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# Tactics of host manipulation by intracellular effectors from plant pathogenic fungi

Melania Figueroa[1], Diana Ortiz[2] and Eva C. Henningsen[3]

**Abstract**

Fungal pathogens can secrete hundreds of effectors, some of which are known to promote host susceptibility. This biological complexity, together with the lack of genetic tools in some fungi, presents a substantial challenge to develop a broad picture of the mechanisms these pathogens use for host manipulation. Nevertheless, recent advances in understanding individual effector functions are beginning to flesh out our view of fungal pathogenesis. This review discusses some of the latest findings that illustrate how effectors from diverse species use similar strategies to modulate plant physiology to their advantage. We also summarize recent breakthroughs in the identification of effectors from challenging systems, like obligate biotrophs, and emerging concepts such as the 'iceberg model' to explain how the activation of plant immunity can be turned off by effectors with suppressive activity.

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**Keywords**

Effector, Fungi, Host, Recognition, Immunity, Plant.

**Abbreviations**

Avr, avirulence; bZIP, basic leucine zipper; DON, deoxynivalenol; EAR, ethylene-responsive element binding factor−associated amphiphilic repression; EBE, effector binding element; ER, endoplasmic reticulum; ERF, ethylene response factor; ETI, effector-triggered immunity; HMA, heavy metal associated; HR, hypersensitive response; ID, integrated domain; JA/ET, jasmonate/ethylene; MAPK, mitogen-activated protein kinase; MAX, *Magnaporthe* Avrs and ToxB−like effectors; NIS1, necrosis-inducing secreted protein 1; NLR, nucleotide-binding site leucine-rich repeat receptor; PAMP, pathogen-associated molecular pattern; PM, plasma membrane; PRR, pattern recognition receptor; PTI, PAMP-triggered immunity; R, resistance; RLP, receptor-like protein; ROS, reactive oxygen species; SA, salicylic acid; SCF, Skp1−Cullin−F-box; SnRK1, SNF1-related kinase; TPL/TPR, TOPLESS/TOPLESS related.

## Introduction

Pathogens secrete effector proteins to subvert plant immune responses and colonize the host [1]. The role of these molecules in plant−microbial compatibility has been demonstrated in diverse pathosystems (biotrophic, necrotrophic, and hemibiotrophic), including those responsible for serious fungal crop diseases [2,3]. Pathogen effectors were initially recognized as being the targets of recognition in plant immunity, but the field of effector biology has since provided many important insights into the broad range of mechanisms that plants use to resist disease and how pathogens overcome plant defense. One layer of plant immunity, pathogen-associated molecular pattern (PAMP)-triggered immunity, involves pathogen recognition by plasma membrane−associated proteins known as pattern recognition receptors and is a common target of effectors to suppress host defense [1,4,5]. However, effector recognition occurs via intracellular nucleotide-binding site leucine-rich repeat immunoreceptors (NLRs) or plasma membrane−localized receptors (similar to pattern recognition receptors), which is the basis of effector-triggered immunity as a second layer of plant immunity [6,7].

Bacterial effectors have been studied for decades, and much detail is known about their biological functions [8,9]. Work on oomycete effectors has expanded greatly in recent years and has been thoroughly reviewed previously [10−12]. However, our understanding of fungal effector repertoires, particularly from obligate biotrophs, is less extensive. Here, we review recent findings on subcellular localization of intracellular proteinaceous fungal effectors and their modes of action to manipulate host machinery. We highlight parallels and contrasts with bacterial effectors, emerging concepts in the field, as well as recent breakthroughs in the identification of effectors from obligate biotrophic fungi, which has been a significant research bottleneck. In acknowledgement of exceptions to the standard effector-triggered immunity/PAMP-triggered immunity dichotomy [6,7,13], diagrams included in this review adopt the 'spatial

