Plant molecular and cell biology

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The synergy afforded by the application of cutting-edge research in molecular biology and in cell biology is apparent in the study of plant science at SCRI. Publication in prestigious journals, top ranking in research assessment exercises, major external contracts, and the generation of intellectual property, all demonstrate the health and vigour which has characterised our efforts in this area. Underpinning these are the talent and enthusiasm of scientific and support staff. We must ensure the consolidation of areas of excellence and explore all opportunities to emulate these successes.

Gene expression and RNA processing Precursor messenger RNA (pre-mRNA) splicing is one level at which gene expression is regulated. The Gene Expression/RNA Processing Group has been studying two inter-related areas of plant splicing: exon scanning and mini-exon splicing. Significant contributions to understanding the process of splicing have been made. Firstly, it has been shown that exons can be defined by interactions between factors assembled at each end of the exon, aiding our knowledge of how splice sites are chosen. Secondly, splicing elements required for correct splicing of a small (nine nucleotide) potato invertase mini-exon have been identified. These sequence elements are also able to promote splicing of heterologous exons of only one nucleotide in length. Splicing is very efficient, reflecting the strength of the splicing signals. Initial experiments suggest that these sequences can act as a splicing enhancer to increase the efficiency of splicing. Whether this will also lead to an increase in levels of gene expression is still to be investigated, but identification of splicing enhancers or expression enhancers will be of value to biotechnologists for the development of improved plant material. The various RNA and protein molecules involved in splicing are often encoded by gene families within the plant genome. We have

developed several novel approaches to the determination of expression profiles of individual members of plant gene families. These have revealed tissue-specific or constitutive expression patterns for individual gene family members, indicating potential uses for the promoters of these genes. Further methods for the subsequent isolation of these unique promoters from large complex genomes, such as tetraploid potato and hexaploid wheat, have yielded promoters eminently suited for biotechnological application for which intellectual property rights have been obtained.

Using these methods, we have confirmed constitutive expression of several genes from the U1A and U2B'' gene families in potato, with one expressed at 65% of the level of the CaMV 35S promoter. Promoters such as this are of increasing importance, given concerns over gene silencing resulting from promoter duplication and the use of promoters derived from viruses or other plant pathogens. Among the best-characterised tissue-specific promoters we have cloned are those for the family of genes encoding cell wall invertases in potato (see pp. 82-85). For each of these four promoters, we have generated a series of transgenic plants expressing a reporter enzyme under the control of the potato promoter, allowing detailed histochemical analysis of expression profiles. One promoter determines gene expression at the axial node in the stem under the axillary bud. Expression is also seen in an analogous region of the root. The axial nodes have major importance in potato - this is where the stolon, which will give rise to the tuber, is initiated, and, in the tuber itself, it is the region which forms the 'eye' from which the sprout will emerge. This promoter may be applied to modulation of development of the plant, or pathogen control strategies. Two promoters govern gene expression in the vascular bundles of the potato stem (there are three major and three minor bundles). One of these is preferentially expressed in the internal phloem of the bundle while the second is preferentially expressed in external phloem. These promoters may find application in insect control strategies, or in the modulation of stem nutritional content. There are early indications that expression of one may also be induced in roots by nematode attack, suggesting a further use in nematicidal strategies. The fourth promoter governs expression in developing pollen cells of the anther. This promoter may have application in pollen ablation for male sterility - we have shown that it is active in potato and tobacco and its utility in gametic transformation protocols is also being assessed.

Plant transformation remains an area of active interest. Cereal transformation using the biolistic approach has been achieved with the generation of transgenic wheat containing constructs bearing genes for coat protein or movement protein from soil-borne wheat mosaic virus. In this EU-funded project, with the goal of engineering resistance to the major viral pathogen of wheat in the under-developed world, both model cultivars and cultivars of importance in Chinese agriculture have been transformed. The genotypic dependency of transformation is a current focus of our work on barley transformation, which has revealed significant differences in regeneration frequencies within the pedigree of cv. Golden Promise, the model cultivar routinely used for transformation. Difficulties in the regeneration phase remain the primary problem in the facile transformation of barley cultivars in current use in UK agriculture.

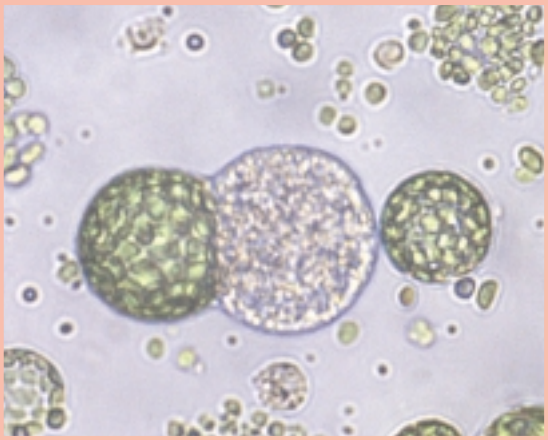

Figure 1 A tobacco-barley heterokaryon resulting from protoplast electrofusion (photograph courtesy of Julie Wardrop).

