Mechanisms & Processes

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All three programmes: Gene Expression (GE), Cell-to-cell Communication (CCC) and Plant Pathogen Interactions (PPI) have seen significant achievements in the last year. A series of "firsts" in plant and pathogen biology demonstrate the excellence of science and technology development carried out within the theme. In particular, the high quality molecular and cell biological expertise has been applied to understanding the subtlety and complexity of gene expression in plants and their pests and pathogens, and the interactions between these organisms.

The phenotype of a plant, whether at the macro-level of plant architecture or organ development or at the micro-level of the plant cell, is governed by regulated changes in gene expression. How plant growth and metabolism is altered under different conditions and how a plant responds to infection or attack by pests and pathogens again is determined by complex patterns of gene expression. Gene expression is regulated mainly at the transcriptional and post-transcriptional levels. The availability of fully determined genome sequences from some plants and pathogens, and the ever-increasing genomics and sequence information of other species, underpins much of plant and crop science and is widely used in the research of the theme.

The last 4 – 5 years has seen a remarkable increase in the importance of post-transcriptional gene regulation with the discovery of silencing and small interfering RNAs, microRNAs involved in many aspects of developmental control, RNA processing and alternative splicing events in development and plant responses, and the dynamic role of the nucleus and nuclear bodies in many of these processes. Research at SCRI is contributing to the international effort in these areas, but more importantly, the unique mix of expertise at SCRI allows us to integrate knowledge and skills and provide new scientific insights for exploitation.

Post-transcriptional control of gene expression in higher eukaryotes involves areas of RNA processing such as alternative splicing and messenger RNA (mRNA) turnover. Alternative splicing of precursor messenger RNAs (pre-mRNAs) in humans is an extremely important source of functional diversity where around 70% of genes are alternatively spliced and over 150,000 – 200,000 proteins can be generated from 30,000 – 40,000 human genes. Although alternative splicing is less prevalent in plants, we estimate that a significant proportion of plant genes (up to 20 – 25% or around 6,000 – 7,000 genes) are alternatively spliced.

Alternative splicing is regulated by short sequences in pre-mRNA transcripts (called splicing enhancer and silencer sequences), and by the levels of *trans*-acting proteins which interact with such sequences. Levels of these proteins are different in different cells and are affected by external stimuli such as light, temperature and other biotic and abiotic stresses. Through the study of genes undergoing alternative splicing and the consequences in terms of protein function, we will provide novel insights into plant responses to environmental conditions and stresses. This year, Craig Simpson (GE) has identified one of the first plant exon splicing enhancer sequences and a splicing regulator protein (PTB) has been cloned to address control of alternative splicing in plants (see following article by Simpson, C.G. *et al*).

Compartmentalisation of components of gene expression in different nuclear structures is of increasing importance in gene regulation, and dynamic movement of components is known to occur in response to changes in cell metabolism or in response to environmental conditions. Recently, a number of plant nuclear bodies or structures have been shown to be involved in RNA processing and in signalling responses to, for example, light and growth hormones demonstrating another level of complexity in expression regulation and plant response. The most prominent nuclear body, the nucleolus, has many different functions in the metabolism and biogenesis of various RNAs and in processes such as the cell cycle, aging and sensing of cellular stress. One way of addressing the functions of such nuclear bodies is to analyse their protein complement (proteome). In association with colleagues at the John Innes Centre, the University of Dundee and the University of Southern Denmark, we have identified over 200 plant nucleolar proteins as well as potentially novel functions for the plant nucleolus in mRNA export from the nucleus and mRNA decay (see following article by Brown, J.W.S. *et al*). The unique opportunity to compare the proteomes of the nucleolus of plants and humans highlights differences which are of value to understanding the function of the nucleolus in both systems. Finally, the plant nucleolar proteome provides the basis for studying the involvement of the nucleolus in response to stresses at the molecular and cellular levels.

A major challenge of the theme is to understand the interactions of plants and pathogens and how they affect each other's gene expression and viability. One of the most interesting developments is the bringing together of studies on the biology of plant viruses and plant RNA metabolism processes. In particular, plant viruses have been extremely important in the study and dissection of one of the most important phenomena in biology, namely RNA silencing. Silencing or the targeted destruction of foreign RNAs is one form of plant defence and when plant viruses infect cells the plant produces siRNAs which promote destruction of the viral RNA. However, viruses have evolved proteins (called silencing suppressors) as a counter-defence against silencing. Three areas of work in the GE programme provide insights into the function of such proteins and how they interact particularly with normal RNA metabolism processes and organisation within the plant cell. In the first case, Stuart MacFarlane and Tomas Canto (GE) demonstrated that the P19 protein of *Tomato bushy stunt virus* (TBSV) interacts with and causes the relocalisation of a protein called ALY/Ref which is involved in the export of RNAs from the nucleus to the cytoplasm. In the second case, Misha Taliansky and Sang Hyon Kim (GE) showed that the ORF3 protein of *Groundnut rosette virus* was localised to the nucleolus and that this localisation or trafficking through the nucleolus is essential for virus infection throughout the plant. In the third case, Peter Palukaitis (GE) and colleagues in Israel have shown that the *Cucumber mosaic virus* (CMV) 2b silencing suppressor has two, independent nuclear localisation signals, and uses the keryopherin α protein nuclear transport system. These intriguing observations demonstrate how plant viruses have evolved proteins which may either interfere with or hijack normal cellular functions to favour conditions for viral replication.