**Table 1**

**Summary of fugal effectors mentioned in this review to illustrate their diverse subcellular localization and function if applicable.**

| Effector | Function | In planta subcellular localization | Species | Lifestyle | Disease name | Host | Reference |
|---|---|---|---|---|---|---|---|
| Avra7, Avra9, Avra10, and Avra22 | Unknown | Unknown | *Blumeria graminis* f. sp. *hordei* | Biotroph | Powdery mildew | Barley | [79] |
| CSEP0064/ BEC1054 | RNase-like protein that binds to nucleic acids and prevents degradation of host ribosomal RNA induced by ribosome-inactivating proteins | Cytoplasm and nucleus | *B. graminis* f. sp. *hordei* | Biotroph | Powdery mildew | Barley | [69] |
| SvrPm3a1/f1 | Suppresses recognition of AvrPm3a2/f2 mediated by the Pm3 wheat resistant gene | Unknown | *B. graminis* f. sp. *tritici* | Biotroph | Powdery mildew | Wheat | [33,45] |
| ChEC89 | Unknown | Peroxisome | *Colletotrichum higginsianum* | Hemibiotroph | Anthracnose | Cruciferous plants | [19] |
| ChEC21 | Unknown | Golgi apparatus | *C. higginsianum* | Hemibiotroph | Anthracnose | Cruciferous plants | [19] |
| NIS1 | Disrupts basal immunity by binding to BAK1 and BIK1 | Cytosol | *Colletotrichum orbiculare* *C. higginsianum* *Magnaporthe oryzae* | Hemibiotroph | Anthracnose and rice blast | Cucumber, cruciferous plants, rice, and so on | [72●●] |
| Avr1(Six4) | Suppression of I-2–and I-3–mediated disease resistance | Unknown | *Fusarium oxysporum* | Hemibiotroph | Fusarium wilt, foot, root, or bulb rot | Tomato, cotton, banana, melon | [44] |
| Osp24 | Induction of proteasome-mediated degradation of its host target | Cytosol | *F. graminearum* | Hemibiotroph | Fusarium head blight | Wheat and barley | [32●●] |
| HvEC-016 | Unknown | Unknown | *Hemileia vastatrix* | Biotroph | Coffee leaf rust | Coffee | [82] |
| AvrLm1 | Alterations to MAPK signaling | Cytosol | *Leptosphaeria maculans* | Hemibiotroph | Black leg | Canola | [38] |
| MoCDIP4 | Disruption of mitochondrial dynamics | Endoplasmic reticulum | *M. oryzae* | Hemibiotroph | Rice blast | Rice | [30●●] |
| MoHTR1 and MoHTR2 | Transcriptional reprogramming of genes related to plant immunity | Nucleus | *M. oryzae* | Hemibiotroph | Rice blast | Rice | [40●●] |
| AvrPiz-t | Inhibition of RING E3 ubiquitin ligases APIP6 and APIP10, modulation of a K+ channel, targeting of Nup08 (the homolog of APIP12) and preventing accumulation of PR transcripts, and suppression of activity of the bZIP-type transcription factor APIP5 | Cytosol | *M. oryzae* | Hemibiotroph | Rice blast | Rice | [51–54] |
| AVR-Pia | Unknown | Cytosol | *M. oryzae* | Hemibiotroph | Rice blast | Rice | [60,62] |
| CTP1 | Unknown | Chloroplast, mitochondria | *Melampsora larici-populina* | Biotroph | Poplar leaf rust | Poplar | [28] |
| CTP2, CTP3 | Unknown | Chloroplast, | *M. larici-populina* | Biotroph | Poplar leaf rust | Poplar | [28] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| MLP124017 | Unknown, member of nuclear transport factor 2–like protein superfamily | Nucleus and cytosol | *M. larici-populina* | Biotroph | Poplar leaf rust | Poplar | [28,37] |
| PtrToxA | Host-selective toxin, induces necrosis | Chloroplast | *Pyrenophora tritici-repentis* | Necrotroph | Tan spot of wheat | Wheat | [25–27] |
| PtrToxB | Host-selective toxin, induces chlorosis | Apoplast | *P. tritici-repentis* | Necrotroph | Tan spot of wheat | Wheat | [27,64] |
| AvrSr50 | Suppression of cell death in *Nicotiana benthamiana* | Cytosol | *Puccinia graminis* f. sp. *tritici* | Biotroph | Stem rust | Wheat, barley | [34●●] |
| AvrSr35 | Unknown | Endoplasmic reticulum | *P. graminis* f. sp. *tritici* | Biotroph | Stem rust | Wheat | [75●●] |
| AvrSr27 | Unknown | Unknown | *P. graminis* f. sp. *tritici* | Biotroph | Stem rust | Wheat | [76●●] |
| Jsi1 | Transcriptional reprogramming of genes involved in JA/ET signaling | Unknown | *Ustilago maydis* | Biotroph | Corn smut | Maize | [35●●] |
| VdSCP41 | Interference with transcription factors which are regulators of immunity | Nucleus | *Verticillium dahliae* | Hemibiotroph | Verticillium wilt | Cotton, tomato, pepper, cabbage, and so on | [39] |

JA/ET, jasmonate/ethylene.

invasion model' to portray plant immunity as a process controlled by cell surface and intracellular immunoreceptors [14,15].

