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P4–TRANSCRIPTIONAL REGULATION OF PLANT MEMBRANE TRANSPORT

Organised by A. Amtman and F.J.M. Maathuis for the Plant Membrane Transport Group

P4.1 Regulation of expression of plant sulphate transporters

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The co-ordinated expression of a gene family for sulphate transporters regulates and manages whole plant sulphur-nutrition. Analysis of the *Arabidopsis* genome has indicated that the gene family may consist of up to 14 isoforms showing homology to one another. Phylogenetic analysis of the gene or amino acid sequences indicates that there are 5 or more distinguishable clusters within the family. Analysis of functional characteristics and patterns of regulation together with localisation data, suggests that these groups have specific roles, such as high affinity uptake in the root, translocation in vascular tissues and cell to cell transfer in leaves and seeds. Expression in relation to sulphur supply has been demonstrated for many of the isoforms. Transporter activity measured by radiotracer influx studies, transporter protein abundance in the plasma membrane and mRNA pools have been shown to be correlated to the sulphur-nutritional status of the plant. Under sulphur limiting conditions expression increases, possibly as a result of the relief of feedback inhibition by downstream reduced S-compounds potentially combined with an activation effect by the cysteine precursor, *O*-acetylserine. Re-supply of sulphur represses gene expression within hours and a rapid turnover of the sulphate transporter protein is observed as a decrease in both activity and protein levels. Differential expression of the gene family is observed in response to fluctuating sulphur-supply, indicative of the local nutritional status but also specific transcriptional regulation.

P4.2 Regulation of nitrate transporters

S. Filleur, M. Murphy and B.G. Forde

The family of high-affinity NRT2 transporters play a major role in plants by capturing nitrate from the soil. NRT2 genes have been identified in barley [1] and *Arabidopsis*. The expression of *AtNRT2.1*, the major gene

controlling the high-affinity nitrate uptake, is rapidly induced by external nitrate, down-regulated in the presence of reduced forms of nitrogen and regulated by endogenous signals related to the N and C status of the plant. Of the six other *Arabidopsis* NRT2 genes, *AtNRT2.2* and *AtNRT2.4* are also nitrate-inducible, while the remainder are regulated in other ways [2].

Little is known about *cis*- and *trans*-acting factors involved in the regulation of NRT2 genes. Using bioinformatics, we have identified a number of conserved motifs in the *AtNRT2.1* promoter that may be involved in its regulation. The challenge we face in identifying the DNA-binding proteins that may bind to these conserved motifs is one that is becoming increasingly common. For example, cluster analysis of microarray data is being used to predict groups of co-regulated genes, and analysis of the promoter sequences of these genes can identify over-represented motifs that are potential *cis*-acting elements [3]. We will discuss how the application of novel techniques, such as Surface Plasmon Resonance, may help in the identification of a transcription factor(s) that bind to conserved sequence motifs.

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P4.3 Evolutionary conservation of phosphate transport across the fungus-plant interface in mycorrhizal symbioses

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As few as about only 150 species of arbuscular mycorrhizal fungi (AMF) live in symbiosis with about 80% of land plants forming a mycorrhiza, *i.e.* a root colonized by a symbiotic fungus. Recently, AMF were placed into a new monophyletic group, the phylum *Glomeromycota*, which probably originated from the same ancestral

group as the *Ascomycota* and *Basidiomycota* and is much older than the earliest land plants. Arbuscular mycorrhizae represent ancient symbioses, thought to have originated about seven hundred million years ago, in the roots of plants pioneering the colonization of terrestrial habitats. In these associations, a key process is the transfer of phosphorus as inorganic phosphate to the host plant across the fungus-plant interface via transport proteins. Mycorrhiza-specific phosphate transporter genes, and their regulation are conserved in phylogenetically distant plant species and are selectively activated by fungal species from the *Glomeromycota*. To obtain information on the spatiotemporal expression pattern of the potato phosphate transporter *StPT3* (1), a reporter system based on the Fluorescent Timer, a mutant form of the DsRed fluorescent protein, was used. The Fluorescent Timer protein shifts fluorescence color from green to red over time due to slow fluorophore maturation (2). The results indicate that phosphate transfer occurs at different symbiotic structures including not only arbuscules but also thick coiled hyphae. Overall, our data indicate that induction of *StPT3* expression is a temporally controlled cell-autonomous process occurring upon cell-to-cell contact between the two symbionts.

