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

Melania Figueroa<sup>1</sup>, Diana Ortiz<sup>2</sup> and Eva C. Henningsen<sup>3</sup>

## 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.

## Addresses

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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* Avr 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 fungal 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	<i>Blumeria graminis</i> f. sp. <i>hordei</i>	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	<i>B. graminis</i> f. sp. <i>hordei</i>	Biotroph	Powdery mildew	Barley	[69]
SvrPm3al/f1	Suppresses recognition of AvrPm3a2/f2 mediated by the Pm3 wheat resistant gene	Unknown	<i>B. graminis</i> f. sp. <i>tritici</i>	Biotroph	Powdery mildew	Wheat	[33,45]
ChEC89	Unknown	Peroxisome	<i>Colletotrichum higginsianum</i>	Hemibiotroph	Anthracnose	Cruciferous plants	[19]
ChEC21	Unknown	Golgi apparatus	<i>C. higginsianum</i>	Hemibiotroph	Anthracnose	Cruciferous plants	[19]
NIS1	Disrupts basal immunity by binding to BAK1 and BIK1	Cytosol	<i>Colletotrichum orbiculare</i> <i>C. higginsianum</i> <i>Magnaporthe oryzae</i>	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	<i>Fusarium oxysporum</i>	Hemibiotroph	Fusarium wilt, foot, root, or bulb rot	Tomato, cotton, banana, melon	[44]
Osp24	Induction of proteasome-mediated degradation of its host target	Cytosol	<i>F. graminearum</i>	Hemibiotroph	Fusarium head blight	Wheat and barley	[32 ● ●]
HvEC-016	Unknown	Unknown	<i>Hemileia vastatrix</i>	Biotroph	Coffee leaf rust	Coffee	[82]
AvrLm1	Alterations to MAPK signaling	Cytosol	<i>Leptosphaeria maculans</i>	Hemibiotroph	Black leg	Canola	[38]
MoCDIP4	Disruption of mitochondrial dynamics	Endoplasmic reticulum	<i>M. oryzae</i>	Hemibiotroph	Rice blast	Rice	[30 ● ●]
MoHTR1 and MoHTR2	Transcriptional reprogramming of genes related to plant immunity	Nucleus	<i>M. oryzae</i>	Hemibiotroph	Rice blast	Rice	[40 ● ●]
AvrPiz-t	Inhibition of RING E3 ubiquitin ligases APIP6 and APIP10, modulation of a K <sup>+</sup> 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	<i>M. oryzae</i>	Hemibiotroph	Rice blast	Rice	[51–54]
AVR-Pia	Unknown	Cytosol	<i>M. oryzae</i>	Hemibiotroph	Rice blast	Rice	[60,62]
CTP1	Unknown	Chloroplast, mitochondria	<i>Melampsora larici-populina</i>	Biotroph	Poplar leaf rust	Poplar	[28]
CTP2, CTP3	Unknown	Chloroplast,	<i>M. larici-populina</i>	Biotroph	Poplar leaf rust	Poplar	[28]

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

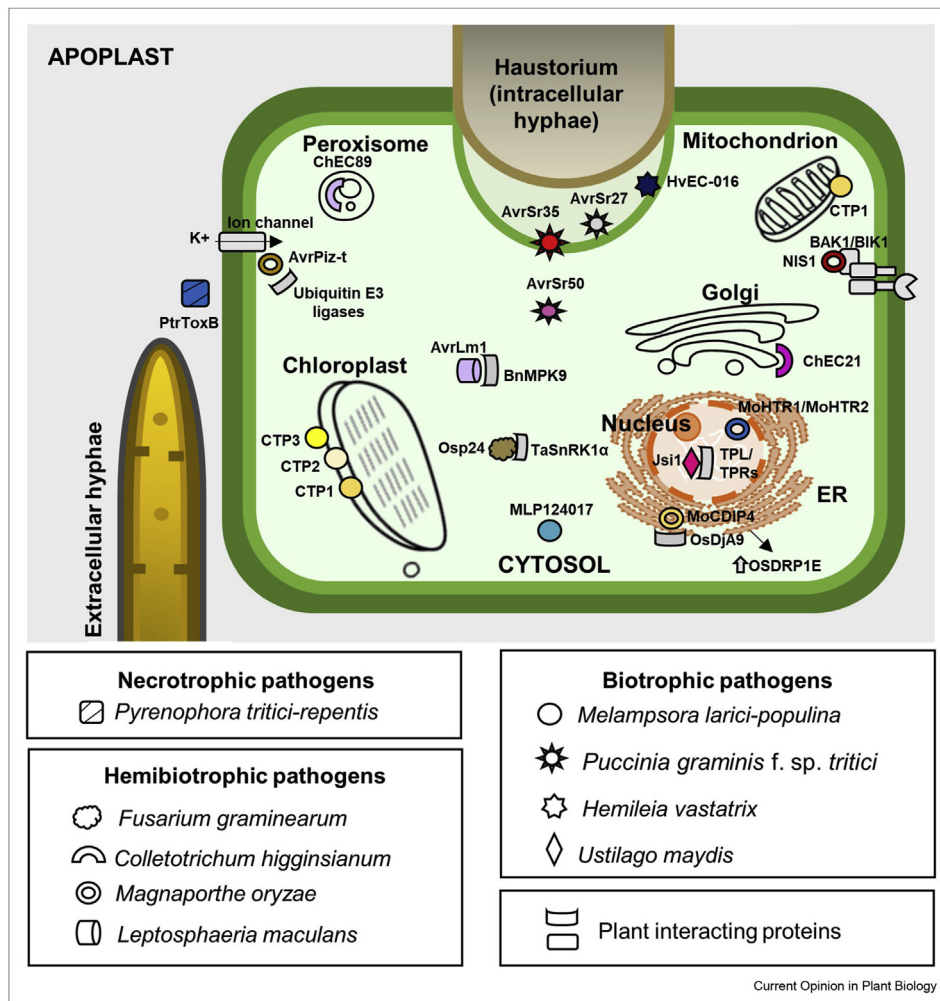
of the dynamin-related protein OsDRP1E. The competition between MoCDIP4 and OsDRP1E interferes with OsDRP1E degradation, resulting in an overaccumulation of OsDRP1E in rice cells. Ultimately, this causes aberrant mitochondrial fission and size reduction, which increases susceptibility to *M. oryzae*. 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 TaSnRK1 $\alpha$ , 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, TaSnRK1 $\alpha$  contributes to resistance to *F. graminearum* by an unknown mechanism and Osp24 promotes proteasomal degradation of TaSnRK1 $\alpha$  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 TaSnRK1 $\alpha$  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