## Subcellular targeting of intracellular proteinaceous fungal effectors

Intracellular proteinaceous effectors exert their function in the cytosol or travel to other subcellular compartments. Many pathogenic fungi secrete hundreds of effectors [16], which have evolved to target key host survival processes and diverse plant cell compartments like those in bacterial systems. A significant advance in the field is the use of prediction tools for protein secretion and subcellular localization such as EffectorP and LOCALIZER [17,18], which can guide experimental analyses (e.g. fluorescence microscopy, heterologous expression systems). Results from this work show the numerous cellular compartments (i.e. nucleus, the Golgi apparatus, chloroplasts, mitochondria, etc.) that effectors target [12,19,20] (Table 1, Figure 1). Nevertheless, linking effector localization and function is a challenging task because of the genetic intractability of some fungi or the subtlety, or even absence, of phenotypes in effector mutants resulting from functional redundancy or minor effects [21].

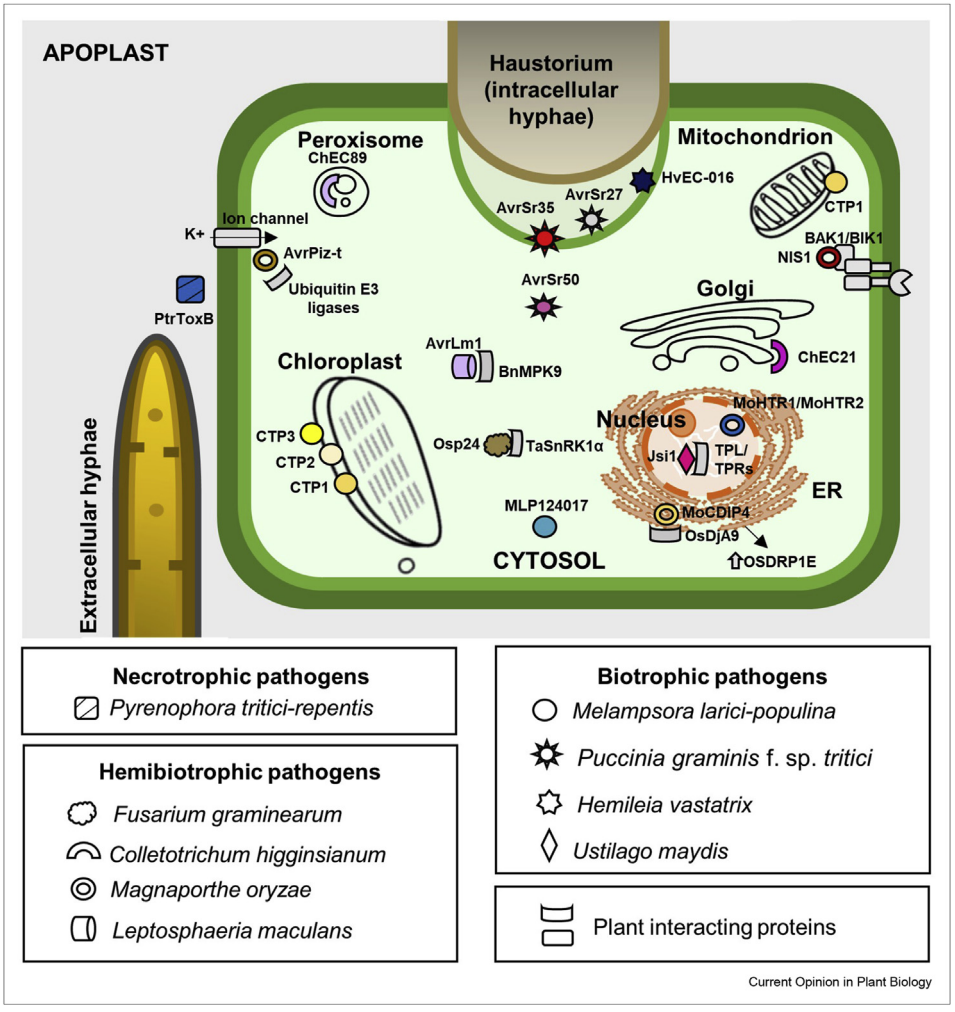
Chloroplasts and mitochondria play important roles in plant immunity and stress responses through the activation of key defense signals such as nitric oxide, reactive oxygen species, and salicylic acid [22–24]. Early studies in *Pyrenophora tritici-repentis* demonstrated the chloroplast as a fungal effector target [25–27]. Since then, effector localization screens have shown chloroplasts and mitochondria are common target sites. Petre et al. [28] determined that the three secreted proteins (CTP1, CTP2, and CTP3) from the poplar rust fungus (*Melampsora larici-populina*) accumulate in the chloroplast by exploiting cellular sorting processes. Their trafficking depends on the in planta cleavage of an N-terminal signal sequence which mimics plant chloroplast targeting sequences. Similarly, targeting-sequence mimicry has been found in *Puccinia graminis* f. sp. *tritici* (wheat stem rust) effectors that show chloroplast localization [17]. Further evidence comes from *Puccinia striiformis* f. sp. *tritici* (wheat stripe rust fungus); the effector protein Pst_12806 contains a chloroplast transit peptide and localizes to the chloroplast, where it perturbs photosynthesis and basal immunity [29]. In some cases, effector localization and phenotype changes occur in different organelles. Xu et al. [30●] demonstrated that *Magnaporthe oryzae* can disrupt mitochondrial fission and fusion cycles, which are required for cell homeostasis and plant immunity. The dynamin-related proteins which mediate mitochondrial fission and fusion cycles are impacted indirectly. First, the *M. oryzae* effector protein MoCDIP4 binds to the DnaJ chaperone protein OsDjA9, which normally promotes degradation

