Functional Analysis of a Potato Cyst Nematode (*Globodera pallida*) Effector Protein

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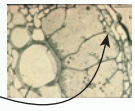
Plant Parasitic Nematodes (PPN)

- PPN is of huge economic importance - $125 billion damage yr $^{-1}$ to world wide agriculture
- The most damaging nematodes are biotrophic sedentary endoparasites:
  - Root-Knot Nematodes RKN (*Meloidogyne*)
  - Cyst (***Globodera*** and *Heterodera*)

Globodera pallida
cysts on potato roots

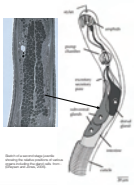
Potato Cyst Nematodes (PCN)

- PCN induce a feeding site (**Syncytium).** Here they live as a biotroph for several weeks. It is therefore essential that they **suppress host defences** using effector proteins.



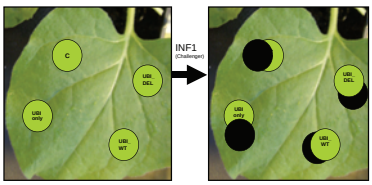
Nematodes Effectors

- Produced in oesophageal gland cells (subventral and dorsal).
- Secreted via the stylet into the host cell.
- Range of roles including: Migration through root, initiation and protection of the feeding site



Method

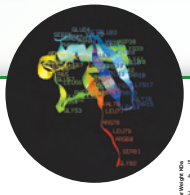
- **DNA was extracted** from selected and non-selected nematode lines. The UBI-EP gene was cloned.
- **To determine if the UBI-EP is cleaved**, GFP fusions were made at the C and N terminus of the protein. The proteins were extracted and analysed via Western blotting.
- Confocal image analysis of the UBI-EP-GFP fusions was used to reveal the **cellular localisation** of the protein domains once cleaved.
- To determine if the UBI-EP **suppresses host defences**: Agro-infiltration was conducted using various 'challengers' to provoke a HR.
- The experimental model was as follows (Control refers to an empty vector, UBI_DEL is the allele with the 3 amino acid deletion in the C-terminal extension, UBI_Only is just the UBI domain - this is not naturally occurring and UBI_WT is the full version of the gene):

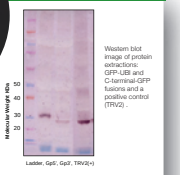


- Assay for PTI used INF1 as the challenger.
- Assay for ETI used AVR3aKI and R3a as the challenger combination.
- Infiltration zones were scored based on a 4 point scoring system: 0 = no suppression of HR (hypersensitive response), 1 = little suppression of HR, 2 = evidence of suppression, 3 = very high suppression of HR (a crescent of HR may be seen outside of the gene of interest infiltration zone).

G. pallida effector: Ubiquitin Extension Protein (*G.p.*UBI-EP)

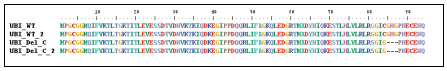
- It is known that similar UBI-EP are present in other cyst nematodes, but not in RKN.
- The lysine amino acids within the *G.p.*UBI-EP ubiquitin domain (UBI) are located at positions 6, 11, 27, 29, 33, 48 and 63. The UBI ends with two glycine amino acids (GG), both of these features are consistent with normal functioning ubiquitin proteins.
- There are 3 amino acid substitutions in UBI domain compared to the highly conserved poly-ubiquitin sequence: If 1 is start of UBI domains, V for I (23), T for S (28) and M for L (56).
- The C terminal extension (12 amino acids) is unique – No Blast hit**!**
- Western blotting analysis reveals the UBI domain and C-terminal extension are cleaved from each other *in planta.*





Western blot image of protein extractions: GFP-UBI and C-terminal-GFP fusions and a positive control (TRV2) .

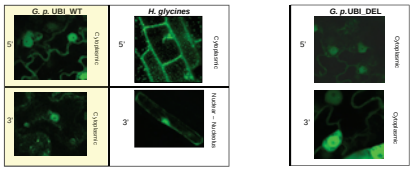
Variation in C – Terminal Extension: Two Isoforms of the Effector



- 3 A.A. **deletion** (this isoform will be referred to as UBI_DEL**)**
- **SG** replaces **GG**
- UBI_DEL is selected for in virulent populations.
- Early statistical analysis suggests there may be a fitness penalty for carrying UBI_DEL (V. Blok)

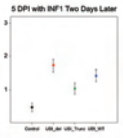
Localisation of *G. pallida* and *H. glycines* UBI-EP

Confocal images used to determine cellular localisation of *G. pallida* and *Heterodera glycines* GFP-UBI and C-terminal-GFP fusions. Note the green nucleolus in the *H. glycines* 3' picture, free-GFP is excluded from the nucleolus (*H. glycines* - images obtained by J.Jones).



G. p. UBI-EP Suppresses INF1 Induced defence responses

- **Control Vs UBI_DEL, UBI _WT: P<0.001, P<0.001 (Mann-Whitney test)**
- Control Vs UBI_Only: No statistical difference.
- Therefore as a conclusion: The presence of the **C-terminal extension is important in suppressing INF1 induced defence responses**.

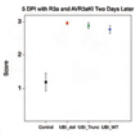


Graph representing the mean and SE of the mean for each population of the G.p.UBI-EP isoforms five days post infiltration (DPI) with INF1 applied two days after effector infiltration.

G. p. UBI-EP Suppresses AVR3aKI Induced defence responses

- **Control Vs ...All infiltration sites statistically significant: P<0.001 (Mann-Whitney test)**
- UBI_OnlyVs UBI_DEL, UBI_WT: No statistical difference.
- **UBI domain important for suppressing ETI induced by AVR3aKI**

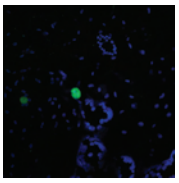
N. benthamiana 5 DPI with R3a and AVR3aKI two days after effector infiltration.



Graph representing the mean and SE of the mean for each population of the G.p.UBI-EP isoforms five days post infiltration with AVR3aKI and R3a applied two days later after effector infiltration.

*G.p.*UBI-EP Infiltration Does not Inhibit Further *Agrobacterium* Transformation

Transient expression of *G. pallida* SPRYSEC-GFP fusion in *N. benthamiana* leaves that has been previously Agro-infiltrated with *G.P.*UBI_WT. This shows further transformation was possible after *G.P.*UBI_WT infiltration.



Conclusion

- The *G.P.*UBI –EP suppresses host defences (PTI and ETI).
- The presence of the C-terminal extension is important in suppressing INF1 induced defence responses (PTI) but is not required in the suppressing R3a-mediated ETI induced by AVR3aKI.
- UBI_DEL allele frequency in nematode populations correlates with the presence of *R* genes (*H3* or *Gpa5*) in the host.

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