References:

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P4.4 Expression and functional analysis of cyclic nucleotide gated channels

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Cyclic Nucleotide Gated Channels (CNGCs) are non-selective ion channels found in membranes of both plant and animal cells. CNGCs are integral membrane proteins composed of 6 transmembrane spanning domains, with a pore domain between the 5th and 6th transmembrane span that allows the movement of ions such as K⁺, Na⁺ and Ca²⁺ (Talke et al 2003; Köhler et al 1999; Sunkar et al 2000; Leng et al 1999). CNGCs are activated by binding of cyclic nucleotides which causes a conformational change. In addition, channel activity is modulated by calmodulin. In *Arabidopsis* there are 20 members of the CNGC family and orthologs have been found in many other plant species (Talke et al 2003). To get insights into the physiological role of plant CNGCs we used a microarray method to study changes

in CNGC expression in response to various conditions. GFP reporter gene fusions and promoter-GUS constructs were made to determine tissue specific and sub cellular expression patterns of CNGCs. We also phenotypically characterised null mutants in all CNGC isoforms.

References:

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- Leng Q, Mercier RW, Yao WZ, Berkowitz GA (1999) Cloning and first functional characterization of a plant cyclic nucleotide-gated cation channel. *Plant Physiology* **121** p753–761

P4.5 Perception of plant K status at the transcriptional level

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Plant K⁺ homeostasis relies on the existence of different functional types of channels and transporters and their differential expression in membranes, cell types and environmental conditions. Complete genome information from *Arabidopsis thaliana* has revealed that there are approx. 100 genes (10 gene families) with putative function in K⁺ transport [1]. Furthermore, coordinated transport of K⁺ requires electrochemical gradients (~80 genes encoding primary pumps), charge balance (~50 genes encoding anion transporters) and water flux (~40 genes encoding aquaporins), as well as systems for the perception and signal transduction of external and internal K⁺ concentrations. Previous cation stress experiments realised on mature plants revealed that very few transporter genes responded to K⁺ starvation [2, 3].

We have employed *Arabidopsis* full genome microarrays to examine how plants perceive K⁺ and execute a coordinated transcriptional response to fluctuating K⁺ supply. Global gene expression of *Arabidopsis* seedlings during K⁺ starvation and short term re-supply was studied independently in roots and shoots and analysed using a new statistical method based on calculation of rank products [4]. Our experimental conditions, verified by root and shoot ion measurements, allowed us to identify genes, which were reversibly regulated by K⁺ starvation and re-supply. Among these, the more striking con-

cerned the jasmonate signalosome and related metabolic pathways such as polyamine synthesis and defence mechanisms. Important changes were also observed for transcripts encoding structural and regulatory cell wall proteins. Surprisingly, the only known K^+ transport system that responded quickly and consistently to changes in K^+ supply was the high-affinity transporter HAK5. Among the other classified transmembrane proteins, aquaporins, multidrug and toxin efflux (MATE) transporters and nitrate transporters were the most highly represented families in our dataset. An overview of the K^+ -status dependent transcriptome will be presented in the context of implicated physiological pathways and related transport mechanisms.

References:

- [1] Mäser *et al.* (2001), *Plant Physiol.* 126, 1646–1667.
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- [3] Maathuis *et al.* (2003) *Plant J.* 35, 675–692.
- [4] Breitling *et al.* (2004) See: http://www.gla.ac.uk/functionalgenomics/tp/affy_analysis.html.

P4.6 Transcriptional and post-transcriptional regulation of aquaporins in plant roots under stress

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Aquaporins form a large multigenic family in plants, with 35 members in *Arabidopsis thaliana*. Aquaporins play an important role in the uptake of soil water [1] and mediate the regulation of root hydraulic conductivity (L_p) in response to a large variety of environmental stresses including salinity and anoxia. An inventory of plasma membrane aquaporin isoforms was performed using a proteomic approach [2] and expression of aquaporin genes in roots was followed by RT-PCR, *in situ* hybridization and macro-array hybridizations. The latter technique revealed that most of the highly expressed aquaporin genes showed a 60–75% decrease in their expression level, in the 4–6 h following exposure to salt. This result is consistent with the long-term down-regulation of L_p by salt. However, down-regulation of aquaporin genes lagged behind inhibition of L_p , suggesting that other mechanisms such as salt-induced post-translational modifications of aquaporins may be involved in the early phase of L_p inhibition. In another study, the whole root and cell bases for inhibition of root water uptake by anoxia were delineated and linked to cytosolic acidosis [3]. A novel molecular mechanism for aquaporin gating by cytosolic pH was uncovered, which permits coordinate inhibition of plasma membrane aquaporins and, as a consequence, a general block of root water transport.

