Mechanisms & Processes

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Knowledge of how plants work is essential to safe and rational strategies for their protection and exploitation. This knowledge must extend from the gene, to the cell, to the plant in its environment, and to how the plant reacts in its environment in response to stress and under attack from pests and diseases. At one end of this spectrum lie fundamental biological systems such as plant and pathogen gene expression, cellular responses to pathogens and intercellular communication. To dissect these requires skills in molecular and cell biology, physiology and plant pathology. These disciplines have been assembled across the three Programmes within this Theme. Each has a focus of activity: on gene expression, on the molecular mechanisms underlying pathogen attack and the plant response, and on intercellular communication. The mechanisms driving these processes have already been shown to have key components that operate across them – these are crucial areas from which interdisciplinary projects can derive synergy.

Gene Expression Regulation of gene expression underpins plant metabolic processes, growth and development, and responses to and interactions with pathogens, pests and environment. The Gene Expression Programme seeks to delineate the mechanisms and components that govern the expression of plant genes, and how the functions they encode determine, for example, susceptibility to viruses and developmental gene expression in selected biological systems. The regulation of plant gene expression can occur both at transcriptional level and post-transcriptionally. The focus of activity at the post-transcriptional level is on pre-mRNA splicing and alternative splicing, snoRNA gene organisation and expression from multigene families in plants. A unique splicing system has been used to dissect splicing signals and trans-acting factor function. The novel potato invertase mini-exon system has for the first time allowed a quantitative assessment of the contribution of individual nucleotides to the strength of splicing signals. This work is an essential basis for future studies of alternative splicing. Up to 70% of human genes undergo

alternative splicing to greatly increase proteomic complexity. Current estimates for plants are between 7% and 20% of genes which undergo alternative splicing such that although less prevalent than in humans, it still represents a very significant regulatory process. The mini-exon system has also yielded the first characterisation of plant intron branchpoint and polypyrimidine tract sequences, and is being exploited to study splicing factors. This work has found practical application in the generation of a novel resistance to nematode infection in a project involving both the RNA processing and nematology groups. By targeting an antisense version of a splicing factor gene in the nematode feeding site in potato roots, from a nematode-induced promoter, 80% resistance to nematode infection was achieved. Future stacking of other genes expressed from other promoters could give full resistance to nematode pests.

The work on RNA processing requires description of RNA-protein and protein-protein complexes, and nuclear and nucleolar architecture and distribution. These structures also feature in our studies of viral gene expression. These have two main thrusts: silencing and suppression of silencing, and virus molecular biology and viral movement proteins. The molecular mechanisms involved in gene silencing and its suppression, and viral movement, and the functions of silencing suppressors and movement proteins are being unravelled using unique systems and tools. For example, we are using cells from the fruit fly, *Drosophila melanogaster,* to assay silencing suppression. Many plant viruses encode silencing suppressors that act at different stages of the silencing process and it is sometimes difficult to demonstrate silencing suppression activity *in planta*. The *Drosophila* system has helped identify potential silencing suppressors and the stage at which they may act. These can be important tools to dissect the silencing mechanism (the study of which is most advanced in *Drosophila*). In addition, a collaboration with the Roslin Institute has been initiated to explore the utility of these suppressors in animal and human cells.

A second area of virus research centers on the development of amplicon systems that induce silencing. An amplicon [in our case based on *Potato leafroll virus (PLRV)* is a transgene that comprises a full-length biologically active copy of a viral genome. In plants that contain an amplicon, transgene-derived transcripts replicate like RNA viruses and this process could potentially take place in every cell of the plant. However, replication of the transcribed amplicon induces very effective silencing of the transgene (see Annual Report article page 111. This system, based on replicating viral RNA, is likely to more accurately mimic RNA silencing caused by virus infection than are alternative silencing systems that use non-viral transgenes or transient gene expression via *Agrobacterium* infiltration. More recently an amplicon has been developed that comprises a full-length cDNA of PLRV expressing the green fluorescent protein (GFP), and this construct is starting to prove very useful in our studies.