of the dynamin-related protein OsDRP1E. The competition between MoCDIP4 and OsDRP1E interferes with OsDRP1E degradation, resulting in an overaccumulation of OsDRP1E in rice cells. Ultimately, this causes aberrant mitochondrial fission and size reduction, which increases susceptibility to *M. oryzae*. Interestingly, these interactions occur in the endoplasmic reticulum, but the phenotype is exerted in the mitochondria. Further investigation of how fungal effectors interfere with organelle function is likely to be a fruitful area of research.

## Manipulation through subversion of host machinery

Fungal effectors, as well as those from oomycetes, often subvert host machinery/processes by binding to host targets [12], rather than modifying the target via an intrinsic enzymatic activity, as commonly observed for bacterial effectors [8,31]. For instance, Jiang et al. [32••] showed that the effector protein Osp24 from *Fusarium graminearum* physically associates with TaSnRK1α, a wheat homolog of the *Arabidopsis thaliana* SNF1-related kinase (SnRK1). SnRK1 plays a pivotal regulatory role in plant metabolism, growth, and immunity and is regulated by a Skp1–Cullin–F-box ubiquitin ligase complex and 26S proteasome [33,34]. In wheat, TaSnRK1α contributes to resistance to *F. graminearum* by an unknown mechanism and Osp24 promotes proteasomal degradation of TaSnRK1α during fungal infection [32••]. In an interesting case of arms-race evolution, another wheat protein TaFROG is induced by detection of the *Fusarium* toxin deoxynivalenol and competes with Osp24 for binding to TaSnRK1α to prevent its degradation and maintain resistance.

**Figure 1**




**Examples of subcellular localization of fungal effector proteins.** During infection, fungi deliver effector proteins into the apoplast and/or host cell. This figure illustrates examples of fungal effectors that are translocated to the intracellular space and localize to diverse subcellular compartments (i.e. cytosol, Golgi apparatus, chloroplast, mitochondrion, peroxisome, and nucleus) and their host targets. The apoplastic effector PtrToxB from *Pyrenophora tritici-repentis* shares a similar structure to the intracellular effector AvrPiz-t from *Magnaporthe oryzae*. Additional information and examples are presented in Table 1.

