Functional analysis of pioneer effectors from *Globodera pallida*

Lee Robertson[1], Rakesh Radnakrishnan[2], Peter J. Cock[2], Vivian C. Blok[2], Ailsa Smith[2], Eric Grenier[3], Martin Hunt[4], Matt Berriman[4] & John T. Jones[2]

[1]Museo Nacional de Ciencias Naturales, Dpdo Biodiversidad y Biología Evolutiva, C/ Jose Gutierrez Abascal 2, Madrid 28006, SPAIN
[2]SCRI, Invergowrie, Dundee DD2 5DA, UK
[3]INRA UMR Bio3P, Domaine de la Motte;BP 35327, 35653 Le Rheu cedex; FRANCE
[4]Wellcome Trust Sanger Institute, Genome Campus, Hinxton, Cambridge CB10 1SA, UK

The potato cyst nematode, *Globodera pallida*, has complex interactions with its hosts (Figure 1).

These interactions are mediated by secreted proteins (effectors) that induce the feeding site and suppress host defence responses.

Genome sequencing projects and EST projects have led to the identification of putative effectors, many of which are pioneers that have no similarity to characterised proteins.

Here we describe our attempts to investigate the functions of two pioneers from *G. pallida* - IA7 and IVG9. These genes were originally identified by Blanchard *et al*. (2007) in work designed to identify genes responsible for host range differences between *G. pallida* and *G. mexicana*.

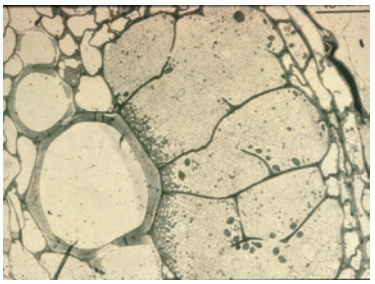


Figure 1 - syncytium induced by *G. pallida*

Gene families

The current assembly of the *G. pallida* genome sequence was searched to investigate the extent and structure of the IA7 and IVG9 gene families

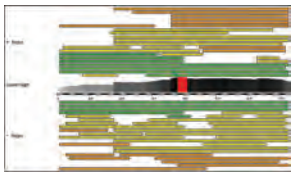


Figure 3 - IA7 matches in the *G. pallida* genome sequence

A large family of IA7-like genes is present in *G. pallida*. Thirteen very similar genes are present as well as 8 more distantly related genes. Each has a predicted signal peptide and a domain similar to the ShK-like domain. Only 2 of these genes have an intron.

A smaller family of IVG9-like genes is present; three close relatives (each with a signal peptide and each containing two introns) as well as four more distantly related sequences.

Expression profiles

Expression profiles of IA7 and IVG9 were examined on a *G. pallida* microarray.

Although they are expressed in different gland cells, both showed similar expression profiles - highly upregulated in J2 as compared to eggs and 7dpi parasitic nematodes (right).

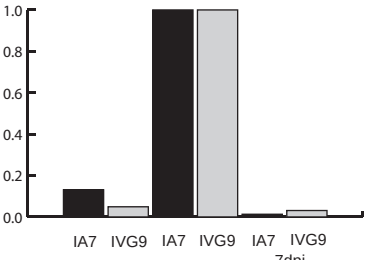
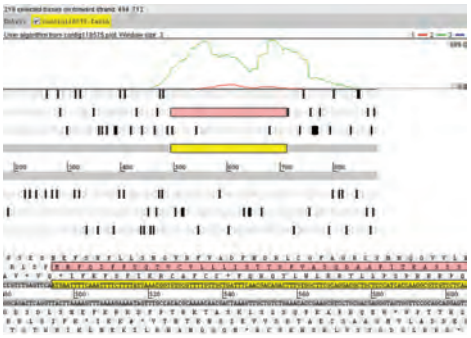


Figure 4 - average expression of IA7 and IVG9 on microarrays in eggs and 7dpi parasitic nematodes normalised against J2 expression.



