

Multiple Avirulence Paralogues in Cereal Powdery Mildew Fungi May Contribute to Parasite Fitness and Defeat of Plant Resistance

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Powdery mildews, obligate biotrophic fungal parasites on a wide range of important crops, can be controlled by plant resistance (*R*) genes, but these are rapidly overcome by parasite mutants evading recognition. It is unknown how this rapid evolution occurs without apparent loss of parasite fitness. *R* proteins recognize avirulence (*AVR*) molecules from parasites in a gene-for-gene manner and trigger defense responses. We identify *AVR_{a10}* and *AVR_{k1}* of barley powdery mildew fungus, *Blumeria graminis* f sp *hordei* (*Bgh*), and show that they induce both cell death and inaccessibility when transiently expressed in *Mla10* and *Mlk1* barley (*Hordeum vulgare*) varieties, respectively. In contrast with other reported fungal *AVR* genes, *AVR_{a10}* and *AVR_{k1}* encode proteins that lack secretion signal peptides and enhance infection success on susceptible host plant cells. *AVR_{a10}* and *AVR_{k1}* belong to a large family with >30 paralogues in the genome of *Bgh*, and homologous sequences are present in other formae speciales of the fungus infecting other grasses. Our findings imply that the mildew fungus has a repertoire of *AVR* genes, which may function as effectors and contribute to parasite virulence. Multiple copies of related but distinct *AVR* effector paralogues might enable populations of *Bgh* to rapidly overcome host *R* genes while maintaining virulence.

INTRODUCTION

Obligate biotrophic parasites cause the most serious and widespread diseases of crop plants but are challenging to investigate because they cannot grow outside their host. The three major groups of biotrophic filamentous parasites are the powdery mildew and rust fungi and the downy mildews, which are oomycetes. Powdery mildews cause economic losses to most crops in temperate areas, infecting >9000 dicot and >650 monocot plant species (Chaure et al., 2000). Infection of host plants during the growing season results from wind-dispersed conidiospores that are the asexual (haploid) state of the fungus. Many powdery mildews also have a sexual phase resulting in the formation of ascospores (Figure 1A). Most powdery mildews show a high degree of host specialization, a feature well exemplified in *Blumeria graminis* f sp *hordei* (*Bgh*), which displays gene-for-gene interactions with its host plant, barley (*Hordeum vulgare*) (Schulze-Lefert and Panstruga, 2003).

In gene-for-gene interactions (Flor, 1971), the recognition of an avirulence (*AVR*) molecule by a host resistance (*R*) protein triggers a localized cell death, known as the hypersensitive

response, and other defense responses that prevent further parasite growth (Greenberg and Yao, 2004; Skamnioti and Ridout, 2005). Bacterial *AVR* proteins are introduced into host cells by the type-three secretion system and can contribute to successful infection in susceptible host varieties (Alfano and Collmer, 2004; Janjusevic et al., 2006). Thus, bacterial *AVR* proteins are often described as effectors since they have both elicitor (avirulence) and virulence activities. Some fungal *AVR* proteins could potentially function as effectors. For example, *Avr4* from *Cladosporium fulvum* binds to chitin, so it may function to protect the fungus from plant chitinases during infection (van den Burg et al., 2003). *Avr2*, also from *C. fulvum*, binds to *Rcr3*, a Cys protease required specifically for the function of the resistance gene *Cf-2* (Rooney et al., 2005). However, there is no proof that these or any other fungal *AVR* proteins contribute directly to infection success.

More than 25 independent *AVR* genes have been described in *Bgh* isolates (Brown and Jessop, 1995; Jensen et al., 1995), but none are yet isolated. Although *AVR* genes are distributed throughout the *Bgh* genome, a cluster containing *AVR_{k1}*, *AVR_{a10}*, and *AVR_{a22}* is linked by 1 to 2 centimorgans (cM) (Brown and Jessop, 1995; Jensen et al., 1995; Caffier et al., 1996). More than 85 barley *R* genes, each conferring resistance to specific *Bgh* *AVR* elicitors, have been described, including *Mlk1* and 28 alleles at the *Mla* locus on barley chromosome 5 (Jørgensen, 1994). The six isolated *Mla* alleles (*Mla1*, *Mla6*, *Mla7*, *Mla10*, *Mla12*, and *Mla13*) are predicted to share >90% amino acid sequence identity (Zhou et al., 2001; Halterman et al., 2003; Shen et al., 2003; Halterman and Wise, 2004). *Mla* proteins have

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