Transgenic barley (Golden Promise) is also being used in a novel radiation hybrid approach to gene mapping in barley. Radiation hybrid mapping is based on the generation of a panel of hybrid callus lines derived from the fusion of herbicide-resistant barley protoplasts to tobacco protoplasts (see Fig. 1). Selection for herbicide-resistance under conditions otherwise unfavourable to the barley cells, encourages the addition of segments of the barley genome (including the herbicide-resistance gene) to the stable tobacco genome. Analysis of co-transfer of linked markers then allows a physical map of the barley genome to be assembled. This, and other *in vitro* mapping approaches based on physical fragmentation of the barley genome, its dilution into fractions of less than a haploid equivalent, then reamplification of the frac-

tionated sub-genome (HAPPY mapping), should allow the construction of physical maps complementary to barley genetic maps based on molecular markers - an area in which SCRI has a world lead.

These novel mapping technologies will also allow the creation of an expression map of the barley genome. We are in the process of characterising several thousand expressed sequence tags (ESTs) from the developing and malted barley grain. This gene discovery project aims to generate a more complete understanding of the patterns of gene expression which underlie the physiology and biochemistry of the developing endosperm and the barley grain during the malting process. Mapping of these sequences will allow their correlation to areas of the barley genome known, through QTL analysis, to contribute to characteristics such as malting quality. Ancillary data from tissue and temporal specificity, allelic diversity and its relation to the trait of interest, and information on metabolic pathways can all help to refine this candidate gene approach. Marker-assisted breeding or transgenesis will then be applied to test the function of the candidate sequence.

This approach relies heavily on the efficiency of the DNA sequencing facility which continues to provide an excellent service to researchers across the Institute despite an ever-increasing workload.

Cell biology Considerable progress has been made in the non-invasive imaging of virus movement in plants. Using viruses expressing GFP, the systemic pattern of invasion of potato virus X was studied in leaves undergoing the sink-source transition. Further studies of this developmental phenomenon have led to the discovery of two distinct populations of plasmodesmata, the channels through which viruses pass when infecting cells. Simple plasmodesmata, which occur only in sink tissues, permit the passage of macromolecules, while branched plasmodesmata, which form later in source leaf tissues, permit the passage of small solute molecules only. These developmental changes are correlated with the import/export transition of the leaf and demonstrate a major role for plasmodesmata in regulating assimilate fluxes in the plant (see pp. 76-79).

Collaborative research with Dr C. Hawes has been funded by the BBSRC and through the SOAEFD flexible fund, and is aimed at dissecting the secretory pathway in plant cells using virus-based vectors. This work has produced the first *in vivo* tags for the Golgi apparatus, an organelle involved in the sorting and redistribution of proteins within the cell, and is exploring the regulatory steps in the secretory pathway.

A collaborative venture between SCRI and Biosource Technologies Inc., a Californian-based biotechnology company, is exploring the use of viral vectors for the expression of foreign proteins in plants. This work involves a multidisciplinary approach utilising skills in molecular biology, virology, cell biology, and imaging. Eleven new appointments have been made to facilitate this major research programme.

A non-invasive study of the development of blackcurrant fruits from flower to maturity has been achieved by NMR microscopy, and the images compared with those derived from low temperature scanning electron microscopy (LTSEM) and resin histology. The entire living tissues of the specimen, still attached to the growing bush in some experiments, were imaged in three dimensions. By reference to *http:www.scri.sari.ac.uk* and clicking 'Special Topics', it is possible to see the animation of serial slices in selected planes and rotations of 3D projections to reveal internal structures of the flower and fruit, such as the ovaries, aril, vascular bundles and the mature seed. Because the NMR signal intensity is a function of mobile proton concentration and relaxation rate, it allows the generation of a variety of contrast patterns to reveal different aspects of structure without the use of stains. This work on the dynamics of fruit development is an example of the interdisciplinary nature of many advances in botany made possible by investment in new technologies at SCRI. The contrast patterns produced non-invasively by NMR imaging represent tissue features distinguished by entirely different physico-chemical processes from those revealed by light or electron microscopy and, at this stage in the development of NMR, it is important to make reference to illustrations produced by conventional histology.