Effector protein translocation in the *Phytophthora infestans* – potato infection

Stephen C. Whisson, Petra C. Boevink, Leighton Pritchard, Anna O. Avrova, Juan G. Morales, Paul R.J. Birch

Disease of plants caused by oomycetes are among the leading causes of crop loss and ecosystem damage worldwide. This is exemplified by the late blight pathogen of potato, *Phytophthora infestans*, which is best known for its role in precipitating the Irish potato famines in the mid-1840s. Today, late blight is still a significant problem in potato production and is considered a threat to global food security; resistance and chemical control can be overcome by pathogen variants. The mechanisms underlying how *P. infestans* invades its hosts, and how resistance is triggered have been significantly advanced in recent years. A major advance in the understanding of *P. infestans*–potato interactions came with the isolation, at SCRI, of the first avirulence gene (*Avr*3a) from this pathogen (Armstrong *et al.,* 2005); the protein products of avirulence genes are recognised and trigger resistance responses in plants carrying the cognate resistance gene. *Avr*3a shares a similar organisation with the other three oomycete avirulence genes identified to date (Rehmany *et al.,* 2005): an N terminal signal peptide for secretion, peptide motifs of RxLR and EER within the first 70 amino acids, followed by the portion of the protein that is recognised by the matching

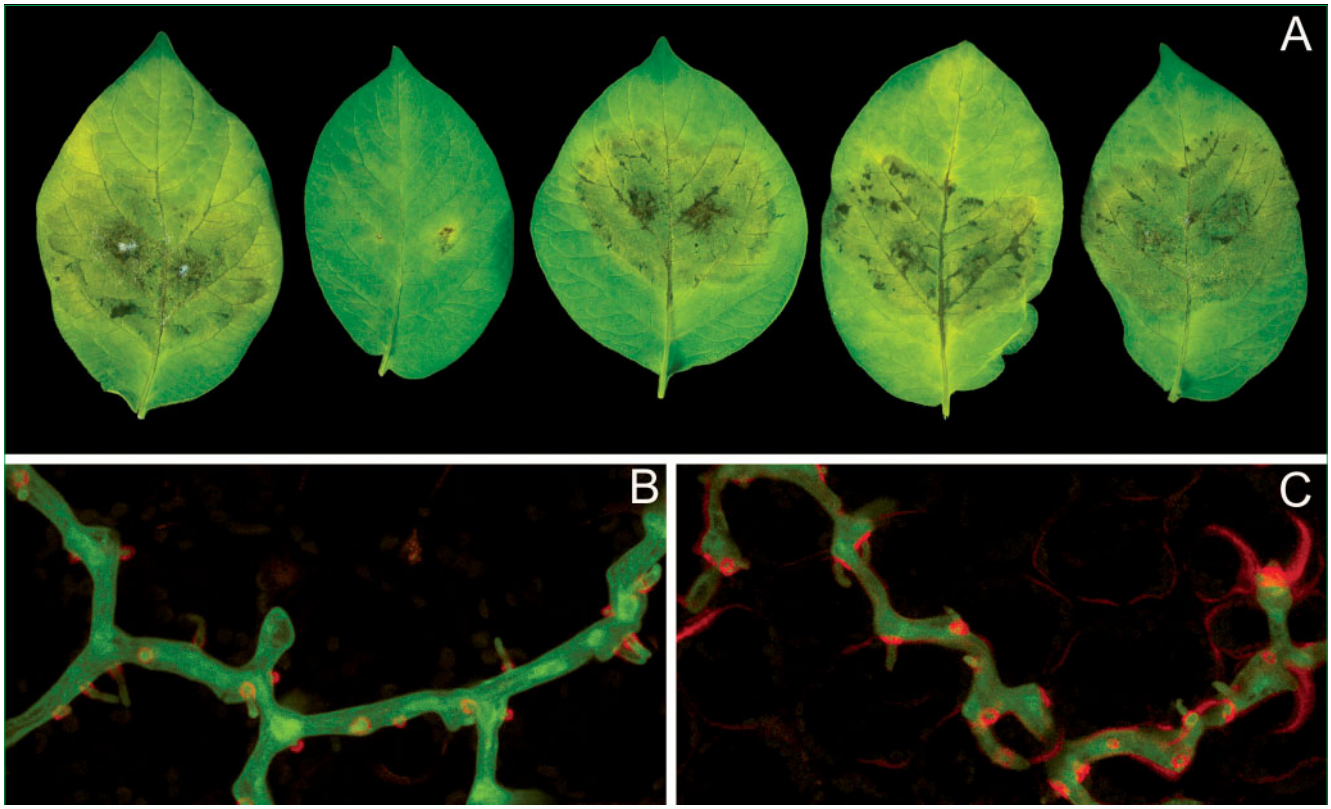

Figure 1 (A) Potato cultivar Pentland Ace (*R3a*) leaves infected with (from L – R): untransformed *P. infestans* expressing the virulent allele of *avr*3a, transformant expressing native avirulent allele *Avr*3a with intact RxLR-EER, transformant expressing alanine replacement of *Avr*3a (AAAA-EER), transformant expressing alanine replacement of *Avr*3a (RxLR-AAA), transformant expressing alanine replacement of *Avr*3a (AAAA-AAA). Transformants were all fully pathogenic on potato cultivars lacking late blight resistance genes. (B) Confocal microscopy image of a *P. infestans* transformant expressing a native AVR3a::mRFP fusion during infection of potato, showing AVR3a secretion (red fluorescence) only from finger-like haustoria. (C) As in B but with the RxLR-EER of AVR3a replaced by alanine amino acids; the red fluorescence is no longer restricted to the haustoria.

resistance protein in the host plant. The function of the RxLR-EER motifs has been speculated to be functionally similar to the host targeting signal (RxLxE/Q) in secreted proteins of malaria parasites (Haldar *et al.,* 2006). The role of these dual motifs in translocating secreted proteins of *P. infestans* was recently demonstrated at SCRI (Fig. 1A). AVR3a is only recognised inside potato cells that express the R3a protein and the RxLR-EER is not required for the recognition