Phytohormone signaling processes are also manipulated by fungal effectors. The *Ustilago maydis* effector Jsi1 targets jasmonate/ethylene signaling by interaction with members of the TOPLESS/TOPLESS-related corepressor family. This activates jasmonate/ethylene signaling by transcriptional reprogramming of genes related to the ethylene response factor branch [35••]. In another case of molecular mimicry, Jsi1 contains a DLNxxP type EAR motif, similar to those conserved in plant ethylene response factor transcription factors that naturally activate this pathway [36]. Similarly, the candidate effector MLP124017 from *M. larici-populina* interacts with the TOPLESS-related protein 4 (PopTPR4) from poplar and *Nicotiana benthamiana* [28,37]; however, the functional consequence of this interaction has not been determined.

Fungi also target host plant signaling cascades, as evidenced by AvrLm1 from *Leptosphaeria maculans*, which like some bacterial and oomycete effectors, targets the mitogen-activated protein kinase (MAPK) signaling cascade to modulate plant immunity [38]. AvrLm1 interacts with the *Brassica napus* MAPK 9, which leads to accumulation and phosphorylation of MAPK9. This results in cell death induction which may support the pathogen's transition from a biotrophic to necrotrophic phase. Another example of subversion of host machinery is given by the effector VdSCP41 from *Verticillium dahliae*, which interferes with the function of the *Arabidopsis* transcription factors CBP60g and SARD1 and cotton GhCBP60b, which serve as regulators of immunity [39].

Some effectors directly bind to DNA and use the host transcriptional machinery to alter gene expression and defense responses. Recently, Kim et al. [40•] characterized two *M. oryzae* effector proteins, MoHTR1 and MoHTR2, which are translocated into the nucleus, where they bind to so-called effector binding elements of genes related to plant immunity regulation. Changes in the transcriptional profile caused by MoHTR1 and MoHTR2 impact how rice responds to additional pathogens. Transgenic rice lines expressing MoHTR1 or MoHTR2 display increased susceptibility to hemibiotrophic pathogens *M. oryzae* and *Xanthomonas oryzae* pv. *oryzae*, but show enhanced resistance to *Cochliobolus miyabeanus*, a necrotrophic pathogen (Figure 2). This highlights an interesting observation that host manipulation by the effector repertoire of one pathogen can modify the outcomes of other plant–microbe interactions.

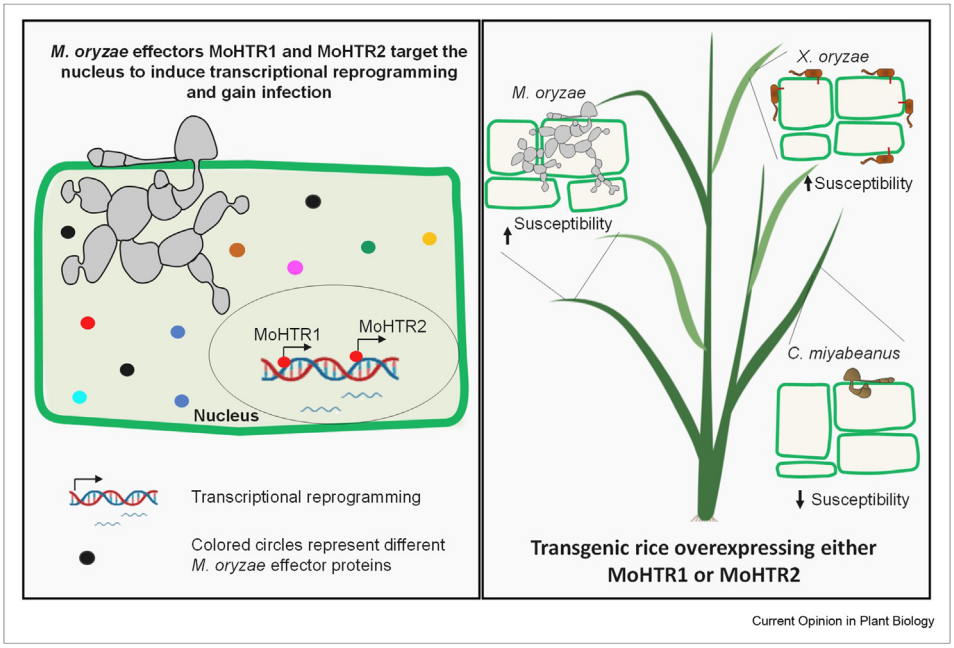
## Emerging concepts and unanswered questions for intracellular fungal effectors