References:

- [1] Javot *et al.*, 2003, *Plant Cell*, 15: 509
- [2] Santoni *et al.*, 2003, *Biochem. J.* 373: 289
- [3] Tournaire-Roux *et al.*, 2003, *Nature* 425: 393

4.7 Single cell microarray expression patterns of Arabidopsis membrane transporters

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Plants have large gene families for ion and solute transporters and different members of the same family are often expressed in different cell types. Functional genomics can provide an understanding of how the temporal and spatial expression of transporter genes control developmental, metabolic and physiological processes and increasingly this information is needed at the level of the individual cell. Recently, techniques have been described for examining the expression of a few genes within an individual cell (e.g. Laval *et al.* 2002). Using a modification of the single cell sampling technique and a new 3' end amplification method (Micro-Expression Amplification; Patent Pending), we can obtain 100 μ g of mRNA fragments from the 1 pg mRNA extracted from a single cell. With these methods, we can analyse not only the expression patterns of all members of a transporter gene family in different cell types, but have enough RNA for microarray analysis. We are currently applying these methods to examine nitrate-dependent expression patterns of genes in *Arabidopsis thaliana*, including nitrate transporters, as well as the effect of salt on the expression patterns of glutamate-like-receptors (AtGLRs) in leaf cells. The techniques involved and the progress made will be described.

Laval, K. *et al.* (2002) *Planta* 215: 287–292.

P4.8 Global expression analysis reveals key transport processes across membranes of nematode induced giant cells

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Nematodes are root pathogens that cause an estimated \$70–80 billion in crop losses worldwide. Root knot nematodes (*Meloidogyne* spp.) are responsible for about 50% of this damage. Upon entry to roots the root knot

nematode selects several plant cells, which are induced to grow into giant cells. The giant cells serve as essential feeding sites for the nematode throughout their lifecycle. Giant cells have many of the hallmarks of transfer cells including: dense cytoplasm, numerous small vacuoles, invaginated cell walls, large numbers of mitochondria and increased metabolic and transport activity. Because the giant cell plasma membrane is highly invaginated with few plasmodesmatal connections to surrounding cells, membrane transporters in the plasma membrane will play an important role in the uptake of specific nutrients required for nematode growth and development.

To identify the key transporters in the giant cell membranes we used the Affymetrix GeneChip Arabidopsis ATH1 Genome Array. We identified many genes encoding membrane transport proteins that are upregulated 1, 2 and 4 weeks after nematode infection. The results were verified using real time PCR. The expression of selected genes was localized to the infected and uninfected parts of the root. The selected transporters fell into three different categories according to where they were expressed (evenly throughout the entire root, higher expression in feeding sites, or higher expression in the root outside feeding sites). The results from the analysis of the GeneChip experiments will be presented with emphasis on the biological processes that may be indicated by the changes in transporter gene expression.

P4.9 Transcriptional regulation of *Arabidopsis* sucrose transporters

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Arabidopsis has 9 genes encoding sucrose transporters or sucrose transporter-like (= functionally uncharacterized) proteins (*AtSUC1*: At1g71880; *AtSUC2*: At1g22710; *AtSUC3*: At2g02860; *AtSUC4*: At1g09960; *AtSUC5*: At1g71890; *AtSUC6*: At5g43610; *AtSUC7*: At1g66570; *AtSUC8*: At2g14670; *AtSUC9*: At5g06170). Recent analyses suggest that two of these genes, *AtSUC6* and *AtSUC7*, are pseudogenes, which reduces the total number of functional, plasma membrane-localized sucrose transporters to 7 in *Arabidopsis*. One of the encoded transporters, *AtSUC2*, is responsible for phloem loading and expression of the corresponding gene is restricted to companion cells of mature (= source) leaves. No *AtSUC2*-promoter activity was detected in any other cell type. Due to the physiological importance of the *AtSUC2* protein and due to the extreme cell and developmental specificity of the very strong *AtSUC2* promoter we got interested in the transcriptional regulation of this gene.

Deletion analyses of the *AtSUC2* promoter identified a short fragment of about 120 bp that seemed to contain

all *cis*-elements necessary for expression of GUS or GFP with the correct cell and developmental specificity, when analyzed in a minimal promoter construct. Linker scanning of the 120-bp fragment identified two *cis*-elements, which were both necessary for the correct *AtSUC2* promoter activity. These fragments were then used in different screenings for transcription factors.

