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Research Focus

Trafficking arms: oomycete effectors enter host plant cells

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Oomycetes cause devastating plant diseases of global importance, yet little is known about the molecular basis of their pathogenicity. Recently, the first oomycete effector genes with cultivar-specific avirulence (AVR) functions were identified. Evidence of diversifying selection in these genes and their cognate plant host resistance genes suggests a molecular 'arms race' as plants and oomycetes attempt to achieve and evade detection, respectively. AVR proteins from *Hyaloperonospora parasitica* and *Phytophthora infestans* are detected in the plant host cytoplasm, consistent with the hypothesis that oomycetes, as is the case with bacteria and fungi, actively deliver effectors inside host cells. The RXLR amino acid motif, which is present in these AVR proteins and other secreted oomycete proteins, is similar to a host-cell-targeting signal in virulence proteins of malaria parasites (*Plasmodium* species), suggesting a conserved role in pathogenicity.

Oomycete plant pathogens

Oomycetes, despite apparently sharing morphological features with some fungal plant pathogens (including hyphae, appressoria, haustoria and spores), belong to the kingdom Stramenopiles and are, therefore, more closely related to brown algae and diatoms. Plant-pathogenic oomycetes are responsible for economically and environmentally devastating epidemics such as the 1846 Irish potato famine (caused by *Phytophthora infestans*) and the current sudden oak death epidemic (caused by *Phytophthora ramorum*) in California, USA [1]. Oomycetes can be host specific or can exhibit a wide host range. A biotrophic mode of nutrition that requires access to living host plant cells at an early stage in the establishment of infection is characteristic of most *Phytophthora* species and all Peronosporaceae (downy mildews) and Albuginaceae (white rusts). Peronosporaceae and Albuginaceae are obligately biotrophic and cannot be cultured easily.

Oomycetes share with many bacterial, fungal and nematode plant pathogens the requirement for living host tissue for at least part of the infection cycle. To establish infection, these pathogens must evade, suppress or manipulate host defenses. This biotrophic requirement presents a point of vulnerability when all such pathogens are detected because invaded plant cells can induce resistance responses, including a localized programmed cell death called the hypersensitive response (HR) [2]. In this article, we highlight recent discoveries that improve the understanding of how oomycetes manipulate plant hosts and that reveal a dynamic evolutionary battle between plant and pathogen to achieve and evade detection, respectively.

Plant–pathogen battle sites: the host cytoplasm and apoplast

After 15 years of forward genetic studies, many resistance (R) proteins have been identified that function as a surveillance system to detect pathogen effectors [3]. Once detected, these effectors are termed avirulence (AVR) proteins. Each AVR protein is believed to be detected by a specific R protein in what is known as the gene-for-gene interaction, which often triggers the HR [2].

Many bacterial plant pathogens synthesize effector proteins and deliver them into host cells where they manipulate host defenses, including the HR [4]. Delivery

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is often carried out by specialized structures such as the type III secretion system (T3SS) of Gram-negative bacteria, which is inserted through plant cell walls to translocate proteins across the host plasma membrane into the host cytoplasm. T3SS effectors have a range of biochemical activities that alters host cytoplasmic targets [4]. Other bacterial effectors that represent a diverse array of virulence determinants are also delivered to the plant apoplast through the pathogen type II secretion system [5].

Fungi deliver effectors to both the inside and the outside of plant cells. AVR2 from the fungal plant pathogen *Cladosporium fulvum* binds to and inhibits the tomato extracellular cysteine protease RCR3. This molecular interaction is detected by the extracellular domain of the tomato R protein Cf-2, leading to an HR [6]. By contrast, other fungal effectors such as AVR-Pita [7] from *Magnaporthe grisea* and AvrL567 [8] from the flax rust fungus *Melampsora lini* are recognized in the host cytoplasm. AvrL567 [8] and Uf-RTP1 [9] (a secreted protein from the rust fungus *Uromyces fabae*) are delivered into host cells through haustoria, which are specialized biotrophic infection structures that invaginate host cells and make near-direct contact with the host plasma membrane. This indicates that fungi, similar to bacteria, translocate effectors to the inside of plant cells, although the molecular basis of such trafficking is unknown. The identification of the first oomycete AVR proteins suggests that these pathogens also translocate effectors into plant cells.

