

## MicroCommentary

# Let there be blight: functional analysis of virulence in *Phytophthora infestans*

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An article in this issue of *Molecular Microbiology* demonstrates that a G-protein  $\alpha$ -subunit plays diverse roles in pathogenesis-related development of the oomycete pathogen *Phytophthora infestans*. This report not only identifies one of the key signalling pathways involved in regulating the activity of motile zoospores and appressorium-mediated plant infection, but also validates the use of homology-dependent gene silencing as a method for studying virulence in oomycete pathogens.

The genus *Phytophthora* contains some of the world's most economically important and devastating pathogen species. Among these are the causes of important diseases of soybeans, oil palms, cocoa, numerous tree species (including oak, which suffers from the currently significant sudden death disease), as well as cucumbers and strawberries (Rizzo *et al.*, 2002; van West *et al.*, 2003). Of all the *Phytophthora* diseases, however, the one that everyone who has ever lifted a plant pathology text book knows about is potato late blight, caused by *Phytophthora infestans*. This was the disease responsible for causing the Irish potato famine in the 1840s, a humanitarian disaster with very considerable long-term socio-economic consequences, some of which are still apparent even today (Donnelly, 1996; Braa, 1997). But this disease is not simply a historical footnote – it is still widespread throughout potato-growing regions of the world, and it is virtually impossible to grow potatoes effectively in temperate climates without some form of late blight disease control. The persistence of late blight as a problem, coupled with the difficulty of breeding for durable disease resistance in the host, means that understanding the biology of *P. infestans* is of paramount importance, not only as a means of developing the next generation of chemicals with which to control the disease, but also in the hope of developing completely novel means of disease control

(Kamoun, 2003). In this issue, Latijnhouwers *et al.* (2003) report the identification of a G-protein  $\alpha$ -subunit involved in regulating pathogenesis-related development in *P. infestans*, revealing one of the likely signalling pathways responsible for host plant infection.

*Phytophthora infestans* is an interesting and distinctive microorganism, because it grows and develops much like a filamentous fungus, making thread-like cells called hyphae that invade plant tissue, but is in fact closely related to the brown algae, its ancestors having diverged from true fungi at the same time as those of land plants (van West *et al.*, 2003). This taxonomic division means that many of the genetic tools available to study fungal pathogens (Gold *et al.*, 2001) are simply not accessible for the study of *P. infestans*. Targeted gene replacement, for example, which is widely used in fungal pathogens to determine the likely function of individual genes in pathogenesis (Idnurm and Howlett, 2001), has not proved possible in *P. infestans* in spite of exhaustive attempts. *P. infestans* is also a diploid organism, a fact that makes targeted gene deletion even more difficult because of the necessity of replacing both alleles of a particular gene (as is performed in the human pathogen *Candida albicans* for instance; see Gow *et al.*, 1994). An alternative strategy, first described in 1999 in *P. infestans*, but not effectively duplicated until now, is homology-dependent gene silencing, which is carried out by the introduction of a second copy of a particular gene (van West *et al.*, 1999). The mechanism underlying homology-dependent gene silencing is not fully understood but involves loss of the corresponding mRNA transcript, and is therefore likely to be related to RNA silencing reported in plants, animals and true fungi (van der Krol *et al.*, 1990; Romano and Macino, 1992; English *et al.*, 1996; Fire *et al.*, 1998; Catalanotto *et al.*, 2000; Kadotani *et al.*, 2003). There are important differences, however, because gene silencing in *P. infestans* is genetically dominant and can be spread to nuclei lacking a transgene. The process is therefore likely to involve a *trans*-acting factor, perhaps a protein or RNA species (van West *et al.*, 1999).

*Phytophthora infestans* brings about plant infection primarily using zoospores. These are motile, flagellated cells that are released from sporangia when they are incubated

at temperatures below 12°C. Zoospores swim towards host cells in a process that involves negative geotaxis (zoospores tend to swim towards the surface of the water they are enclosed within) and may also require chemotaxis or electrotaxis to locate the host plant surface (for reviews, see Kamoun, 2003; van West *et al.*, 2003). Once in contact with the host, zoospores encyst, a process involving the development of a cell wall, attachment to the host cell surface and loss of motility. The cyst then germinates, producing a short germ tube, which differentiates into a swollen infection structure called an appressorium, and it is this cell that is used to breach the host cuticle sending a penetration hypha into the underlying plant tissue.