Solexa RNA sequence plots against the *G. pallida* genome confirmed this expression pattern (left). Signal was detected from J2 (green) only, with little signal from eggs (red) and no signal from later stages (blue)

Subcellular localisation and effects on nuclear size

The subcellular localisation of the IA7 and IVG9 proteins was examined using N and C terminal fusions with GFP expressed from TRV.

IA7 was localised in the cytoplasm in both leaves and roots (Figure 5a, 5b).

IVG9 formed unusual clumps of fluorescent material in roots and leaves (Figure 5c). A single clump of material formed in each cell but counterstaining with diethidium bromide showed that these clumps were not associated with the nucleus (Figure 5d).

IA7 and IVG9 were also expressed without a fusion protein from the pMDC32 vector in plants constitutively expressing GFP targeted to the endoplasmic reticulum. These plants allow nuclear size to be measured (Figure 5e).

IA7 induced a small but statistically significant increase in size of the nucleii compared to the controls (p= 0.001). IVG9 did not induce a statistically significant change in nuclear size.

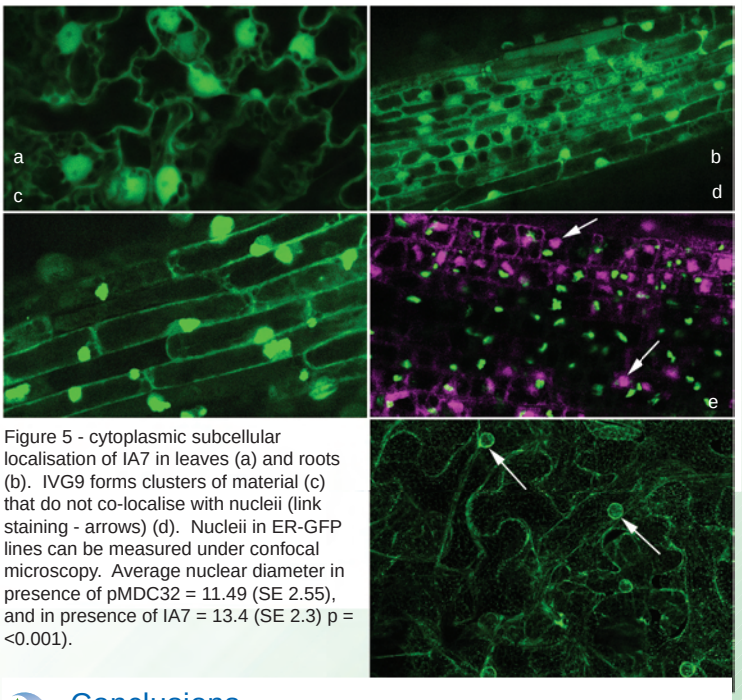
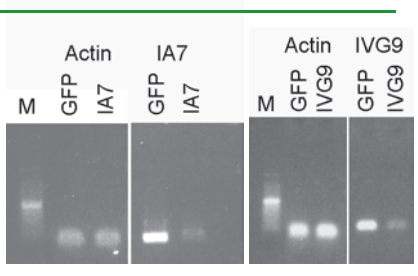


Figure 5 - cytoplasmic subcellular localisation of IA7 in leaves (a) and roots (b). IVG9 forms clusters of material (c) that do not co-localise with nucleii (link staining - arrows) (d). Nucleii in ER-GFP lines can be measured under confocal microscopy. Average nuclear diameter in presence of pMDC32 = 11.49 (SE 2.55), and in presence of IA7 = 13.4 (SE 2.3) p = <0.001).

RNAi

We used RNAi to silence IA7 or IVG9 in J2s of *G. pallida*.

RT-PCR showed that in both experiments target gene expression was reduced compared to J2s soaked in dsRNA from GFP (Figure 6).



J2s were subsequently allowed to infect potato roots and examined 5 and 14 days after infection. No differences were observed in infection rates or development of the nematodes. This may be a reflection of functional redundancy within the gene families.

Conclusions

Functional characterisation of pioneers, particularly those present in gene families, remains a major challenge!

The potential role of IA7 in inducing changes in plant nuclei will be further examined.

This work was funded in part by an STSM from COST 872