Yeast 2 Hybrid Screening to Identify Plant Targets of Oomycete Effector Protein AVR3a

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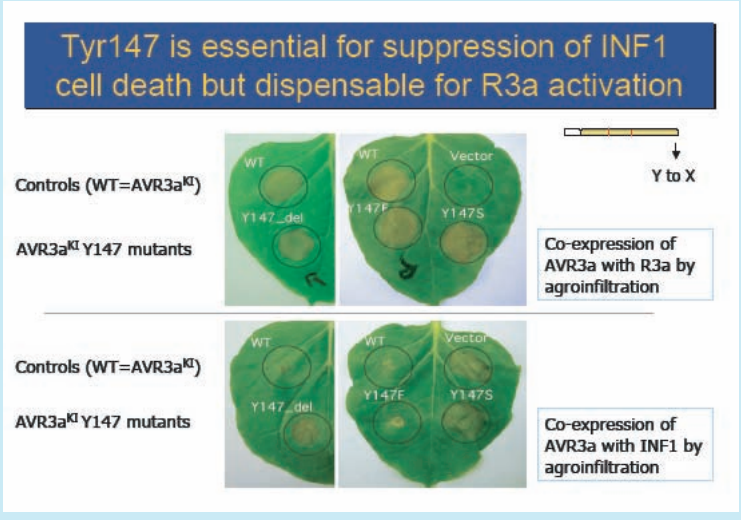
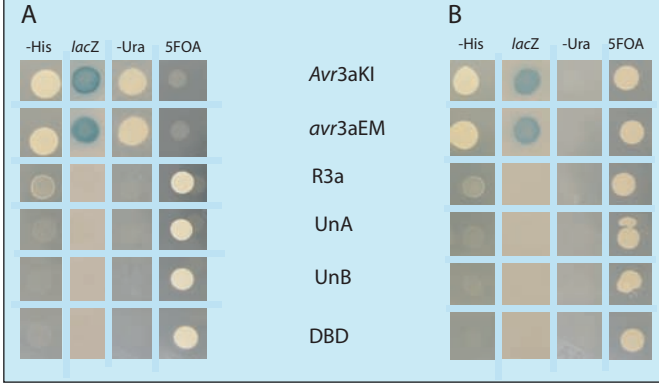
The observation below suggests a virulence function for the KI allele of AVR3a in the suppression of defence signalling leading to the hypersensitive response.

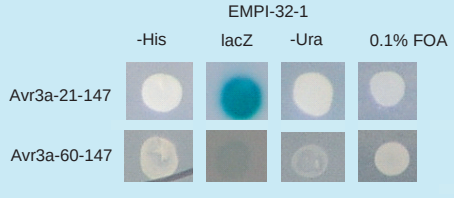


These data also indicate that Avr3aKI INF1 suppressor and R3a activation functions are uncoupled, presenting the possibility that Avr3aKI might associate with multiple, functionally distinct proteins.

A Y2H library, made from infected leaf material, was screened with both the KI and EM alleles of AVR3a. Fourteen potential interactors (PIs) were identified. An example of a strong and a weak interacting protein are shown below.



The 14 PIs were further screened against a truncated AVR3a construct in which the RXLR targetting domain had been removed. In the example below the strong induction of all three reporter genes is much reduced in the truncated construct suggesting that the interaction may involve the targetting domain. Six of the 14 PIs exhibited this effect and have thus been prioritised as candidates for involvement in the targetting process.



Acknowledgments

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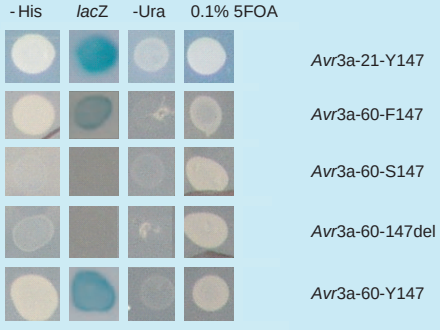
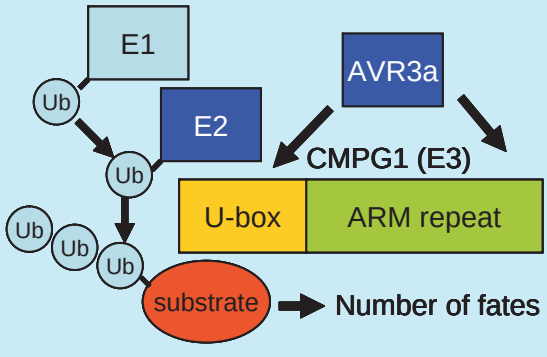
Background

The oomycete Phytophthora infestans causes late blight, the potato disease that precipitated the Irish famines of 1846 and 1847. It represents a re-emerging threat to potato production and is one of over 70 oomycete species which, collectively, are arguably the most devastating pathogens of dicotyledonous plants. Plant pathogens such as *P. infestans* are known to secrete effector proteins into plant cells that act as virulence factors. One possible function of such molecules would be the suppression of plant defence signalling. Here we use Y2H to identify the plant targets of AVR3a, with the aim of inferring the virulence function/s of this effector.

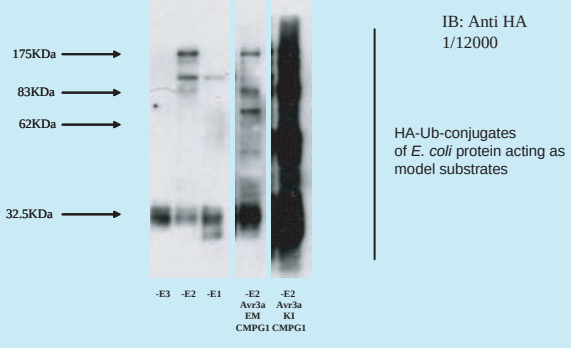


One of the 14 PIs is similar to CMPG1, a E3 ubiquitin ligase known to be essential for Cf-9/Avr9, Pto/AvrPto and INF1 HR induction. The interaction between mutants of AVR3a and CMPG1 corresponds to the ability of those mutants to suppress the INF1 HR.

What effect does the binding of AVR3a to *St*CMPG1 have on E3 activity?



The KI allele of AVR3a appears to function as an E2



E1: yeast
E2: Hubc5b
E3: *St*CMPG1