Results on the characterization of *AtSUC2* *cis*-elements and on possible transcription factors will be presented.

P4.10 Transcriptional profiling of *Alyssum* species to identify transporters involved in metal hyperaccumulation

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Metal hyperaccumulation is the ability of certain plants to accumulate potentially toxic elements to exceptionally high concentrations in their above-ground biomass. Research is needed on the genetic basis of hyperaccumulation to provide insights into cellular mechanisms of metal-ion transport and homeostasis, and would help in the development of phytoremediation technologies for the clean-up of metal-contaminated soils. The aim of this work is to identify genes involved in Ni transport in *Alyssum*, a large genus in the family Brassicaceae containing 170 species, of which 48 are known to hyperaccumulate Ni. Using *Arabidopsis thaliana* whole-genome oligonucleotide arrays, we compared the transcriptome of *A. lesbiacum* (a hyperaccumulator) with that of *A. montanum* (a non-accumulator). Experiments were performed separately on both root and shoot tissues. Hybridization was detected for about one-third of the genes represented on the array, of which approximately 10% were more highly expressed in *A. lesbiacum* than in *A. montanum*. A similar comparison between a second hyperaccumulator species, *A. pintodasilvae*, and *A. montanum* revealed a subset of genes more highly expressed in both Ni-hyperaccumulator species than in the non-accumulator species. This approach reduced the number of candidate genes potentially involved in Ni hyperaccumulation by an order of magnitude. Tests of selected genes indicated that results from microarray analysis were in good agreement with transcript levels assayed by RNA gel-blot analysis and RT-PCR. Members of several classes of membrane transporters were observed amongst the transcripts highly expressed in the hyperaccumulator species, some of which may represent novel candidate transporters for nickel.

P4.11 Transcriptional regulation of caesium accumulation in Arabidopsis

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Caesium (Cs) is a Group I alkali metal with chemical properties similar to potassium (K). The radionuclides ^{134}Cs and ^{137}Cs are of environmental concern due to their long half-lives, emissions of β and γ radiation during decay and rapid entry to the food chain through plants. Theoretical models predict that Cs^+ enters plants through voltage-independent cation channels (VICCs), possibly encoded by the *CNGC* and *GLR* genes, and the H^+/K^+ cotransporters encoded by the *KUP* genes (White and Broadley, *New Phytologist* 147, 241–256, 2000). These predictions are consistent with (a) the identical pharmacology of VICCs and Cs^+ uptake by K-replete plants, (b) the changes in pharmacology of Cs^+ uptake as KUPs are induced by K starvation, and (c) the contrasting phenotypes of Arabidopsis mutants lacking the dominant K^+ channel involved in K nutrition (AKT1), or certain CNGCs or KUPs. Caesium is also toxic to plants at (unnaturally) high concentrations. It is thought that Cs toxicity may result from K starvation, because Cs inhibits AKT1. However, there are both commonalities and differences in the transcriptional profiles of Cs-intoxicated and K-deficient plants, suggesting that Cs intoxication was not perceived genetically solely as K starvation. Furthermore, when Arabidopsis were grown on agar containing various concentrations of Cs and K, their growth was better related to the shoot Cs/K concentration ratio rather than shoot K or Cs concentration.

P4.12 The Role of Transporters in the Light Regulation of Arabidopsis Seedling Development

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The transition from the etiolated seedling to the photoautotrophic seedling is accompanied by extensive changes in seedling morphology. Associated with this is a re-distribution of photoassimilates and other vital compounds required for seedling development. It is likely that these processes require changes in expression of a wide variety of transporter genes. To identify these genes we have investigated the expression of transporters in response to various light treatments using the Arabidopsis Membrane Transporter (AMT) microarray (Maathuis *et al.*, 2003, *Plant J.* 35, 675–692) and data

from previous published microarray studies. Analysis of expression profiles in response to far-red light (FR), mediated by phytochrome A, has identified more than 20 putative light-regulated transporter genes representing a broad range of transporter classes. Examples of FR-induced genes include the monosaccharide transporter *STP1* and a gene encoding a member of the ACA family of Ca^{2+} ATPases. In contrast, the amino acid transporter *CAT4* and a H^+ ATPase gene were repressed by FR. We have confirmed these results using RT-PCR and/or quantitative real-time PCR. Examination of these genes under other light conditions has shown that some are specific to FR, such as the *ACA* gene, while others are also affected at other wavelengths. For example, *STP1* is more strongly induced by blue light. We are currently examining knockout mutants of these genes to further characterise their role in seedling development.

