Characterising pathogen-induced signal transduction pathways in plants - opening Pandora's box

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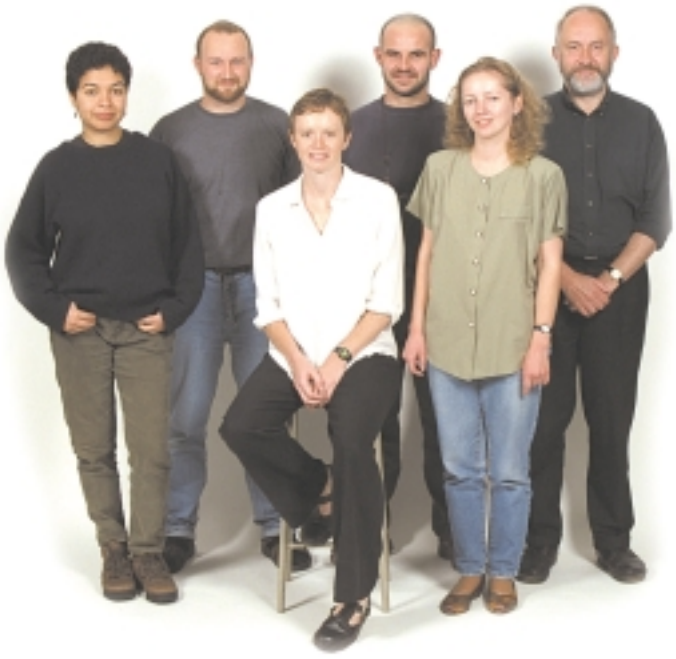
One of the most significant developments in biology in recent years is the world-wide commitment to fully sequence a number of genomes, including mammals such as human and rat, plants such as *Arabidopsis thaliana* and rice, and microbial genomes such as *Escherichia coli* and yeast. To date, some 21 genomes or chromosomes have been published and a further 83 microbial genomes are being sequenced. Many biotechnology companies are keen to exploit these new resources by isolating genes or expressed sequence tags (ESTs), identifying a function, initially through comparison with known genes in international databases, and exploiting novel applications with their use. One important focus of such genome studies in both plants and animals is to gain an understanding of resistance to invading pathogens. With the recent development of new molecular techniques to target the isolation of ESTs specific to a particular tissue, developmental stage or process, it is now possible to better characterise signal transduction pathways involved in disease resistance.

We are currently working to characterise the molecular bases of resistance to two important potato pathogens, *Phytophthora infestans*, the cause of late blight, and *Erwinia carotovora* subsp. *atroseptica* (*Eca*), the cause of blackleg. *P. infestans* is a fungal pathogen that has an initial biotrophic phase, later becoming necrotrophic. It shows a high degree of specificity in that it occurs as distinct races which elicit a hypersensitive response (HR) in potato cultivars possessing appropriate major resistance (R) genes. In contrast, *Eca* is a bacterial pathogen that does not show the same high degree of specificity with potato, does not occur as distinct races, and does not induce an HR. A study of plant signalling in response to infection by these two quite different pathogens enables us to obtain a better understanding of many aspects of plant-microbe signalling, including the nature of specificity and factors involved in hypersensitivity. In addition, these two pathogens cause important economic diseases of potato within the UK and are amongst the three most important pathogens of potato world-wide.

Specificity in cell signalling can arise at several levels, from the receptor, through modulation of signalling kinetics, interactions of different signalling pathways, and at the level of tissue-specific downstream regulators. Thus, tissue-specific transcriptional complexes could allow similar upstream signals to regulate different sets of genes in different tissues. For this reason, if we are to fully understand the molecular bases of disease resistance, we should look at all the molecular processes associated with induced resistance.



We compared the potato cultivars Stirling, which possesses both horizontal resistance and major (R gene) resistance to *P. infestans*, and Bintje, which is highly susceptible and does not exhibit an HR. We used recently developed PCR-based methods for isolating differen-

tially expressed genes, cDNA-AFLP and suppression subtractive hybridisation (SSH), to generate a cDNA library enriched for *P. infestans*-induced genes specific to Stirling at a time-point of 24 hours post-inoculation. cDNA prepared from the infected Bintje was used as a driver to remove all common sequences from the infected Stirling via the subtraction procedures associated with SSH. The remaining material was amplified and cloned. One thousand clones prepared from this cDNA were screened by hybridisation to remove repetitive sequences such as ribosomal RNA. One hundred of the remaining clones were sequenced and compared to databases using both FASTA (DNA-DNA) and BLASTX (DNA-protein) searches. Fifty per cent matched sequences in international databases. Of these, approximately 60% showed similarity to previously characterised stress-, defense-, or senescence-associated genes of plant origin. In addition, a number of identified genes are implicated in programmed cell death, including signal transduction (serine palmitoyltransferase and phosphatidylinositol-4-phosphate-5-kinase), protein degradation (ubiquitin, ubiquitin carrier protein and cysteine protease), DNA degradation (cyclophilin) and metal ion chelation (metallothionein), and may thus be involved in the HR[1].