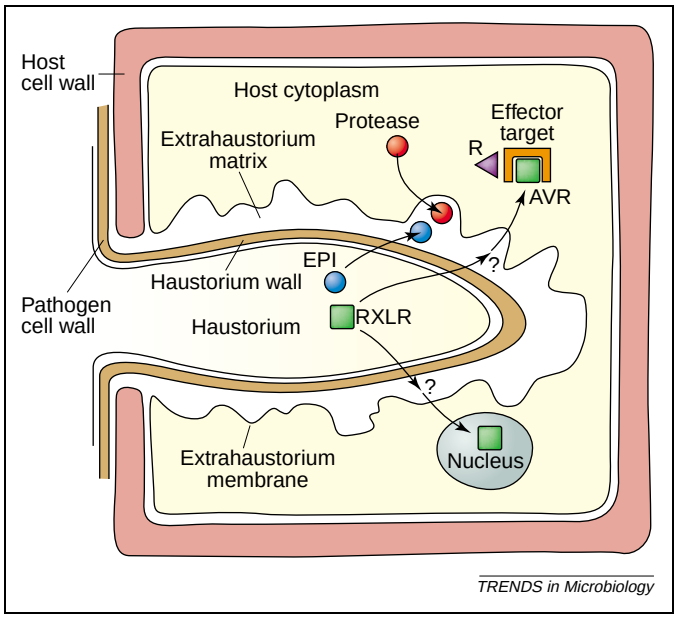

Figure 1. The interaction of a *Phytophthora infestans* haustorium with a potato cell. The potential sites of action of recently identified *P. infestans* effector proteins, including AVR proteins, are shown. Extracellular protease inhibitor (EPI) proteins (blue circles) target extracellular host-defense-associated proteases (red circles). RXLR-motif-containing proteins (green squares) such as AVR3a are secreted but it is unknown how they are translocated to the inside of host cells and which host proteins they target (effector targets, orange) to manipulate host defenses, potentially enabling recognition by 'guarding' R proteins (purple triangle) in the cytoplasm. One (as yet unnamed) RXLR-containing protein has a functional nuclear localization signal that could localize it to the host nucleus during infection (T.D. Kanneganti *et al.*, unpublished).

Oomycete AVR proteins are recognized inside host cells

It is thought that oomycetes accomplish parasitic colonization by molecular reprogramming of host defense circuitry, specifically by introducing an array of effectors that functions in the plant apoplast and cytoplasm. Similar to *C. fulvum* [6], *P. infestans* secretes inhibitors that target defense proteases in the plant apoplast [10,11] (Figure 1). Effectors such as glucanase inhibitors and cell death elicitors also function in the host apoplast [1]. Recently, four oomycete *Avr* genes were identified: *Avr1b-1* from the soybean pathogen *Phytophthora sojae* [12], *ATR13* [13] and *ATR1*^{NdWsB} [14] from the *Arabidopsis* pathogen *Hyaloperonospora parasitica* and *Avr3a* from *P. infestans* [15]. ATR13, ATR1^{NdWsB} and AVR3a [13–15] are detected by cognate R proteins in the host cytoplasm, which is consistent with the hypothesis that oomycetes deliver effectors to the inside of host cells, potentially through haustoria (Figure 1). Sequence alignment of oomycete AVR proteins revealed a conserved motif (RXLR) within 32 amino acids of the predicted signal peptides [14]. The RXLR motif is similar to the host-cell-targeting signal RXLX(E/Q), which is required for the translocation of proteins from malaria parasites (*Plasmodium* species) into host erythrocytes [16,17]. This prompted the hypothesis that RXLR functions as a signal that mediates trafficking into host cells [14].