Both plant and viral gene expression overlap in the requirement to study protein components and their interaction with RNA and other proteins in functional complexes. The Programme aims to link the molecular biology of plant and viral gene expression, silencing and suppression of silencing through cell biological and biochemical approaches. This will require analysis of the expression of specific sets of genes identified by conventional or genomics approaches. Such analyses will be promoted by interactions with two new scientific appointments made under a new tranche of outer core funding supplied by SEERAD. Methods for parallel gene expression analyses are being developed by this funding – these will support functional analysis, hypothesis testing and germplasm enhancement, both within the Programme, and through links with other Programmes. Parallel gene expression analysis will provide a broader view of gene expression in a variety of biological systems (e.g. effects of silencing suppressors, germinating barley, alternative splicing, host responses).

We are also developing genetic and cell biological techniques to gain enhanced insight into the function of gene sets. Radiation hybrid mapping utilises fusion of somatic cells from barley, which have been subjected to radiation to induce chromosomal fragmentation, and tobacco. The resulting selected hybrid cells will contain a tobacco genome containing random chromosomal fragments of barley, providing a panel of cell lines for molecular marker analyses of the barley fragments. The establishment and application of this technology allows the physical mapping of the expressed gene (EST) set of barley, linking through genetic maps to phenotype and function. Also under investigation is the potential of male gametic cells, microspores, for the development of gene targeting in plants – the most sophisticated manifestation of transgenic biology, permitting gene knock-outs, down-regulation and over-expression, and allelic substitutions.

Cell-to-Cell Communication Research in the Cell-to-Cell Communication Programme is multidisciplinary, ranging from basic molecular studies of plasmodesmal structure and function to whole-plant studies of virus movement. Non-invasive imaging approaches are a hallmark of the Programme, and several innovative strategies have been developed for studying macromolecular transport in plants based on confocal laser scanning microscopy. This Programme involves extensive interactions with plant pathologists, molecular biologists and biochemists at SCRI to study the basic cell-to-cell signalling processes that underpin plant-defence responses.

The structure/function relationships of plasmodesmata, the small pores that interconnect plant cells, are one focus of activity within the Programme. Plasmodesmata are essential conduits for small solutes and systemic signalling molecules, playing an essential role in plant development, and recent work has shown that they behave as regulatable channels whose permeability is dependent on a number of intracellular cues. A major discovery (published in the prestigious journal *Cell)* reveals that plasmodesmata can exist in different functional forms, some with the ability to traffic extremely large molecules. Plasmodesmata can also be modified by plant viruses to allow the passage of viral genomes between cells. Cell-cell virus movement has been used as a model system for understanding the regulation of plasmodesmata in response to viral invasion. The study of virus movement in plants using GFP-based technologies has led to the development of new models for virus movement in plants. A highlight is a new functional model for the intercellular movement of tobacco mosaic virus, the most extensively studied of the plant viruses. Unique, non-invasive techniques have been developed for studying virus movement and other basic transport processes in plants and the group is renowned for novel imaging techniques based on confocal laser scanning microscopy.

In addition, considerable contract work is undertaken, most notably with Biosource Genetics Corporation (BGC; California, USA), through Mylnefield Research Services Limited. This joint research programme is aimed at understanding and improving the movement of plant-viral vectors for the production of therapeutics in plants. Stable viral vectors, produced by DNA shuffling of the viral movement protein, represent the first demonstration of this technology using a plant virus. Isolation of novel genes in plants using a viral-based high-throughput functional genomics approach is also under way. In this work, random cDNA-GFP gene sequences are expressed within viral infection sites to identify novel proteins in different plant organelles. To date, several hundred novel proteins have been discovered in plant cells that were of previously unknown function and location. This approach is also identifying novel plasmodesmatal proteins that play a role in regulating intercellular transport of macromolecules and viruses. These studies are currently generating substantial intellectual property that is being exploited via Mylnefield Research Services.

There are further extensive collaborations with other research centres world-wide in joint studies of cell-to-cell communication in plants, exemplified by an ongoing collaboration with Professor Chris Hawes (Oxford Brookes, UK) that has been exploring the use of plant-viral expression vectors for studying the endomembrame system in plant cells. A new initiative, funded by SEERAD under outer core funding and undertaken in collaboration with the Gene Expression programme, is examining the utility of viral vectors as tools to determine protein function in post-genomics applications.