A similar approach, using SSH, was carried out with potato Stirling one hour after infiltration with *Eca*, using uninfected Stirling as a driver. The intention here was to generate a cDNA library highly enriched for early response genes, including those involved in signalling. Analysis of the *Eca*-induced cDNA library showed that it is highly redundant, i.e. it contains only a few different genes, and confirmed that it does indeed contain a number of genes related to transcriptional regulation and signal transduction. These include genes encoding a WRKY-like transcription factor, a protein phosphatase (PP2A) regulatory subunit, and a ubiquitin-specific protease. The expression profiles (Northerns) of these genes were studied at different times after inoculation with either *Eca* or *P. infestans* (compatible and incompatible interactions) and confirmed their early, pathogen-induced up-regulation. Other genes in the *Eca*-induced cDNA library included matches to previously reported genes of unknown function. These include a dehydration-induced gene ERD15, a phosphate-induced gene *phi-1* and a *Meloidogyne*-induced giant cell gene. Sequences matching a gypsy-like retrotransposon were also identified. No function could be ascribed to approximately 50% of the sequences as they show no similarity to known genes.

Both cDNA libraries described above contain a number of chloroplast-associated genes. This may reflect a non-specific induction, possibly through changes in redox potential, which is known to be a regulator of chloroplast gene transcription, or a more specific induction possibly involving free radical production associated with the oxidative burst.

As each isolated gene is putatively identified via database searches, it is positioned within a model of the infected plant cell (Fig. 1) which is being developed as knowledge of signalling processes emerges in the literature. This allows us to hypothesise about the roles of gene products within the plant-pathogen interaction and stimulates the design of future experiments to test the functions of these genes.

As we continue to sequence clones from these sub-

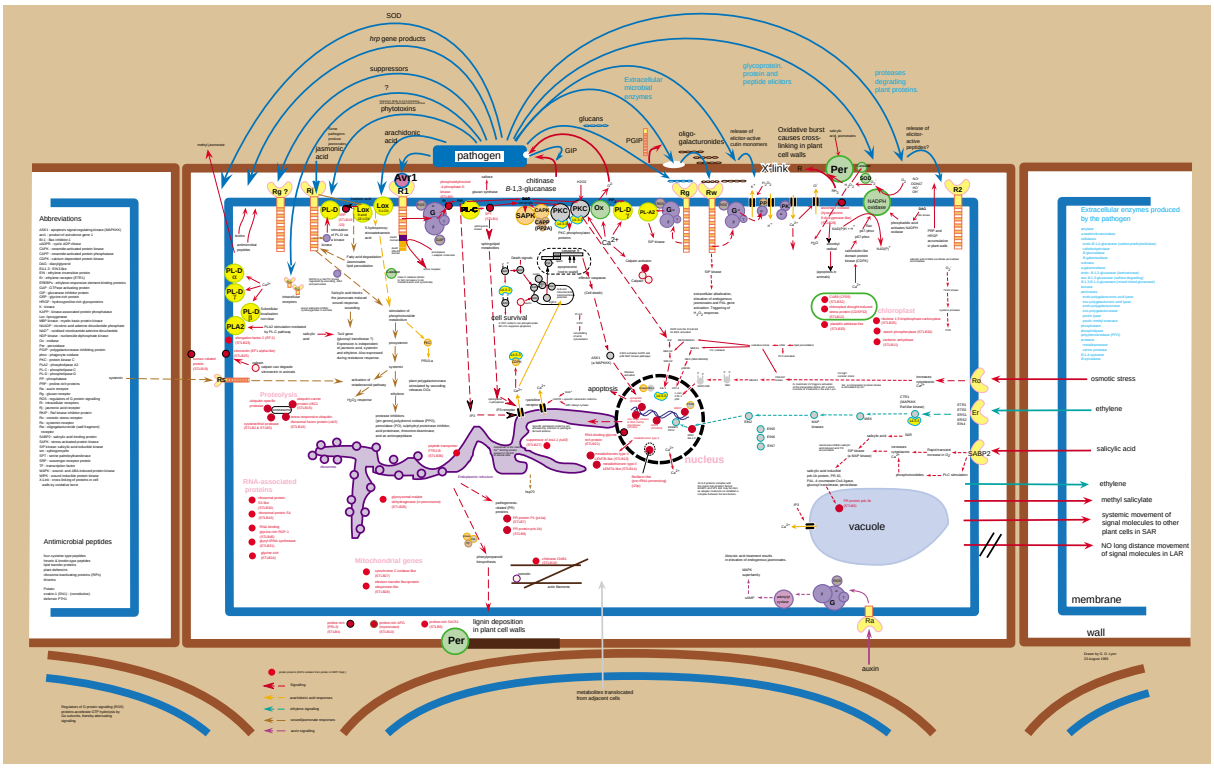


Figure 1 Cell signalling in resistance - a hypothesis.

tracted cDNA libraries, gain more information about their profiles of expression, and identify their function through, for example, transformation studies, we hope to form a picture of the signalling pathways involved in resistance to late-blight and in the HR in general. With an understanding of such processes, there will be increased potential to engineer resistance cascades in order to modify resistance responses. In addition, promoters will emerge that respond rapidly to general pathogen attack and may thus be useful in strategies to engineer broad-spectrum resistance. Furthermore, as efforts to understand cell signalling increase worldwide, a picture is emerging of the considerable conservation in programmed cell death processes between plants and animals.

Reference

1 Birch, P.R.J., Avrova, A.O., Duncan, J.M., Lyon, G.D., Toth, R.L. (1999). *Molecular Plant-Microbe Interactions* 12, 356-361.