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Taking the stage: effectors in the spotlight

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Plant pathogens are a serious threat to agriculture and to global food security, causing diverse crop diseases which lead to extensive annual yield losses. Production of effector proteins by pathogens, to manipulate host cellular processes, is central to their success. An understanding of fundamental effector biology is key to addressing the threat posed by these pathogens. Recent advances in 'omics' technologies have facilitated high-throughput identification of putative effector proteins, while evolving cellular, structural and biochemical approaches have assisted in characterising their function. Furthermore, structures of effectors in complex with host factors now provide opportunities for applying our knowledge of effector biology to influence disease outcomes. In this review, we highlight recent advances in the field and suggest avenues for future research.

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Introduction

Adapted pathogens of plants secrete effector proteins to facilitate host colonization [1]. These effectors typically act to perturb plant immune responses, but can also interfere with host cell physiology to benefit the parasite. Plants can also detect the presence and/or activity of effectors as 'non-self', re-instating immunity [1–3]. A general description of effectors defines them as pathogen secreted factors that alter the interaction with the host either in the apoplast or inside cells. Here, we are only able to consider host-translocated proteins, and therefore we use the general term 'effector' to mean host-translocated effectors.

Effectors are under selective pressure to evade detection by the host immune system, but also maintain their virulence-promoting activity, and potentially evolve new functions. This has led to diverse sets of effectors evolving in bacterial and filamentous plant pathogens. Understanding the virulence-promoting activities of effectors, and how they are recognized by the plant immune system, remain major themes in the study of plant–microbe interactions.

In this review, we focus on recent advances in the area of identifying effectors, discuss some of the approaches taken to identify the host molecules that interact with these proteins, and highlight what has been discovered about the biological activity of effectors through these studies.

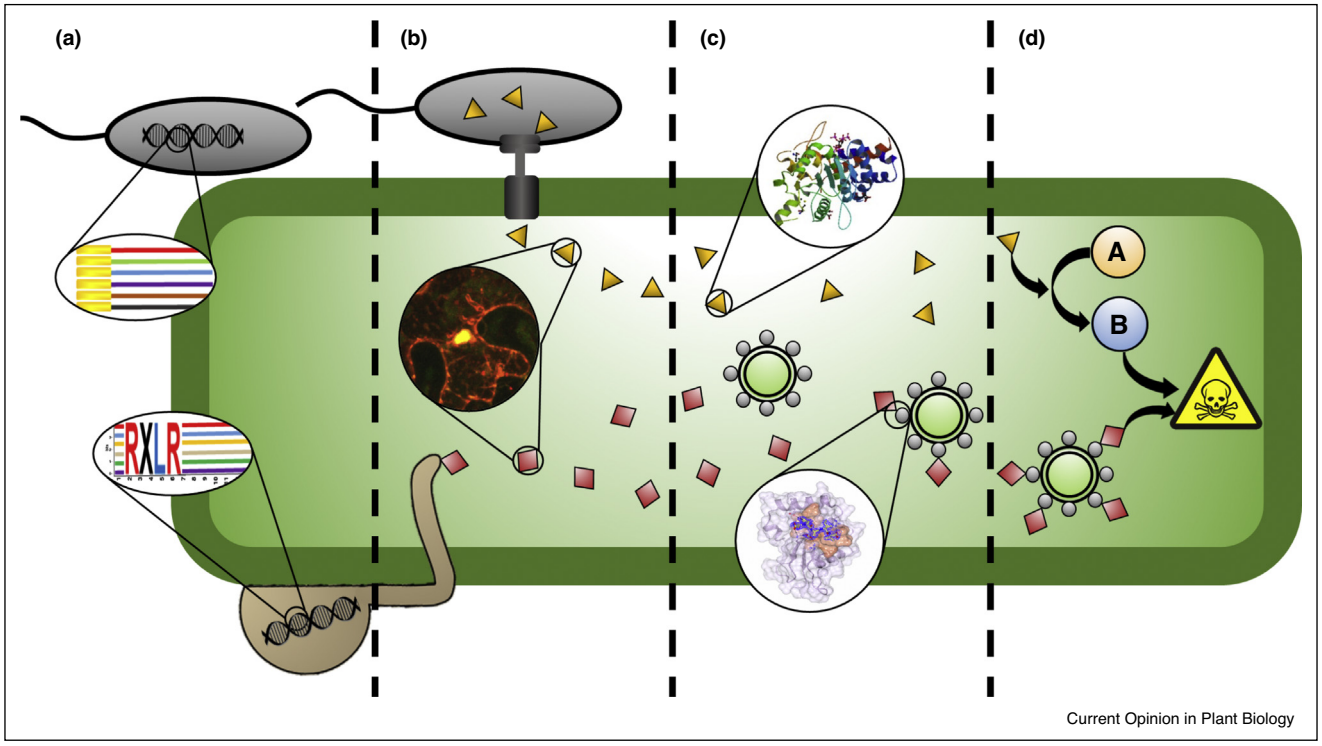
Genomics and bioinformatics to identify effectors: an extensive back catalogue