P.4.13 Taking the P out of plants – Understanding plant responses to P deficiency

JP Hammond and PJ White, HRI, Warwick; MR Broadley and MJ Bennett, Biosciences, Nottingham

Phosphorus (P) is an essential macronutrient required by plants. Plants respond to P deficiency through morphological, physiological and biochemical changes. These responses are effected by altered gene expression. Since plants take up P as phosphate and little phosphate is available in most soils, phosphate fertilisers are used to ensure product quality and yields. However, phosphate fertiliser use is unsustainable and causes pollution. To ensure phosphate fertilisers are used wisely there is a need for, i) a better understanding of the mechanisms by which plants sense fluctuations in phosphate availability, and ii) more precise methods to monitor crop P status, such as sensor plants and diagnostic microarrays, so that phosphate fertiliser applications can be managed efficiently.

A proof-of-concept for sensor plants was performed using transgenic Arabidopsis in which the P-sensitive promoter from *SQDI* controlled the expression of β -glucuronidase (*GUS*). Gus activity in these plants responded to their P status, demonstrating the feasibility of this approach. However, this approach requires the generation of transgenic plants.

Monitoring gene expression directly in crop plants in the laboratory would remove the need for transgenic crops. Using microarrays, it is possible to monitor changes in the expression of thousands of genes simultaneously. A transcriptional profile for P deficiency can be produced. The detection of this transcript profile in the leaves of

crop plants would indicate a physiological P stress and the need for remedial phosphate fertiliser application. Indeed, this technique could be used to monitor crop responses to many biotic and abiotic stresses simultaneously, providing a diagnostic report for overall crop health.

P4.14 Identification and characterisation of a new family of *Arabidopsis* ion channels

E.P. Thompson, V. Demidchik, M. Oliynyk, B. Glover and J.M. Davies

P2X purinoceptors are ATP-gated, Ca^{2+} -permeable cation channels that are found in many eukaryotic cells. The receptors are divided into subgroups by pharmacology, and typically contain two transmembrane domains joined by a large extracellular loop upon which numerous cysteine residues are found.

Extracellular ATP signalling is well-studied in animal cells, e.g., in synaptic transmission (neuron P2X receptors), vasoconstriction (vascular smooth muscle cell P2Xs), and in immune response and cell death (a P2X has been linked with immature thymocyte apoptosis). [Atkinson et al] A P2X receptor was also found in the nuclear envelope of rat hippocampal neurons, potentially allowing regulation of nuclear Ca^{2+} in response to cytoplasmic ATP.

Whereas similar potassium channels and glutamate receptors occur in plants and animals, P2Xs have not been identified in plants. A role for P2X ionotropic receptors might be foreseen in signalling by extracellular nucleotides and regulation of cytosolic calcium, however, just as in other organisms. Preliminary work showed that extracellular ATP does elicit an increase in cytoplasmic Ca^{2+} in *Arabidopsis* root cells, caused in part by Ca^{2+} influx across the plasma membrane. Further, this influx was sensitive to P2X antagonists. Bioinformatic study of the *Arabidopsis* genome also predicts proteins with similar structural characteristics to animal P2Xs. Although high sequence homology would not be expected between family members in divergent organisms, hydropathy and domain analysis identified some proteins with the familiar transmembrane helices, large loop and key cysteine residues. Here we report our investigation of these P2X-like proteins using T-DNA mutants and molecular biology to explore expression patterns and activity.

P4.15 Abstract not supplied

P.4.16 Microarray Analysis of Circadian Transcription in Guard Cells of *Arabidopsis thaliana*

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In *Arabidopsis* the concentration of cytosolic free calcium free ($[\text{Ca}^{2+}]_{\text{cyt}}$) oscillates with a period of 24 h in constant conditions. However, it is not known whether these circadian oscillations of $[\text{Ca}^{2+}]_{\text{cyt}}$ are a core component of the circadian clock, a regulatory input signal, or an output that serves to transduce temporal information. We are investigating the hypothesis that oscillations of $[\text{Ca}^{2+}]_{\text{cyt}}$ are an output of the circadian oscillator and act in the circadian signalling network to regulate physiology. As part of our investigations we are characterising the circadian regulation of transcript abundance in stomatal guard cells using whole genome oligonucleotide arrays. The data will be used to identify putative components of the circadian calcium signalling network in guard cells. Reverse genetic tools will be used to investigate the importance of these components in the control of biological rhythms.