Plant-Pathogen Interactions The interactions between plants and microbial pathogens are specific, complex and dynamic. They involve recognition and response in the plant leading to signalling cascades and the up- or down-regulation of numerous genes, and in the pathogen to adaptation or evasion. This Programme focuses on plant and pathogen interactions at the cell and molecular level. The aims are to

understand and exploit the complex processes involved by using genomics-based approaches to study transcriptional and proteomic changes that occur at key stages of the interaction (recognition, signalling and defence response). The objectives are to discover and functionally characterise plant and pathogen genes involved in pathogenicity and plant defence, to understand the communication mechanisms (elicitation, recognition, response) between plant and pathogen, and to describe the identity and functions of the proteins involved in these processes.

Pathogen characterisation and the molecular basis of pathogenicity involve the identification and functional analysis of pathogen genes associated with pathogenicity, host specificity, avirulence and survival. New approaches to discover novel pathogenicity and host range related genes have been developed. Sample sequencing of selected regions of the *Erwinia* genome has allowed a detailed analysis of the hrp gene cluster and has uncovered a number of novel gene homologues from other animal and plant pathogens. Molecular analyses of the *Phytophthora infestans* genome have led to the construction of physical maps across loci containing avirulence genes and the isolation of infection stage specific genes. Genes expressed by potato cyst nematodes following invasion of roots have been isolated and sequence analyses reveals expression from novel nematode genes as well as host genes, many of which are typical of response genes to pathogen attack. Notable among these is the cloning and characterisation of a chorismate mutase from *Globodera pallida*. The gene encoding chorismate mutase appears to have been obtained by horizontal gene transfer from bacteria. This protein is secreted from the gland cells and may have an important role in the host parasite interaction. Global gene characterisation is also under way with near completion of sequencing of the entire genome of the plant pathogen *Erwinia carotovora ssp. atroseptica*, with a major bioinformatic annotation exercise to follow.

Description of the plant response to infection requires the discovery, characterisation and functional analysis of plant genes involved in surveillance or activated in response to pathogen invasion. Profiling of differential gene expression has revealed signalling pathways specific to either R gene mediated or field resistance to *Phytophthora infestans* in potato. This information, set in the context of related published research, has been made available by the development of DRASTIC, a web-searchable database of genes up- or down-regulated in plants in response to infection or application of resistance-modifying compounds for the analysis of signal transduction in cells (http://www.drastic.org.uk). Similar catalogues of pathogen–induced plant genes have been assembled for nematode infection where the isolation and functional characterisation of a broad-spectrum potato cyst nematode resistance gene (*Hero*) of tomato features.

Increasingly, functional characterisation will turn to protein interactions and the role of proteins in disease processes. Physico-chemical analysis of key proteins will be required and protein-protein and protein-nucleic acid interactions will be studied by a variety of methods. Subcellular localization and protein interaction studies have revealed new insights into the coordinate mode of action of *Potato mop-top virus* triple gene block movement proteins. The lipid binding protein, identified in surface secretions of nematodes and known to counteract the plant defence response, has been purified after recombinant expression for 3D and quaternary structure studies. Further novel regulatory and avr proteins will soon follow. The knowledge gained will help us to understand the molecular bases of disease processes that can be exploited to produce durable resistance to plant diseases. For example, the discovery of common and disease specific responses to pathogens can be used to design novel broad-spectrum control strategies. Already, a number of diagnostic sequences have been made available to aid complementary studies in other Programmes on the co-evolution of plants and parasites at the level of crops and pathogen populations. A new method has been developed using 16 S rDNA decreasing the time taken to identify the soft rot erwinias from 14 days to 1 day. Novel recombinant synthetic antibody assays (ELISA and stem printing) to detect *Potato leafroll virus* were devised and shown to work as well as tests that utilise reagents obtained by animal immunization. Microsatellite markers have been used with individual nematodes from *Globodera pallida* populations to distinguish UK populations and a further PCR based diagnostic has been used to distinguish trichodorid nematodes of economic importance in the UK (*Paratrichodorus pachydermus*, *P. macrostylus, Trichodorus similis*, *T. primitivus*). We expect that the genes and mechanisms discovered in this programme will be invaluable to analyses of the durability of resistance and the influence of environmental factors in studies of host-pathogen co-evolution.