The importance of gene regulation at the post-transcriptional level has been demonstrated clearly in the regulation of genes involved in the control of flowering time in research carried out by Gordon Simpson (GE). His research will continue to investigate the genetic pathways of flowering control in both the model plant, *Arabidopsis*, and in barley.

Plants display a range of cellular and molecular responses to infection and disease. In animals, one response is programmed cell death or apoptosis where damaged or infected cells are destroyed. Key components in apoptosis in animals are a family of caspase proteases which digest other cellular proteins. Despite plants having an analogous apoptotic process, the hypersensitive response, no caspases have ever been identified. However, this year, Misha Taliansky (GE) along with colleagues at Moscow State University discovered for the first time, caspase-like proteins in plants (see following article by Kim, S.H. *et al*). In a related study, Paul Birch (PPI) and Christophe Lacomme (CCC) identified other apoptotic ortho-

logues active in the hypersensitive response in potato. These discoveries will contribute greatly to our understanding of plant defence.

An integral part of understanding plant-pathogen interactions is the identification and functional characterisation of pathogen genes expressed during infection. Greatest progress in this area is, of course, possible when the entire genome sequence is available. A milestone for SCRI was the generation of the first *Erwinia* genome sequence by Ian Toth and Paul Birch (PPI) which has resulted in a major increase in information about this intriguing bacterium. Bioinformatics tools, developed by Leighton Pritchard (PPI), help manage the information and aid analysis (see following article by Pritchard, L. *et al.*). The analysis has already revealed the presence of a number of novel proteins and chemicals involved in blackleg disease including phytotoxins, and exported necrosis inducing factors.

An important novel finding in the area of late blight research has been the identification by Paul Birch and Steve Whisson (PPI) of the *Avr3a* avirulence gene from *Phytophthora infestans*. In addition, collaborative research with HRI-Warwick has demonstrated that *Avr3a* lies in a genomic region that is conserved with a locus containing an avirulence gene, $ATR1^{NdWsB}$, in another oomycete, *Hyaloperonospora parasitica* (*Arabidopsis* downy mildew). Although the gene order is conserved at these loci in the two species, the avirulence genes are very different from each other. Such evolutionary relationships reflect events in pathogen evolution and diversity, and host range and specificity. In a similar vein, John Jones (PPI) and colleagues have identified genes encoding a family of GHF 45 cellulases (cell wall degrading enzymes) in nematodes that have been acquired by horizontal gene transfer from fungi. While gene transfer from bacteria to nematodes has been described previously, this is the first instance of horizontal gene transfer from fungi. These findings show that horizontal gene transfer has played a key role in the evolution of plant parasitism by nematodes on more than one occasion.

A key goal in understanding plant growth and development, and plant-pathogen responses is to gain knowledge of the function of different genes. This is addressed through a variety of biochemical, genetic, molecular and cell biological techniques such as analysis of mutants, global gene expression, imaging and biochemical interactions. Examining the patterns of gene expression in both plant and pathogens, in different tissues and cells, and the changes that occur during development or in response to environmental conditions or the infection process is a key to developing new strategies for crop improvement and disease control. Two of the major technologies in this area are microarray analysis and virus-induced gene silencing (VIGS), established at SCRI over the last 2 – 3 years as part of the Outer Core programme by Peter Hedley (GE) and Christophe Lacomme (CCC) respectively. Microarray analysis allows the rapid assessment of expression profiles of many thousands of genes at the same time. Changes in expression are detected by comparing mRNA populations from plants and pathogens at different stages in their life cycle and during the infection process. This system is currently being used successfully in a number of areas of research across the Institute including the control of pigment synthesis in potato, dormancy in raspberry, abiotic stress in barley, host responses to disease including regulatory and signalling networks in disease development, and changes in pathogen gene expression during infection cycles. VIGS technology will play an increasingly important role in analysing gene function in all areas of biology in the Institute. Emphasis has been given to developing efficient VIGS systems for potato and barley, and its application to diploid and tetraploid potato was demonstrated for the first time this year. VIGS is currently being used to analyse starch biosynthesis and proteins involved in disease resistance in potato. In addition to silencing plant genes, VIGS will also be extremely important in understanding the function of genes from plant pathogens, and will be assisted by the success of Steve Whisson and John Jones (PPI) in developing gene silencing protocols in *Phytophthora infestans* and cyst nematodes (see following article by Whisson, S.C. *et al*).

