Mechanisms & Processes

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An exciting year of research into the biology of the plant cell, and the pathogens that infect it, has seen landmark achievements, significant new scientific insights, and the establishment of key novel technologies across all three programmes (Gene Expression - GE, Cell to Cell Communication - CCC, and Plant-Pathogen Interactions - PPI) within the theme. Crucial to this success has been the development of productive collaborations between programmes in the theme, across SCRI, and worldwide.

For the first time in the UK, the genome of a plant pathogen, of major economic significance, has been completely sequenced and annotated (PPI, in collaboration with the Sanger Institute). The finished genome sequence of *Erwinia carotovora* subsp. atroseptica has already allowed SCRI scientists to uncover a number of novel putative pathogenicity genes, some of which have been confirmed *in planta*. It has led to the first microarray (PPI and GE) containing all the genes of this organism, allowing novel *in planta* gene expression studies. The sequence is also allowing bioinformatics approaches to uncover novel effector proteins involved in plant interactions, and offers many other possibilities for computational analyses, including a study of the evolution of this pathogen. The sequence will continue to act as a major resource for *Erwinia* biologists both at SCRI and in other groups around the world, with use facilitated through interactive web access.

The targeted sequencing of important loci in other plant pathogen genomes has revealed conservation of two avirulence loci between *Phytophthora infestans* and

Peronospora parasitica (PPI in collaboration with HRI, Wellesbourne). Sequencing around the *Atr1* avirulence locus in *Pe. parasitica* revealed open reading frames similar to ESTs in *P. infestans*. Probing a BAC library of *P. infestans* with these identified overlapping BAC clones and SNPs developed to the ends of these BAC clones have been used in association genetics studies to reveal linkage disequilibrium across a 100 kb region containing the *P. infestans Avr3* gene. This is the first demonstration of conservation of *Avr* loci in plant pathogens other than the *hrp* gene cluster in bacteria.

This work arose from the identification of candidate Avr genes from *Phytophthora infestans* (PPI in collaboration with the University of Ohio, USA). A combination of association genetics, using SNP analyses across a range of candidate *Avr* genes, and functional genomics, expressing the candidate genes in potato differentials using Potato virus X-based viral vector, revealed two candidate *Avr* genes, *Avr3* and *Avr2*. The former resides in a genomic region referred to above in syntenic studies, and the latter comprises a gene encoding a small cysteine rich protein that elicits the hypersensitive response in potato genotypes containing the *R2* gene.

These, and other current gene discovery programmes at SCRI, are producing large amounts of sequence data for which there is an urgent requirement to ascribe function. Following on from a successful collaboration (CCC with Large Scale Biology Corporation) that produced improved viral vectors for cell biological studies and the production of therapeutics in plants, additional vectors have been created to allow targeted gene silencing in plants. Gene silencing allows rapid and facile screens for gene function without the need to produce transgenic plants. A strategy using inverted repeat sequences and hairpin structures has been used to increase the efficiency of silencing, initially using vectors based on *Tobacco Mosaic Virus*. These vectors allow enhanced functional genomics screens that are being incorporated into the bioinformatics analyses that are occurring across the research programmes at SCRI.

Further novel viral vectors that infect diverse species such as *Arabidopsis*, potato and barley have been generated (CCC). This work, funded by the SEERAD 'outer core' programme, is aimed at exploiting viral vectors as functional genomics tools for high-throughput analysis of novel gene sequences arising from SCRI-based genomics programmes on potato and barley. New vectors based on *Barley Stripe Mosaic Virus* and other plant viruses that infect monocotyledonous hosts, together with *Potato Virus X* (PVX), *Tobacco Rattle Virus* and *Potato Leaf Roll Virus* have been created so that gene function can be determined through gene silencing or over-expression.