Improvements in high-throughput sequencing and proteomic technologies have generated extensive new knowledge on the genes, transcripts and proteins from some of the most destructive bacterial and filamentous plant pathogens [4–10]. Exploiting this information for identification and *in silico* prediction of putative effector proteins is important for prioritizing molecular studies of specific plant–pathogen interactions (reviewed in Ref. [11]).

For Gram-negative bacteria, putative effectors can be identified from genome sequences by their N-terminal motif (Figure 1), which directs effectors to the secretion system [12]. However, this alone is not sufficient to confirm translocation to the host cell, as mediated by the Type III secretion system, and further studies are required to confirm a host cell function. One of the more accessible assays is the 'gene-for-gene' (more precisely 'protein-for-protein') phenotype, showing specific recognition by the plant immune system (recent example in Ref. [13•]). Promotion of bacterial growth upon heterologous expression in plants is also a phenotype often used to support function as an effector. Establishing the biochemical activity of effectors in host cells remains more challenging (see section below).

Similarly, in oomycete pathogens, effector prediction has been facilitated by the presence of the RXLR motif (Figure 1), a conserved amino acid sequence signature involved in effector translocation [14]. This motif has enabled the cataloging of hundreds of putative effectors

Figure 1



Effectors in plant-pathogen interactions. (a) An extensive back catalogue: Putative effectors can be predicted from bacterial and filamentous pathogen genomes by searching for conserved sequences (*e.g.* signal peptide), or using machine learning approaches, and these can be combined with structural information when available. **(b) Through the lens:** Cell biology studies revealing informative effector localization may shed light on effector function. **(c) Extreme close up:** Structural studies can unravel the atomic details of effector mechanism (*e.g.* when effectors are enzymes), and the interaction with targets when complexes can be obtained. **(d) and . . . Action!:** Biochemical/biophysical studies can be used to discover and validate effectors' biological functions.

from pathogens of the *Phytophthora* genus [4,8,15,16], and *Hyaloperonospora arabidopsidis* [6].

Prediction of putative effectors from fungal pathogens has proven more challenging. New approaches have included developing machine-learning tools that incorporate common features of fungal effectors discovered to date (including protein size, amino acid content, charge, evidence for diversifying selection). When combined with *in planta* expression data, these tools have provided improved prediction of effectors from fungal secretomes [17,18•].

In recent years, *in silico* prediction of putative effectors from filamentous plant pathogens has also been improved through knowledge of protein structure. For oomycetes, identification of the 'WY-domain' [19,20] suggested another common feature of RXLR-containing effectors. For fungal effectors, specifically those of the rice blast pathogen *Magnaporthe oryzae*, structurally similar but sequence divergent effectors have been identified and termed MAX effectors (Magnaporthe AVRs and ToxB like, [21••]). Analysis of gene expression suggests that the MAX effector family is important during biotrophic infection [21••]. Interestingly, the structure of another *M. oryzae* effector, that was not part of the De Guillen *et al.* study, was also shown to adopt the MAX fold [22••], but this was not predicted by the *in silico* analysis. This demonstrates that caution should be exercised when interpreting *in silico* predictions, as it is possible to exclude genuine effectors.

Similar to phytopathogenic fungi, effectors of cyst nematodes lack an easily identifiable motif in their protein-coding region. A recent *in silico* approach for effector prediction focused on examining promoter regions [23•]. This led to identification of a DNA motif termed the 'DOG box', which is associated with a superset of putative effectors.

The next generation of machine-learning tools for effector identification from pathogen genomes and proteomes will require the integration of gene expression data, structural biology, genomic organization and promoter