Further important functional characteristics of plant proteins are their transport and localisation to different parts of the cell, and interactions with other proteins. In CCC the localisations of many different proteins have been determined by fusing their genes to genes encoding fluorescent proteins (FPs) and visualisation of the FP fusion proteins in living cells by confocal microscopy. A programme funded by the Gatsby Foundation uses a high-throughput screening system to identify proteins localised to different cellular compartments (e.g. chloroplast, mitochondria, Golgi, nucleus etc.) following expression of cDNA-GFP fusion libraries from viral vectors. This programme aims to deliver localisation information of benefit to plant scientists throughout the world. Kenny Bell and colleagues (CCC) have optimised the viral vector expression system and created several cDNA-GFP

fusion libraries, and have already identified valuable novel markers for subcellular components.

The major thrust of the cell biology research is to understand the structure and function of plasmodesmata – channels connecting plant cells through which molecules and macromolecular complexes (including viruses) pass from cell to cell. Plasmodesmata have proven exceedingly difficult to analyse biochemically. A localisation screen, similar to that described above, successfully identified around a dozen potential plasmodesmatal candidate proteins which are currently being analysed.

The acquisition of a new, state-of-the-art, confocal microscope has greatly increased the ability to exploit novel fluorescent proteins, while advanced techniques such as photoactivation, photoconversion, FlAsH, FRAP and FRET have facilitated studies of protein dynamics. Petra Boevink (CCC) has used the photoactivation of a recently developed GFP derivative for real-time analysis of protein movement between cells (see following article by Boevink, P. *et al.*). Fluorescence resonance energy transfer (FRET), a powerful but difficult technique to examine protein-protein interactions *in vivo*, will be used to extend the research into the Golgi matrix proteins identified by Maita Latijnhouwers (CCC) in a highly successful collaborative project between SCRI and Oxford Brookes University (see following article by Latijnhouwers, M. *et al.*). In addition to the trafficking of various cellular molecules, a major question is how viral components target and modify plasmodesmata to facilitate virus movement. A detailed study of the *Tobacco mosaic virus* movement protein (TMV MP) by Kath Wright and colleagues in CCC used a selection of fluorescent markers for co-expression, photoactivation and fluorescence recovery after photobleaching (FRAP) to extend previous research that indicated that the TMV MP did not use microtubules to target the plasmodesmata, and to develop a new model for TMV movement. This work indicates that the TMV MP uses the endoplasmic reticulum to locate plasmodesmata and depends on the actin/myosin system for movement. Similarly, Tomas Canto and Peter Palukaitis (GE) identified sequences important for the CMV 3a movement protein to localise to plasmodesmata and showed that the 3a protein also did not associate with microtubules as thought previously.

The combination of cell biology and molecular pathology has revealed for the first time the association of the plant endocytic pathway in intercellular movement. Movement proteins encoded by the *Potato mop-top virus* were tagged with fluorescent markers and monitored in living cells, and found to associate with components of the secretory and endocytic pathways. In addition, protein interaction analysis revealed that one of the virus proteins (TGB2) interacted with a tobacco protein belonging to the highly conserved RME-8 family of J-domain chaperones that are essential for endocytic trafficking in *Caenorhabditis elegans* and *Drosophila melanogaster* (see following article by Haupt, S. *et al.*).

Scientists from all three programmes are involved in the application of their research to discovering new ways of controlling plant pests and pathogens. For example, John Jones (PPI) and colleagues from GE are developing novel strategies for control of potato nematodes bringing together expertise in molecular plant pathology and RNA metabolism within the theme. Similarly, Hugh Barker (GE) has been developing novel GM routes for controlling viruses *via* gene silencing. Several approaches have been tested to explore the possibility of designing transformation methods that confer resistance to multiple viruses through a single transgene.

In addition to the successes of research groups in the theme and programmes, there have been notable individual achievements. Karl Oparka, the Programme Leader of CCC received the accolade of being made a Fellow of the Royal Society of Edinburgh and was also appointed to Honorary and Visiting Professorships at the University of Dundee and the Oxford Brookes University respectively. Sanjeev Kumar Sharma, a PhD student in GE won three prizes in the last year to attend an international meeting in San Francisco and for outstanding poster presentations. Finally, Steve Whisson (PPI) was awarded the Peter Massalski prize.

The synergy obtained from combining molecular and cell biology, biochemistry and bioinformatics and the collaborations developed among scientists with wide-ranging expertise and knowledge provide the basis for understanding in detail the gene function of plants and pathogens. The knowledge generated at the molecular and cellular levels is essential to understanding processes of growth and development, and the plant's response to environmental and pathogenic challenges, and more importantly to the development of innovative and original ideas and strategies for exploitation. The quality of science and potential for application is demonstrated by the high impact publications and the success in attracting external income and will be increasingly important for the future.