During infection, malaria parasites reside and replicate inside parasitophorous vacuoles within human erythrocytes. Parasite N-terminal signal peptides function to secrete effectors into the parasitophorous vacuole but it is the host-cell-targeting signal that enables translocation of parasite proteins across the parasitophorous vacuolar membrane into the host erythrocyte [18]. If oomycete effectors are delivered through haustoria, a comparable function for the RXLR motif is plausible. Thus, the extrahaustorial matrix (Figure 1) could equate to the malaria parasitophorous vacuole and, in the same way that signal peptides direct AVR secretion into the erythrocyte matrix, the RXLR motif directs AVR translocation into the plant cell. Further experiments that investigate the extent to which oomycete and malaria signals are functionally conserved should help to address the exciting possibility that eukaryotic pathogens of plants and animals share similar mechanisms of effector delivery into host cells.

The effector secretome of plant-pathogenic oomycetes is complex, with hundreds of proteins that manipulate host defenses. Bioinformatic analyses indicate that the RXLR motif is common in *P. infestans*, *P. sojae* and *P. ramorum* and that it occurs within ?100 proteins predicted to be secreted that were identified in the genomes of these organisms (J. Win *et al.*, unpublished). This suggests that these pathogens deliver a complex set of effectors into the host cytoplasm. Another *P. infestans* RXLR-containing protein carries a functional nuclear localization signal and might accumulate in host nuclei during infection (T.D. Kanneganti *et al.*, unpublished).

The 'arms race' for recognition and evasion in oomycete gene-for-gene interactions

Sequenced alleles of plant *R* genes reveal that some protein-encoding domains are highly conserved, whereas

others have high levels of allelic variation, suggesting that there is a selective advantage for generating new versions of these domains in R proteins. This diversifying selection is concentrated in the putative, ligand-binding leucine-rich repeats of many R proteins, which supports proposals that R proteins directly interact with AVR proteins that are evolving to evade recognition [19]. This parallel molecular diversification in R and AVR proteins has been termed an 'arms race'. However, there are fewer *R* genes in plant genomes than there are potential pathogens and associated effector protein complexes, and direct interactions between R and AVR proteins have been demonstrated only rarely. Consequently, it has been hypothesized that many R proteins 'guard' the key host defense proteins that are the binding targets of AVR proteins [20,21]. Rather than direct interaction with AVR proteins, it has been proposed that R proteins detect conformational changes in effector targets that are bound to AVR proteins [20,21].

Diversifying selection seems to have had a role in the evolution of oomycete *Avr* genes. There is marked allelic diversity in both *ATR13* [13] and its cognate resistance gene *RPP13* [22]. $ATR1^{NdWsB}$ has even greater allelic diversity than does *ATR13*, and overlapping sets of allelic products are recognized by distinct host genes at the *RPP1* locus in different *Arabidopsis* accessions [14]. Both *Avr3a* [15] and its cognate gene in potato, *R3a* [23], show evidence of diversifying selection. However, certain alleles of *Avr3a* are becoming fixed, possibly as a result of well-documented genetic bottlenecks in the global distribution of *P. infestans* [15]. *Avr1b* also has considerable allelic diversity and is a member of a large *Avr1b*-like gene family [12]. The distantly related *Avr3a* gene is also a member of a related group of genes, and *Avr3a*-like paralogs in the *Avr3a* locus indicate that gene duplication and divergence have provided an additional mechanism to generate diversity [15].

