Functional genomics tools for the major eukaryotic pathogens of potato

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The greatest losses from disease in potato crops are caused by the independent actions of the oomycete *Phytophthora infestans* causing late blight, and the potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*. Compared with viruses and bacteria, these eukaryotic pathogens have complex life cycles and genetics, and large genomes. Although PCN and *P. infestans* use very different strategies to invade host plants, both establish intimate interactions within the invaded plant tissues. Much research on both pathogens to date has focussed on discovering genes that are specifically up-regulated during their interactions with potato. This has led to the identification of numerous genes that are potentially important in establishing or maintaining a compatible interaction (disease). However, many of the newly discovered pathogen genes are unique and it is difficult to infer any function from sequence alone. A strategy for determining the function of these novel genes is therefore needed.

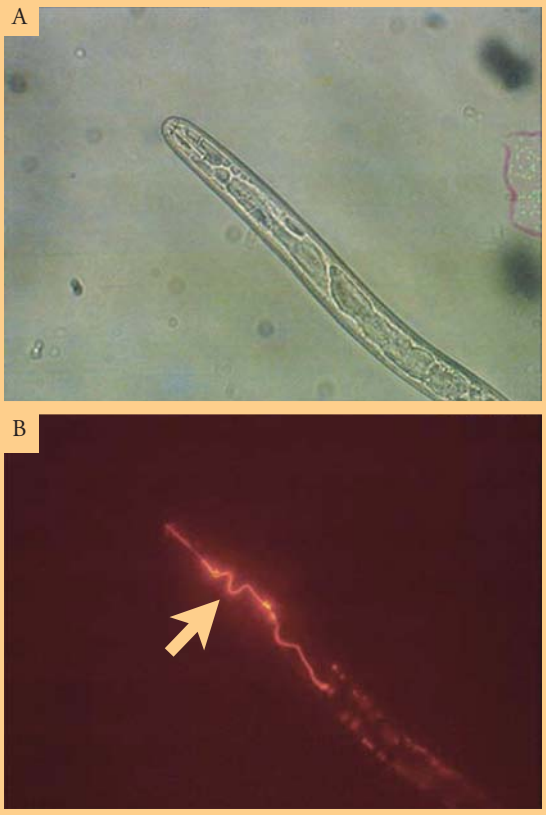

Figure 1 Uptake of dsRNA labelled with Cy3 by a *G. pallida* second stage juvenile. A- bright field image. B- same nematode viewed under fluorescence optics. Labelled dsRNA is visible in the tube that connects the nematode stylet to the digestive system (arrow).

One of the most revealing ways to analyse gene function is to remove its activity through gene silencing. RNA interference (RNAi) has been widely exploited in many organisms as a strategy for determining gene function through silencing. RNAi relies on the degradation of gene-specific double-stranded RNA (dsRNA) molecules into short interfering RNAs (siRNAs) that guide the destruction of the identical endogenous messenger RNA from the gene of interest. RNAi has not been widely applied in determining gene function in eukaryotic plant pathogens. At SCRI, RNAi has been successfully adapted for use in determining gene function in the nematode *G. pallida* (Fig. 1), an organism that has previously been recalcitrant to analysis of gene function. The function of genes potentially involved in sensory perception, invasion and avirulence is currently being studied.

Most recently, RNAi has been adapted at SCRI for use in *P. infestans*. Protoplasts derived from mycelium have been shown to take up dsRNA and exhibit RNAi gene silencing as the mycelium regenerates (Fig. 2A, 2B). dsRNA also triggers a secondary down-regulation

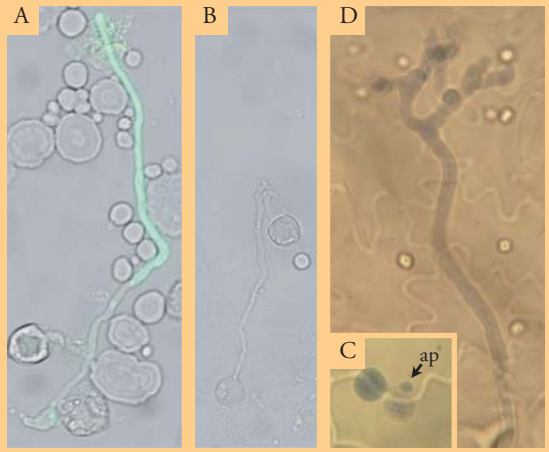

Figure 2 Treatment of transgenic *P. infestans* protoplasts expressing green fluorescent protein (GFP) (A) with *gfp*-dsRNA results in silencing of the *gfp* gene (B) in regenerating protoplasts. Silencing of a *P. infestans* gene expressed in germinating cysts with appressoria (ap arrowed) during the early stages of infection (C) results in abnormal cyst germination and no appressorium formation (D).

of the target gene in *P. infestans* approximately 12 – 15 days after exposure to dsRNA. The mechanism causing this is presently unknown but is being investigated. The significance of this is that it allows the effect of silencing specific genes on all lifecycle stages, including infection, to be investigated. Several *P. infestans* genes have already been studied at SCRI using RNAi. Of these, a striking phenotype was observed with an entirely novel gene that has been shown to be up-regulated prior to invasion, and during the late stages of infection. Silencing of this gene prevented formation of preinfection structures called appressoria, and reduced invasion of potato leaves (Fig 2C, 2D).

Genes that are specific to individual pathogens or groups of pathogens are promising areas for future disease control strategies. However, proof of the role of these genes and their encoded proteins in disease is a crucial requirement. Additional genes will be tested in future to reveal a more complete picture of nematode and oomycete pathogen biology, and provide targets for future control strategies.