process. The RxLR and EER motifs were replaced by alanine amino acids in the AVR3a protein. This experiment demonstrated that both the RxLR and EER motifs are required for transport of the AVR3a protein, as AVR3a was no longer recognised in R3a cells. The role of these dual motifs was further investigated by translational fusion of the native and alanine replacement *Avr*3a genes to the monomeric red fluorescent protein, followed by confocal microscopy (Fig. 1B). This showed that AVR3a is secreted from *P. infestans* haustoria, which are finger-like biotrophic structures that are invaginated into plant cells and are therefore in intimate contact with host cytoplasm. In comparing the cellular localisation of different forms of AVR3a fused to mRFP, the alanine replacement version was intensely fluorescent surrounding the haustoria, and red fluorescence was observed in the plant intercellular spaces, as if accumulating and overspilling the extra-haustorial matrix (Fig. 1C). This is also consistent with a role for the RxLR-EER motifs in protein translocation across host plant membranes.

A bioinformatics search of the *P. infestans* genome sequence, plus 18,000 unique gene sequences, yielded a prediction of 425 RxLR-EER class genes for *P. infestans*. A subset of these, derived from unigenes, showed that all were up-regulated either in preinfection stages or during infection, consistent with the hypothesis that the encoded proteins are involved in establishing and maintaining host plant infection. Screening of these genes using a transient gene silencing strategy developed at SCRI has revealed that some play a role in establishing infection. The central role that the RxLR-EER motifs apparently play in translocating *P. infestans* effectors into plant cells, provides potential targets for novel oomycete disease control strategies.

References

Armstrong, M.R., Whisson, S.C., Pritchard, L., Bos, J.I.B, Venter, E., Avrova, A.O., Rehmany, A.P., Böhme, U., Brooks, K., Cherevach, I., Hamlin, N., White, B., Fraser, A., Lord, A., Quail, M.A., Churcher, C., Hall, N., Berriman, M., Huang, S., Kamoun, S., Beynon, J.L. & Birch, P.R.J. 2005. An ancestral oomycete locus contains late blight avirulence gene *Avr*3a, encoding a protein that is recognized in the host cytoplasm. *Proceedings of the National Academy of Sciences, USA* **102**, 7766-7771.

Haldar, K., Kamoun, S., Hiller, N.L., Bhattacharje, S. & van Ooij, C. 2006. Common infection strategies of pathogenic eukaryotes. *Nature Reviews Microbiology* **4**, 922-931.

Rehmany, A.P., Gordon, A., Rose, L.E., Allen, R.L., Armstrong, M.R., Whisson, S.C., Kamoun, S., Tyler, B.M., Birch, P.R.J. & Beynon, J.L. 2005. Differential recognition of highly divergent downy mildew avirulence gene alleles by *RPP1* resistance genes from two *Arabidopsis* lines. *Plant Cell* **17**, 1839-1850.