Laitijnhouwers and colleagues investigated the role of a G-protein  $\alpha$ -subunit encoded by the *Pigpa1* gene and found that silencing of this gene affected several processes in the prepenetration stage of development of *P. infestans*. Release of zoospores from sporangia, for example, was less efficient in the absence of *Pigpa1* expression. Once released, zoospores also behaved quite differently in *Pigpa1*-silenced strains. Swimming patterns were altered, and the normal autoaggregation of zoospores, a phenomenon in which suspensions of zoospores at a high concentration will spontaneously form visible clumps within 1 min, was also affected. The latter phenotype is demonstrated very strikingly in a movie that accompanies the paper. The ability of zoospores to swim towards a gradient of glutamic acid, a compound that normally attracts the swimming spores and promotes encystment, was also impaired, indicating a role in directional swimming and chemical perception. Once in contact with the host, *Pigpa1* mutants showed reduced appressorium formation, limiting the infection ability and probably contributing to the reduced virulence exhibited by these silenced strains. Taken together, the phenotypes described imply that *Pigpa1* is required for a variety of environmental sensing functions that impact upon subsequent development of the pathogen.

The significance of this result lies not only in the identification of a heterotrimeric G-protein involved in morphogenesis in an oomycete – a role that shows remarkable parallels with filamentous fungi in which G-proteins are also implicated in appressorium development, for example in the rice blast fungus *Magnaporthe grisea* among other species (for a review, see Bölker, 1998) – but also because the mutant provides a key resource for identifying the downstream effectors that mediate the remarkable morphogenetic transitions shown by *P. infestans*. The presence of a heterotrimeric G-protein indicates that an external environmental signal is perceived and responded to by zoospores, and its transduction to induce cytoskeletal changes and elicit gene expression in *P. infestans* is likely to involve one or more of the common downstream

pathways stimulated by G-proteins, such as a MAP kinase cascade, cAMP response pathway or phospholipid signalling. Significantly, phospholipid signalling has already been shown to be involved in zoospore encystment in *P. infestans* (Laitijnhouwers *et al.*, 2002).

A number of functional genomics initiatives are in progress in *P. infestans*, using transcriptional profiling, proteomic analysis or bioinformatics-driven approaches to facilitate gene identification (Avrova *et al.*, 2003; Shepherd *et al.*, 2003; Torto *et al.*, 2003). Currently, such approaches have been limited, however, because of the lack of empirically validated virulence determinants and associated mutants in *P. infestans*, which are vital for comparative or subtractive analysis. Transcriptional profiling or proteomic analysis of *Pigpa1*-silenced strains of *P. infestans* will provide a means of identifying genes encoding determinants of zoospore motility, directional sensing, encystment and appressorium development.

Importantly, the paper also validates the use of homology-dependent gene silencing as a means of gene functional analysis. Although there are still problems with the technique (it is obviously very variable and probably locus dependent in its utility), gene silencing definitely has a future in understanding the biology of *P. infestans*. So what is next? One important consequence of the study should be a systematic analysis of the mechanism by which homology-dependent gene silencing operates in *P. infestans* and how the process could be made more efficient. Comparative analysis with RNA interference in animals and plants and with virus-induced gene silencing would be a good place to start. Do small interfering RNAs mediate silencing in *P. infestans* for example (Hamilton *et al.*, 2002), and what is the nature of the diffusible factor that allows homology-dependent gene silencing to spread from nucleus to nucleus, as reported previously (van West *et al.*, 1999)? Next, the identification of the interacting factors with *Pigpa1* should provide a means of beginning to determine the outline of the G-protein-mediated signalling pathway involved in zoospore behaviour and subsequent development. Finally, and arguably most exciting, will be the application of genomics approaches using *Pigpa1*-silenced mutants to identify and characterize the morphogenetic determinants that regulate and contribute to the remarkable biology of this hitherto intractable microorganism.

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