Translocation of effector proteins from the oomycete *Phytophthtora infestans* into plant cells.

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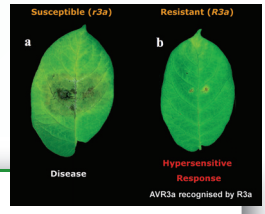

The interaction between the products of avirulence gene *Avr3a* and cognate resistance gene *R3a* can be used as a reporter for translocation: Inoculation of sporangia from virulent *P. infestans* transformed with *Avr3a* causes disease on susceptible potato cultivar (a). The same transformant triggers the hypersensitive response on a resistant cultivar (b), indicating that AVR3a has been translocated into the plant cell, where it is recognized by R3a.

Background

Like bacteria and fungi, the potato blight pathogen *Phytophthtora infestans* translocates effector proteins into host plant cells during infection. Whereas bacteria possess the well characterized type III secretion system, the mechanism used by eukaryotic plant pathogens for delivering effector proteins into the host cell remains unclear. In oomycetes this process depends on a short conserved amino acid sequence (RxLR) located near the signal peptide of many secreted proteins. This motif is specific to oomycetes but resembles the host cell targeting-signal found in virulence proteins from the malaria parasite *Plasmodium falciparum* (RxLxE/D/Q). A recent study showed that the RxLR motif from a *P. infestans* effector protein was sufficient to export the green fluorescent protein (GFP) from *Plasmodium* to the erythrocyte, suggesting a conserved mechanism to deliver effector/virulence proteins into host cells[1]. Potentially, the host targeting signal used by the malaria parasite could function in *P. infestans*. Moreover, RxLR motifs found in avirulence proteins from other oomycetes may also function in *P. infestans*, but these hypotheses still have to be demonstrated.

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P. infestans AVR3a:     MRLAIMLSATAVAINFATCSAIDQTKVLVYGTPAHYIHDSAGRRLLRKNEENEETSEER----------
H. parasitica ATR1:  MRVCYFVLVPSVALAVIATESSETSGTIVHVFPLRDVADHRNDALINRALRAQTALDDDEER----------
H. parasitica ATR13:          MRLVHAVLLPGIIVFVSNGNLLHAHALHEDETGVTAGRQLR----------------------
P. falciparum HRPII:   MVSFSKNKVLSAAVFASVLLLDNNNSAFNNNLCSKNAKGLNLNKRLLHETQAHVDDAHHAHHVAD-----
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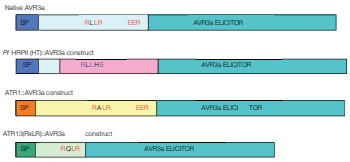
Similarities in sequence and position between the conserved translocation motifs from oomycetes and from *Plasmodium*. Alignment of amino-acid sequences of N-terminal regions, centered on the oomycete translocation motif (in red) and the malarial host targeting signal (in blue), for three oomycete avirulence proteins: AVR3a from *Phytophthora infestans*, ATR1 and ATR13 from *Hyaloperonospora parasitica* and HRPII, a virulence protein from *Plasmodium falciparum*.

Strategy

Recently, our laboratory identified the *Avr3a* avirulence gene from the oomycete *P. infestans,* and showed that the effector protein AVR3a was recognized by the product of resistance gene *R3a* in the host cytoplasm, triggering the hypersensitive response (HR), a form of programmed cell death in the resistant plant[2]. The C-terminal region of AVR3a is sufficient for recognition[3], and there is now evidence that the RxLR-EER motifs are required for the translocation of AVR3a into the host cell[4].

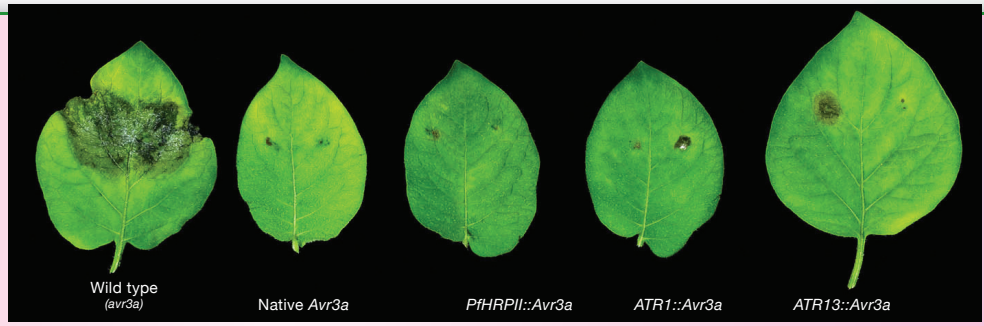
We have used this AVR3a-R3a interaction as a reporter for translocation in *P. infestans* transformants and replaced the RxLR-EER motifs from AVR3a with the motifs from the malaria parasite or from the related oomycete *Hyaloperonospora parasitica* (downy mildew). Stable transformation of *P. infestans* was achieved using a PEG-$CaCl_2$-Lipofectin protocol and gene constructs were cloned into constitutive expression vector pTor.



Schematic representations of AVR3a constructs: The N-terminal region, including the motif RxLR-EER in the native AVR3a, has been replaced by alternative sequences from the malaria virulence protein *Pf*HRPII and from the related oomycete *H. parasitica* avirulence proteins ATR1 and ATR13. The C-terminal domain recognized by R3a remains unchanged for all the constructs.

Results

Transformation of a virulent *P. infestans* isolate with the various *Avr3a* constructs conferred to transformants the ability to trigger the hypersensitive response in plants expressing the resistance gene *R3a*, implying that the alternative sequences are functionally similar to the native RxLR-EER. All avirulent transformants were virulent on susceptible potato (*r*), indicating that transformation did not affect pathogenicity.



Conclusion

Transformants triggered a hypersensitive response similar to that induced by the native AVR3a, indicating that the proteins encoded by the three different constructs are delivered into the host cell during infection.

The RXLR motif alone in the ATR13::Avr3a construct was sufficient to translocate the AVR3a elicitor, suggesting that additional downstream sequences are not always required in this particular effector, and that the sequence upstream the RXLR motif may also function in effector delivery.

The RxLR motif from AVR3a was shown to be functional in the malaria parasite *P. falciparum*[1], and our results support the hypothesis that plant and animal eukaryotic pathogens share a conserved mechanism to deliver effector/virulence proteins into the host cell. Alternatively, these exciting findings may inform more on the flexibility of these protein translocation motifs, and raise the question of whether RxLxE/D/Q and RxLR-EER are evolutionary convergent solutions to the common problem of effector delivery to the inside of host cells.

Future research

Previous work has utilized the β-glucuronidase (GUS) and monomeric red fluorescent protein (mRFP) genes to confirm effector translocation[4]. We are currently using fusions of various effectors to tandem dimer Tomato (tdTomato), a brighter fluorescent protein, to visualize the subcellular targeting of translocated effectors in infected cells.

References

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