Knockout of PCN genes using RNAi

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Many genes have been identified from the potato cyst nematode (PCN) *Globodera spp* that may play a role in parasitism of plants. This information can be used to identify important nematode proteins that might be useful targets for novel control methods. However, before this can be done it is necessary to understand the function of the proteins encoded by the genes. Predicting function of some genes is straightforward. For example, one group of nematode proteins is similar to plant cell wall degrading enzymes. However, many proteins have no similarity to any other genes in the databases. In order to analyse the function of these genes systems for studying nematode gene function *in vivo* are needed. One technique that offers this possibility is RNA inhibition (RNAi). The basis of this technique is that exposure of an organism to double stranded RNA (dsRNA) generated from a gene of interest causes down regulation of the gene and, as a result, levels of the protein encoded by the gene drop dramatically. A recent paper described a method for using RNAi with PCN J2s. We have further developed this technique to allow it to be used with secreted proteins.

Nematodes locate their hosts by chemoreception using two sense organs – the amphids – located at the anterior tip of the nematode. In our previous work we identified a secreted protein expressed in the amphids (Figure a). We have now used RNAi to knock out expression of this gene. The rationale for this was that ablating a sense organ protein should inhibit sensory perception, giving rise to a phenotype that can be scored easily – an inability to locate host roots. Nematodes were soaked in dsRNA generated from the amphid gene (ams-1) (or in soaking solution without dsRNA for the controls) and then split into two batches. One batch was used for invasion studies while the other batch was used in RT-PCR experiments in order to demonstrate that we had successfully knocked out expression of the ams-1 gene without affecting expression of another control gene (actin). These experiments showed that the actin gene could be successfully amplified from both control and dsRNA soaked nematodes whereas the ams-1 gene was far more readily amplified from the control nematodes than from the soaked nematodes, indicating a reduced level of expression of the ams-1 gene in the dsRNA soaked nematodes (Figure b). These results were mirrored in invasion studies in which the dsRNA treated nematodes showed an almost complete inability to infect host roots (Figure c). These experiments have now been repeated using another gene, a secreted cellulase that is thought to be important in invasion and migration. Nematodes treated with this dsRNA also showed a statistically significant reduction in their ability to infect roots (Figure d). Those nematodes that managed to invade roots and establish feeding sites were examined in more detail. The developmental fate of these nematodes was no different to that of the controls suggesting that for this gene the RNAi procedure generated silencing in some nematodes but not others.

This work shows that RNAi can be used to investigate the function of genes implicated in the parasitic process of PCN. We are currently investigating the function of other putative parasitism genes using this technique.

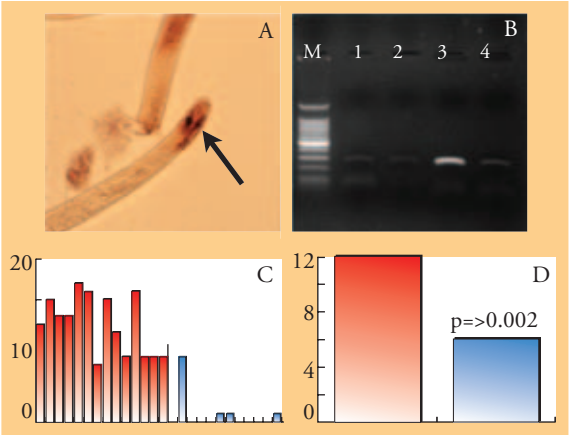

Figure 1 A: *in situ* hybridisation reaction showing expression of the *ams-1* gene in the amphids (arrows) of PCN. **B:** RT-PCR reaction showing PCR products obtained using actin primers from control (lane 1) and ams dsRNA treated (lane 2) samples and PCR products obtained using ams-1 primers from control (lane 3) and ams dsRNA treated (lane 4) samples. The data show that the ams-1 mRNA is less abundant in the dsRNA treated samples but that expression of other genes is unaffected. M = marker. **C:** Infection of potato plants by control (red) or ams dsRNA treated (blue) nematodes. Each bar represents a replicate experiment. Nematodes treated with ams dsRNA are far less able to locate host roots. **D:** Average infection of potato plants by control (red) or cellulase dsRNA treated nematodes. Nematodes treated with cellulase dsRNA are significantly (p= 0.002) less able to infect potato plants.