The genomic era has revealed not only that many fungi harbor hundreds of effector proteins, but also that plants possess large sets of immunity receptors [2,41,42]. Yet, it is intriguing that genetic studies attribute incompatibility between plants and adapted pathogens to only a few receptors and corresponding Avr proteins (interaction units). The iceberg model proposed by Thordal-Christensen [43] seeks to explain this observation as resulting from one effector suppressing the activation of plant immunity mediated by the recognition of another effector (Figure 3). Such interference leads to silent interaction units as the invisible base of the iceberg, whereas we are only able to detect those units that lead to disease resistance at the tip of the iceberg. Consistent with this model, the Avr1 effector from *Fusarium oxysporum* suppresses resistance responses induced by the NLR proteins I-2 and I-3, while activating resistance mediated by I and I-1 in tomato plants [44]. Similarly, the SvrPm3$^{a1/f1}$ effector protein from *Blumeria graminis* f. sp. *tritici* has been shown to suppress cell death triggered on recognition of AvrPm3$^{a2/f2}$ by the resistance protein Pm3 in *N. benthamiana* and in wheat [33,45••]. Cell death suppression activity may also be part of the mechanism of action of effectors deployed by rust fungi [34•,46•,47]. Functional redundancy of effectors [48–50] can also explain why we only seem to detect a few interaction units.

Individual fungal effectors may have more than one function by targeting different host proteins. For instance, AvrPiz-t from *M. oryzae* targets two ubiquitin E3 ligases, a K+ transporter, a basic leucine zipper transcription factor, and a nucleoporin protein Nup98 in rice [51–55]. Conversely, multiple effectors may display common host targets. Variants of AvrPik bind and stabilize rice proteins containing heavy metal–associated (HMA) domains, which act as susceptibility factors to promote disease [56,57••,58]. The HMA domain is also present as an integrated domain in some NLRs, including Pik, where it allows immune recognition of Avr–Pik [59–61]. Two other *M. oryzae* effectors, AVR–Pia and AVR1–CO39, are also recognized by their binding to integrated HMA domains in NLRs [60,62,63]. This suggests that these effectors have all evolved the ability to target host proteins containing HMA domains and consequently driven evolution of NLR receptors with HMA-like integrated domains. These three effectors, as well as AvrPiz-t and the apoplastic effector PtrToxB from *P. tritici repentis* [64] are all structurally related, being members of the MAX (*Magnaporthe* Avrs and ToxB-like effectors) effector family [65]. This indicates that effectors with common structural scaffolds can evolve to quite different functions, which complicates the application of structural information to predict effector function. Likewise, many powdery mildew effectors are predicted to share a common RNase-like fold [66-68,69•], which may serve as a structural scaffold to support multiple functions.

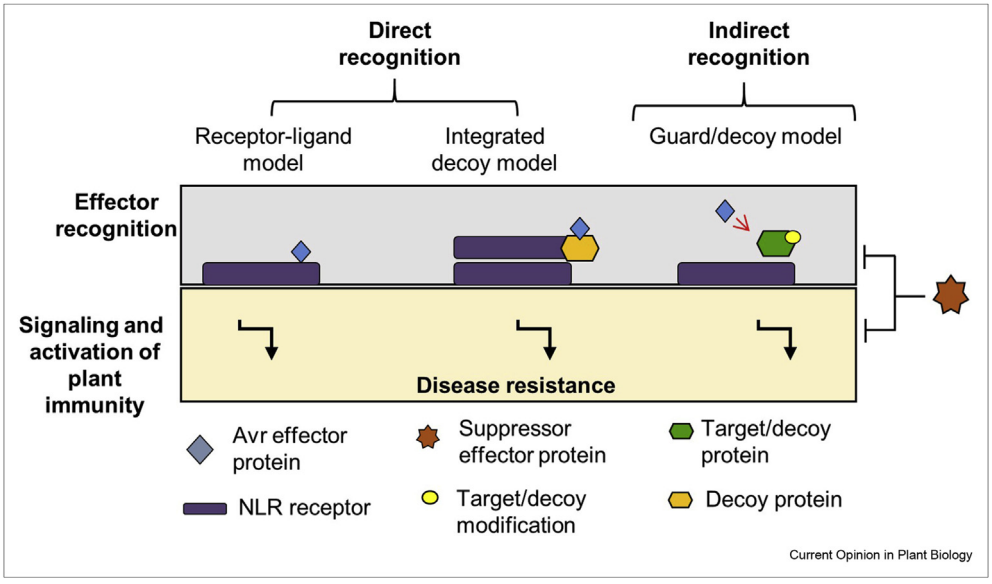
The existence of 'core' effectors that are conserved across pathogens is an intriguing topic [48,70,71]. Although most effectors characterized to date are pathogen specific, it would be interesting to identify the