Using the PVX vector the first gene silencing in potato tubers (PPI, CCC and QHN) has been demonstrated. The endogenous *pds* gene was silenced, leading to a photo-bleaching phenotype in tetraploid cultivars (Desiree, Bintje and Stirling) of *Solanum tuberosum* and in the diploid wild potato species *S. bulbocastanum*. Up to 80 % silencing at the transcriptional level was seen and HPLC revealed accumulation of phytoene (the substrate of phytoene desaturase). Silencing in tubers was maintained through several generations of micro-tuberisation.

A system that allows RNAi to be used as a tool to knock out expression of putative plant parasitic nematode pathogenicity genes has also been developed (PPI). Use of this technique to knock out expression of genes encoding secreted cellulases significantly impaired the ability of the nematodes to invade host roots. Similarly, knock out of a gene encoding a secreted protein of the amphids (the main sense organs in nematodes) almost completely abolished the ability of the nematodes to locate and invade host roots. This system will allow the function of genes potentially involved in nematode feeding site induction to be tested. It will also allow us to assess the effects of disrupting various nematode genes and thus identify suitable targets for novel control methods. A further technology with potential applications in functional genomics and clean GM technology is based around homologous recombination between DNA sequences (GE). Using artificial target sequences presented in the genome in a test system involving the preparation of tobacco microspores and *in vitro* maturation to pollen grains, a rate of homologous recombination around 2% is indicated, which is probably the most efficient in plants to date. Currently the frequency of targeting of endogenous genes, the mechanism of homologous recombination involved in this system, and its transfer to crop plants are being investigated.

Novel proteomics initiatives have also generated significant results. An analysis of the nucleolar proteome of *Arabidopsis* is underway with the identification of over 200 proteins from isolated nucleoli (GE in collaboration with JIC and the University of Dundee). From bioinformatics analysis of the 200 proteins,

complemented by localisation within the cell of a third of the proteins generated from GFP-fusions of full-length cDNAs , >90% of the proteins have nucleolar or nucleolus associated labelling. Currently, a direct comparison of the plant and human nucleolar proteome is being carried out and has identified proteins of unknown function conserved in both organisms, and plant-specific proteins. A novel viral-vector screen has been used to ascribe subcellular localisations to proteins derived from hundreds of random GFP-fused sequences from cDNA libraries leading to the first isolation of plasmodesmal protein genes (CCC). For both research areas the provision of a web-based database for use by the wider research community has been undertaken.

Two areas of research with plant virus proteins, involved in silencing suppression, have demonstrated the link between basal RNA metabolism and RNA silencing (GE, in collaboration with the Sainsbury lab). The proteins, from two completely different viruses, interact with an RNA export factor and a nucleolar protein required for processing and modification of rRNAs. Further studies have continued to characterise the function of the viral movement proteins (MPs) and how they interact with plasmodesmata (CCC). Using novel FRAP (Fluorescence Recovery After Photo-bleaching) methods, and a number of cell biological and chemical inhibitor-based studies, it has been conclusively shown that the MP of *Tobacco Mosaic Virus* does not move on microtubules (contrary to current dogma) and that the endomembrane system is likely to play an important role in the trafficking of viral movement proteins, and endogenous proteins from one cell to another. Similar approaches will be taken in the future to functionally characterise the plasmodesmal proteins that are discovered from the GFP-library screens above. In addition, work on subcellular localisation of *Potato mop-top virus* triple gene block proteins (PPI/CCC) has shown that proteins p2 and p3 utilise the endoplasmic reticulum (ER) and ER-derived compartments for intracellular movement. A motif in p3 that targets the protein to ER membranes may function also as a MP target signal.

The fusion between molecular biology, cell biology and bioinformatics of plants and their pathogens, as illustrated above, provides a powerful tool for the analysis of gene function as plant science matures into a postgenomic era. High impact factor publications and notable successes in the acquisition of external funding for fundamental studies and, for example through DTI-Link and Scottish Enterprise funding, for their application, will ensure the translation of these research discoveries to practical benefit.