Is KIPI30 the main virulence target of the Phytophthora infestans effector protein Avr3a?

Stefan Engelhardt, Miles R. Armstrong, Eleanor M. Gilroy, Paul R.J. Birch

Plant Pathogen Interactions Programme, Scottish Crop Research Institute, Invergowrie, DD2 5DA Dundee.

Abstract

Plant parasitic aphids like Phytophthora infestans secretes diverse classes of effector proteins to modulate host innate immune reactions. The different alleles of Avr3a, which belong to the R3a effector family, are known to be the ED-form, in contrast to the EM-form, recognized by the NBS-LRR-resistance protein R3a (1). To date, the main virulence target of any effector protein is unknown. By means of a Yeast-2-Hybrid screen using Avr3a as bait the strongest attractor of both forms, KIPI30ΔAvr3a and KIPI30ΔAvr3a, turned out to be an E-chromosomally translocated parasite kinase-like protein. Moreover, the transformed potato plants with VIGS caused a significant decrease of the HR response after Agrobacterium tumefaciens-mediated expression of Avr3a (2). Localization studies show that the truncated KIPI30ΔAvr3a is localized in a vesicle-like structure, while the full-length protein is chloroplast-associated. However, co-localization with either form of Avr3a could not be observed. Moreover, in an interaction assay using BiFC, the Yeast-2-Hybrid did not reveal any interaction between either form of Avr3a and the full-length KIPI30ΔAvr3a. Currently we focus our investigation on optimizing the VIGS-conditions and on interaction studies via pull-down assays to figure out if KIPI30ΔAvr3a is a genuine virulence target of Avr3a, and to investigate if it is the mediator in R3a recognition.

Introduction

As shown below, R3a-mediated hypersensitive response occurs only in the presence of Avr3aΔAvr3a. If a form other than Avr3aΔAvr3a is recognized by the NBS-LRR-resistance protein R3a and, in contrast to the ED-form, does not suppress cell death in N. benthamiana triggered by the Pseudomonas syringae pv. syringae (PSPS) (1, 2). It has recently been demonstrated that the deletion of the C-terminal tyrosine (Y147) of Avr3a abolishes the expression of NBS-mediated cell death, but does not affect R3a recognition (2). This feature separation supports the idea that this effector interacts with two different proteins (3). Here we aim at investigating its particular protein from potato, namely KIPI30ΔAvr3a, for its putative role in virulence target and mediator R3a recognition.

Results

We conducted a Yeast-2-Hybrid screen against a cDNA library from N. benthamiana plants. As shown in Fig. 3, the fluorescence caused by YFP-KIPI30ΔAvr3a was exclusively localized to the chloroplasts (a) and nuclear (b) compartments, respectively (not shown). Interestingly, only the full-length alleles harbor a chloroplast-targeting signal.

To investigate the subcellular localization of KIPI30ΔAvr3a, we constructed Yellow-Fluorescent-Protein (YFP) fusions and transiently expressed these via Agrobacterium tumefaciens infiltration in N. benthamiana plants. As shown in Fig. 4, the fluorescence of both CFP-KIPI30ΔAvr3aΔAvr3a fusions (red) is clearly visible at 7 dpi. In contrast, both CFP-Avr3a fusions (red) are located in the cytoplasm and the nucleus, but do not localize to the chloroplasts (green). The expression of the fusion constructs was confirmed by Western blotting (not shown).

Conclusion and Outlook

In order to assay if KIPI30ΔAvr3a is involved in R3a-mediated recognition of Avr3aΔAvr3a, we employed Virus-induced gene silencing (VIGS) followed by agro-infiltration assays. Eye leaf from N. benthamiana transgenic plants were infiltrated with mixtures of Agrobacterium tumefaciens (Fig. 5a-d). However, we did not detect such a consistent specific interaction after co-expression of either Avr3aΔAvr3a indicates by its growth on both dropout-media (Fig. 5 LWH and LWU). As shown in Fig. 6, only yeast cells expressing the NBS-terminally truncated Avr3aΔAvr3a fusions (right lane) is able to grow on dropout-media, whereas yeasts expressing either full-length Avr3aΔAvr3a or Avr3aΔAvr3aΔAvr3a fusions (left lane) do not grow on dropout-media, either. Moreover, in an interaction assay using BiFC, the Yeast-2-Hybrid did not reveal any interaction between either form of Avr3a and the full-length KIPI30ΔAvr3a. Currently we focus our investigation on optimizing the VIGS-conditions and on interaction studies via pull-down assays to figure out if KIPI30ΔAvr3a is a genuine virulence target of Avr3a, and to investigate if it is the mediator in R3a recognition.

References

(2) Bevan et al. 2006. Plant J. 48, pp. 165-179