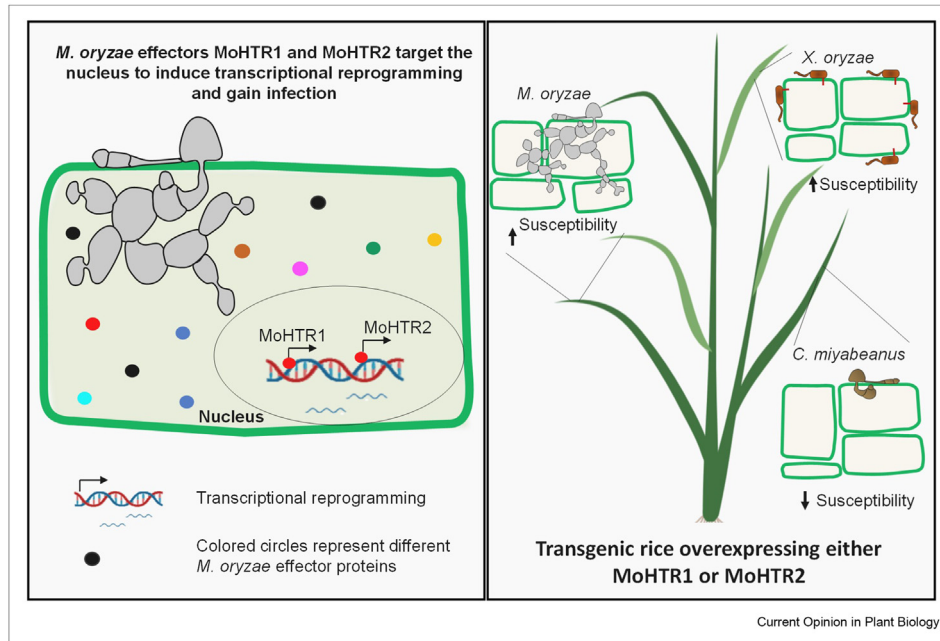
The genomic era has revealed not only that many fungi harbor hundreds of effector proteins, but also that plants possess large sets of immunity receptors [2,41,42]. Yet, it is intriguing that genetic studies attribute incompatibility between plants and adapted pathogens to

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<sup>a1/f1</sup> effector protein from *Blumeria graminis* f. sp. *tritici* has been shown to suppress cell death triggered on recognition of AvrPm3<sup>a2/f2</sup> 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<sup>+</sup> 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* Avr 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.

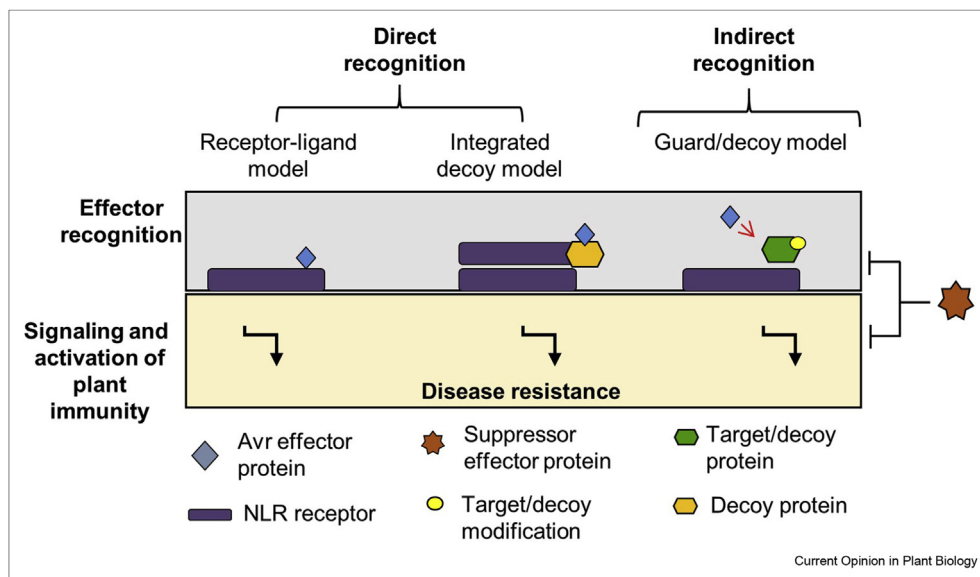
The existence of 'core' effectors that are conserved across pathogens is an intriguing topic [48,70,71]. Although most effectors characterized to date are pathogen specific, it would be interesting to identify the

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 reprogramming 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 outstanding interest
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