If there is no direct interaction between R and AVR partners, what drives their diversification? It is possible that AVR proteins, while maintaining the ability to bind to a host effector target, evolve to alter conformational changes in the target that are caused by binding, thus evading detection by guarding R proteins. It is also possible that pathogen effectors evolve to interact with equivalent target proteins in different host species. The allelic diversity in the small number of oomycete effectors studied so far suggests that searches for conserved functional domains within these rapidly evolving proteins and investigation of the effects of allelic differences on effector protein 3D structure, combined with searches for host target proteins, will be important for understanding the coevolutionary drivers of diversification. To what extent do these effectors determine speciation within oomycetes by such tight coevolution with target proteins that the pathogen becomes host limited?

Comparative oomycete genomics

Analysis of the *Avr3a* locus in *P. infestans* and the $ATR1^{NdWsB}$ locus of *H. parasitica* unexpectedly revealed several common genes in a conserved order [15]. Such colinearity, together with the identification of the RXLR motif, supports the use of comparative genomics to investigate the evolution of pathogenicity in oomycetes and to identify other *Avr* and effector genes. This approach is timely, considering that the genomes of five oomycetes (*Phytophthora capsici*, *P. infestans*, *P. ramorum, P. sojae* and *H. parasitica*) are being sequenced and annotated. Genome-wide comparisons among these oomycetes will enable comparative analyses of the effector secretomes and will reveal insights into the processes of pathogenesis and biotrophy.

Future studies of oomycete effectors

The recent identification of oomycete *Avr* genes raises several key questions that will drive future research in this area (Box 1). Answering these questions will reveal the molecular mechanisms that are used by plant-pathogenic oomycetes to establish infection by overcoming a set of host defense proteins and pathways. After 15 years of forward genetics, a great deal has been learnt about *R* genes but little is understood about resistance mechanisms and how they are regulated. The identification of pathogen effectors and their use as cell biology tools to reveal and characterize defense pathways provide a new strategy for understanding the fundamental bases of plant innate immunity and pathogen host specificity. With the recent advances in the identification of oomycete effector proteins and the impending completion of the genome sequences of five

Box 1. Key questions arising from the cloning of oomycete *Avr* genes

Avirulences and effectors

When and where are oomycete AVR proteins synthesized?
Where are they delivered?
What are their biochemical activities?
How do changes in their 3D structure affect function and detection?

Effector delivery

How do oomycetes achieve effector protein trafficking into host cells?
Is the RXLR motif a key component of a specific transport mechanism?
If so, what is the full complement of effector proteins delivered by this mechanism?
To what extent are effector secretion systems conserved in pathogenic eukaryotes?

Host targets

What are the host protein targets of oomycete effector proteins?
Do the effector targets have a role in resistance?
If not, why are they targeted?
How does this help the oomycete to establish infection?
Are any of the effector targets manipulated by bacterial, fungal or nematode effectors?
Are the effector target proteins species specific or conserved in all plants?

Effector evolution

Does the marked diversity in pathogen proteins that are targeted to the host cytoplasm suggest a mechanism that determines speciation in oomycetes?
How does the coevolutionary 'arms race' operate in conjunction with the guard model of R proteins?

oomycete species, these pathogens are set to have a major role in the future of plant disease research.

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Adaptive evolution by optimizing expression levels in different environments

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Organisms adapt to environmental changes through the fixation of mutations that enhance reproductive success. A recent study by Dekel and Alon demonstrated that *Escherichia coli* adapts to different growth conditions by fine-tuning protein levels, as predicted by a simple cost–benefit model. A study by Fong *et al.* showed that independent evolutionary trajectories lead to similar adaptive endpoints. Initial mutations on the path to adaptation altered the mRNA levels of numerous genes. Subsequent optimization through compensatory mutations restored the expression of most genes to baseline levels, except for a small set that retained differential levels of expression. These studies clarify how adaptation could occur by the alteration of gene expression.

Environmental change and adaptation

All organisms actively maintain homeostasis despite living in changing environments. However, this equilibrium can break down if organisms encounter challenges that exceed their innate adaptations to cope with atypical conditions [1]. Nevertheless, some individuals in a population might have particular genetic mutations that enable them to survive in unfamiliar environmental conditions. Accordingly, these

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