**Figure 2**




**Manipulation of host physiology by effectors changes the outcome of additional plant–pathogen interactions**. The fungus *Magnaporthe oryzae* delivers hundreds of effector proteins into the plant cytoplasm during infection. The effectors MoHTR1 and MoHTR2 (for *Magnaporthe oryzae* host transcription reprogramming 1 and 2) target the nucleus and reprogram the transcription of immunity-associated genes to promote *M. oryzae* infection (left panel) [40••]. Transcriptional reprograming induced by either MoHTR1 or MoHTR2 in transgenic rice plants modifies the response to two additional pathogens, *Xanthomonas oryzae* and *Cochliobolus miyabeanus*, by increasing and decreasing susceptibility, respectively (right panel). This figure was made using BioRender®.

**Figure 3**




**Effectors can mask effector recognition by suppressing the activation of plant immunity**. Pathogenic fungi secrete hundreds of effectors during infection. Intracellular effectors can be recognized by NLRs through various mechanisms [1,59]. Effector recognition through the receptor ligand model is commonly observed for obligate biotrophic systems in contrast to indirect recognition in bacterial systems. Effector proteins with suppressive activity can interfere with either NLR effector monitoring or the disease resistance signaling pathways, which prevents activation of plant immunity.

functions of more broadly conserved effectors, which may represent indispensable pathogenicity functions. The fungal effector necrosis-inducing secreted protein 1, which is widely conserved in filamentous fungi, targets BAK1 and BIK and interferes with signaling derived from cell surface pathogen recognition [72••]. If core effectors often target basal immunity like in the case of necrosis-inducing secreted protein 1, these may also alter outcomes of other plant–microbial interactions.

The inability to culture and transform obligate biotrophic fungi, such as rusts and powdery mildews, has limited the isolation and characterization of effectors in these fungi. However, advances in genomic and genetic resources have led to the identification of several Avr effector genes in wheat stem rust (*P. graminis* f. sp. *tritici*) [34•,73•,74,75•,76••,77,78] and wheat and barley powdery mildews (*B. graminis* f. sp. *tritici* and *B. graminis* f. sp. *hordei*, respectively) [33••,45,78••,79••] (Table 1). Other exciting progress in obligate biotrophic fungi includes the identification of Avr effector candidates in *Puccinia coronata* f. sp. *avenae* [80•], *Uromyces appendiculatus* [81], and *Hemileia vastatrix* [82]. These breakthroughs open new research opportunities to understand effector recognition and function in these systems. For instance, functional characterization of the ribonuclease-like effector CSEP0064/BEC1054 from *B. graminis* f. sp. *hordei* [69••] has revealed inhibition of ribosome-inactivating proteins as a mechanism to prevent cell death and promote susceptibility. An interesting observation is that resistance to obligate biotrophic fungal pathogens generally relies on direct effector recognition by NLRs (receptor ligand model), rather than indirect recognition commonly seen in bacterial resistance (Figure 3) [83,84]. The mechanisms underlying translocation of fungal effectors into the plant cell remain unknown [85]. The sequence diversity of these molecules and lack of conserved motifs present challenges to address this question.

