome, which is about 4 Mb per haploid genome).

These new genetic components of the AON circuit certainly advance our understanding of process (Figure 1), although



**Figure 1**

A model of the genetic components and developmental relations of the autoregulation of nodulation in soybean and medics (*Medicago*). *SN1*, *NOD3* and *RDN1* have not yet been cloned and characterized. *Distans* (now called *Klavier*) is cloned but not yet published (Masayoshi Kawaguchi, personal communication). Arrest of nodulation occurs at different stages in soybean and medics as indicated by the white boxes. This may reflect a difference of determinate (spherical) and indeterminate (cylindrical) nodulating species. Cells are shown as boxes with nuclei indicated as circles. LCO, lipo-chito-oligosaccharide, the nodulation factor synthesized by the *Rhizobium*. Q, the 'cue' from the root to the shoot to indicate that *Rhizobium* attack has commenced; SDI, the shoot-derived inhibitor signal from the leaf to facilitate blockage of further nodulation advance. SDI may attenuate the response of the root to the nodulation factor. For other abbreviations, see text.

cloning and determining the molecular function of the genes will be essential to see how their products fit into the signaling cascade connecting early nodulation events and downstream control of nodule proliferation. The focus of this work is progressively moving towards an understanding of sensing during AON. I presented results from grafts between nodulation mutants blocked in early nodulation-factor perception and wild-type soybean, to evaluate - through time-delayed inoculation - the stage at which the rhizobial attack is perceived. The early pathway leading up to the first cell divisions (but not infection thread formation) appears to be critical.

One of the most highly acclaimed talks was given by Ingo Potrykus (Institut für Pflanzenwissenschaften, Zürich, Switzerland), who described the regulatory burdens surrounding the release of the vitamin-A-biofortified 'Golden Rice'. His team's achievements are an inspiration for most genome researchers, yet there is frustration that despite the defined need and demonstrated utility of the transgenic rice, there is still no open-field trial permitted in Asia. He highlighted a shortcoming in much of our research: namely that the public through its governmental bodies supports scientific discovery but not its conversion to real-world outcomes. Institutions routinely seek commercial backing for conversion of basic science discoveries to commercialization, but often this commercial backing prevents applications in the less-developed world, and environmental groups target genetic modification technology in part because of its profit motive and the related fear factor. Golden Rice, on the other hand, was developed by combined public and private initiatives, and adapted varieties are available to regional farmers with no strings attached; yet it is still under a taboo of non-usage that costs more lives annually than were taken by the recent Asian tsunami. If crop plants derived by genomic technologies are to be developed for the public good, the active support of the UN agencies and influential charities such as the Bill and Melinda Gates Foundation will be needed. Overall, it is clear that the PAG conference has now irreversibly moved on from simply reporting technologies to the application of those technologies to the functional analysis of biological systems.