P4.17 Instigation into the functions of CLC channels in the filamentous fungus *Aspergillus nidulans*

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The voltage-dependent chloride channel (CLC) family defines a large class of anion-permeable membrane proteins present in all Eukaryotes. In the fungal kingdom, our best knowledge of CLC gene function comes from the study of *GEF1*, the sole member of the CLC family present in *S. cerevisiae*. Functional analysis of *Gef1* null mutants reveal a role for the chloride channel in intracellular iron and copper metabolism and transport.

In contrast to the yeast model, we have identified three CLC homologues in the *Aspergillus nidulans* genome. This study reports the characterisation of one of the homologues, AnCLCA. AnCLCA is a 909 amino acid polypeptide which functions as a chloride channel when expressed in yeast *Gef1* null mutant.

To define its role in *Aspergillus*, we created an *AnCLCA* null mutant. This mutant shows:

- * Hypersensitivity to extracellular copper that can be overcome by the addition of N-acetyl cysteine and resulting from an intracellular accumulation of copper.
- * Reduced activity of copper-dependent superoxide dismutase thus exacerbating sensitivity to reactive oxygen species.

* Elevated respiration rates resulting from enhanced copper-dependent cytochrome oxidase activity.

On the basis of these results we have proposed a model for the role of AnCLCA in copper metabolism in filamentous fungi and have compared this function to that of Gef1 in yeast.

The expression patterns of the three *A. nidulans* CLCs and their phylogenetic relationship are also presented.

P4.18 Abstract not supplied

P4.19 New methods for analysing microarray data

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After data collection, biologists analysing a microarray experiment face three main challenges:

1. Identification of differentially expressed genes.
2. Detecting physiologically relevant patterns among those genes.
3. Synthesising these patterns into a comprehensive biological interpretation.

These three tasks are complicated by the large number of genes involved and the fact that micro-arrays tend to reveal interesting information beyond the original question.

We have developed three new techniques that provide fast, easy, and statistically rigorous assistance during that process.

1. Rank Products (RP): A novel and powerful testing statistics for the sensitive detection of significantly regulated genes. RP performs better than previous tests and is particularly strong for a small number of replicates.
2. Iterative Group Analysis (iGA): A statistical approach using functional annotations to detect differentially expressed genes and at the same time provide an automated functional interpretation, including unbiased confidence values.
3. Graph-based iGA (GiGA): An extension of iGA that is more flexible, can be applied to non-annotated genes, and summarises microarray results in an easy-to-navigate graphical format. This summary can be used as a starting point to focus and expedite subsequent literature research.

Validation studies in collaboration with the Functional Genomics Facility at the University of Glasgow show

that RP and iGA lead to a significant improvement and dramatic acceleration of microarray interpretation.

For further details see: http://www.gla.ac.uk/function-algenomics/rp/affy_analysis.html.

P4.20 Cross-species hybridization and Analysis using Affymetrix GeneChip

John Okyere, Guo-an Sun, David Craigon, Janet Higgins and Sean May

The Affymetrix oligonucleotide array uses multiple pairs of 25mer probe sequences (probeset) to interrogate each transcript. The conservation of gene sequences between related species offers an opportunity to use a subset of probe pairs in a probeset to analyze a cross-species transcriptome.

At the Nottingham Arabidopsis Stock Centre (NASC) we have developed a novel technique for analyzing Affymetrix cross-species transcriptome data. We employed two approaches to select orthologous Arabidopsis probes with high homology to Brassica oleraceae and Capsella rubella sequences.

A Brassica oleraceae database was queried with approximately 250 000 Affymetrix Arabidopsis thaliana sequences. Perl scripts were then developed to select orthologous Arabidopsis 25mer probes with 100% identity to Brassica oleraceae sequences. A minimum of two 25mer probe pairs was selected per probeset. This gives a minimum of 50bp orthologous Arabidopsis probe sequences to interrogate the cross-species target. This database approach to probe selection is limited to species with sufficient sequence information in public databases. Capsella rubella suffers this disadvantage.

Perl scripts were developed to select orthologous probes with high hybridization signal following *Capsella* genomic DNA hybridization to the Arabidopsis GeneChip. A minimum of two 25mer orthologous probe pairs was selected per probeset for *Capsella*.

Our database approach to probe selection has other advantages. *Brassica oleraceae* cDNA annotation is assisting putative annotation of previously unknown Arabidopsis thaliana genes. Likewise Brassica GSS annotation will benefit from the Arabidopsis annotation.