## Concluding statements

During the 2019 International Society Molecular Plant–Microbe Interactions conference, our scientific community selected "Why do some pathogens need so many effectors when others need a few?" as one of the top 10 unanswered questions in the field [86]. This question is particularly relevant to fungi, which often contain large effector repertoires compared with bacteria. The more complex lifestyle and evolutionary history of pathogenic fungi may mean that they need to target additional host processes to support successful infection. Their larger genome size may also permit the evolution of an effector repertoire with substantial redundancy to enhance the robustness of pathogenicity mechanisms. The answer to this question may also require looking beyond the individual functions of effectors to consider how their combinatorial effects enhance disease susceptibility.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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- • of special interest
- •• of outstanding interest

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This paper illustrates the tug-of-war between the effector Osp24 from *F. graminearum* and TaFROG to bind to TaSnRK1α in wheat to induce or prevent ubiquitin 26S-proteasome-mediated degradation of TaSnRK1α, respectively. TaSnRK1α plays a role in pathogen resistance and therefore targeting this protein by a cytoplasmic effector from *F. graminearum* is advantageous for fungal colonization. *F. graminearum* produces mycotoxins such as deoxynivalenol (DON) during infection. In presence of DON, TaFROG competes for binding to TaSnRK1α at the same site than Osp24, which stabilizes the protein to prevent ubiquitin 26S-proteasome-mediated degradation.

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In this paper the authors identified two nuclear *M. oryzae* effectors, MoHTR1 and MoHTR2 that function as transcriptional repressors to reprogram the transcription of rice genes associated with immunity. To regulate rice gene expression, MoHTR1 and MoHTR2 bind the promotor of their gene targets, often reducing expression of those genes. Interestingly, transgenic rice plants individually expressing MoHTR1 or MoHTR2 show contrasting responses to pathogen infection. They display an increased susceptibility to the *M. oryzae* and *X. oryzae*, both hemibiotrophic pathogens, and increased resistance to a necrotrophic pathogen, *C. miyabeanus*.

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This article demonstrates that the Pm3 multiallelic race-specific resistance in wheat against *Blumeria graminis* f. sp. *tritici* involves an allele-specific Avr effector, a *R* gene allele, and a pathogen-encoded suppressor of avirulence. The authors cloned the effector gene AvrPm3$^{a2/f2}$, which is recognized by Pm3a and Pm3f alleles, and show that gene expression levels play a role in plant pathogen incompatibility.

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The authors report that the conserved effector NIS1, which is commonly found across members of the Ascomycota and Basidiomycota including the pathogens *Colletotrichum orbiculare* (cucumber anthracnose fungus), *Colletotrichum higginsianum* (crucifer anthracnose fungus) and *Magnaporthe oryzae* (rice blast fungus), suppresses multiple PTI responses by targeting BAK1 and BIK1. This work shows the commonalities between bacterial and fungal effectors and suggests that interference with kinase signaling initiated upon PAMP recognition in the cell wall-plasma membrane interface is a universal infection strategy.

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This paper describes the release of a fully haplotype resolved chromosome level genome assembly for any rust fungi and provides evidence of the role of somatic hybridization in the evolution of rust fungi. The latest gene annotations for predicted effectors in *Puccinia graminis* f. sp. *tritici* are part of this publication.

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Together with Chen et al. [34•] this is the first report of the cloning of the first Avr effector from a cereal rust fungus and demonstrate that direct recognition of Avr-R pairs occur in these organisms. Interestingly, AvrSr35 is not a small secreted protein as most reported fungal effectors.

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The authors use long-read sequencing, high-density genetic mapping and bacterial artificial chromosome sequences to generate the first chromosome level genome assembly for wheat powdery mildew. This represents a valuable resource to study the entire effector repertoire of this important plant pathogen. Interestingly, candidate effector genes of the same family are found in clusters and are enriched around recombination events.

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The authors identified four new effector proteins, $Avr_{a7}$, $Avr_{a9}$, $Avr_{a10}$ and $Avr_{a22}$ in *Blumeria graminis f. sp. hordei* by transcriptome-wide association studies. They also demonstrated that these effectors are directly recognized by their corresponding allelic MLA7, MLA9, MLA10 and MLA22 immunoreceptors. These findings support the idea that unlike the indirect NLR mediated recognition often observed for bacterial effectors, MLA like receptors have evolved the ability to directly recognize fungal effectors. This likely explains the MLA functional diversification observed in the host population.

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The authors report a dramatic shift in population virulence profiles of the oat crown rust fungus between 1990 and 2015, which is associated with substantial genetic differentiation between the two populations. The authors identify seven Avr loci associated with virulence phenotypes on fifteen R genes, with most loci containing a single candidate Avr gene. A selective sweep at a single Avr locus corresponding to six R genes is associated with a complete shift to virulence on these R genes in the 2015 population. This research piece represents the first application of a genome-wide association study to uncover *Avr* effectors in a rust fungus.

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