Functional Analysis of the *Avr3a* gene family of *Phytophthora infestans*

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ABSTRACT

Plant pathogens secrete molecules that promote the infection process and disease. Plants may defend themselves against attack by recognizing these pathogen molecules, resulting in resistance. The plant pathogen *Phytophthora infestans* was responsible for the Irish potato famine in the nineteenth century. This plant pathogen causes late blight on potato and tomato, a devastating disease that leads to billions of dollars in economic losses each year. To understand how this pathogen is able to cause disease on host tomato and potato plants, we aim to identify *P. infestans* molecules that are secreted during infection, and investigate their functions. The *Avr3a* gene of *P. infestans* encodes a small secreted protein that is recognized by R3a in *Solanum demissum* (Armstrong *et al.,* 2005; Huang e*t al.*, 2005). The *Avr3a* gene family is represented by at least two polymorphic members, *Avr3a*_$S^{19}E^{80}M^{103}$ and *Avr3a*_$C^{19}K^{80}I^{103}$ that confer a virulent and avirulent phenotype on *R3a* potato plants, respectively. We performed functional characterization studies aimed at identifying the virulence function of AVR3a to gain insights in the mechanisms by which this protein contributes to plant susceptibility. Eventually, understanding of the mechanisms by which *P. infestans* causes disease will help us to generate more resistant crops and reduce yield loss.

SUMMARY

In plant-microbe interactions recognition of pathogen molecules by plants results in the induction of plant defense responses and resistance. Plant pathogen proteins, encoded by avirulence (*Avr*) genes are recognized by products of plant resistance (*R*) genes. This interaction typically follows the gene-for-gene model, which postulates that the concurrent expression of matching pairs of pathogen *Avr* genes and plant *R* genes results in resistance (Staskawicz *et al.*, 1995; Dangl and Jones, 2001). In the simplest illustration of this model the AVR and R proteins interact directly. However, recent studies implicate the involvement of a third component, the virulence target (Dangl and Jones, 2001; Martin *et al.*, 2003). According to the "Guard hypothesis" the AVR protein interacts with this virulence target in both resistant and susceptible plants (Van der Biezen and Jones, 1998). However, in resistant plants the virulence target is "guarded" by the R protein, which monitors alterations in the target and promotes defense signaling.

Oomycetes, such as *Phytophthora*, downy-mildews, and *Pythium*, form a unique branch of eukaryotic plant pathogens with an independent evolutionary history (Kamoun, 2003). Among the oomycetes, *Phytophthora* species cause some of the most destructive plant diseases in the world. The most notable and best-studied oomycete is *Phytophthora infestans*, the Irish famine pathogen. *P. infestans* causes late blight, a devastating and reemerging disease of potato and tomato (Birch and Whisson, 2001; Kamoun and Smart, 2005). In our working model, *P. infestans* delivers proteins to different cellular compartments of the host where they interact with plant targets to reprogram host defenses and promote susceptibility. To understand the mechanisms underlying disease and susceptibility, we aim to identify and characterize these *P. infestans* proteins that are secreted during infection.

The *Avr3a* gene family encodes two polymorphic secreted proteins that are the main focus of our studies (Armstrong *et al.*, 2005). *P. infestans* isolates that are avirulent on *R3a* potato carry the avirulence gene *Avr3a*, which encodes the mature protein $AVR3a^{KI}$ (containing amino acids K^{80} and I^{103}), whereas virulent isolates carry only the virulence allele *avr3a*, encoding the mature protein $AVR3a^{EM}$ (containing amino acids E^{80}, and M^{103}) (Armstrong *et al.*, 2005). Thus, *P. infestans* isolates that secrete $AVR3a^{KI}$ are recognized by host potato plants that express the R3a resistance protein, which leads to plant resistance. However, in susceptible plants there is no recognition of AVR3a due to the absence of the matching R protein and therefore no induction of defense responses. We aim to find out how AVR3a contributes to this establishment of disease in these susceptible plants. The main questions are: what are the molecular mechanisms by which AVR3a alters host defense responses? What protein features of AVR3a are important for its functions in avirulence and virulence? What plant protein(s) does AVR3a target in its host? Ultimately, knowledge about the molecular basis of plant susceptibility to *P. infestans* will help generate more resistant crops and improved disease management strategies.

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