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Sticking to it: phytopathogen effector molecules may converge on evolutionarily conserved host targets in green plants

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Introduction

The green plant lineage (Embryophytes) evolved from freshwater charophyte green algae over 450 million years ago and has since dominated terrestrial environments [1,2•]. The evolutionary history and diversification of land plant lineages is extensively reviewed elsewhere [3–5], but generally follows that non-vascular gametophyte-dominant bryophytes (liverworts, hornworts, mosses) were the earliest diverging lineage whose ancestor gave rise to sporophyte-dominant vascular plants (tracheophytes) that include lycophytes (clubmosses), ferns, and seed plants, which themselves diverged to gymnosperms (non-flowering) and angiosperms (flowering). Throughout their evolutionary history, plants have been exposed to a diverse range of microbial life forms that had the potential to impact their fitness. To protect themselves from detrimental microbes, land plants utilize a tiered immune system that includes the detection of common microbial motifs (MAMPs, microbe-associated molecular patterns), such as bacterial flagellin or fungal chitin, via membrane-localized PRRs (pattern recognition receptors) as an early line of defence [6,7•]. The recognition of MAMPs by PRRs initiates an intracellular MAP kinase signalling cascade that activates MTI (MAMP-triggered immunity), leading to several well-described molecular and physiological adjustments that limit pathogen ingress [6,8,9]. Conversely, microbes evolved effector proteins that suppress MTI and other host cellular activities to render hosts susceptible and promote disease [10,11,12•]. To date, pathogen effector research is largely performed in angiosperms, which represent an evolutionarily young (albeit diverse) land plant lineage. Below, we introduce key concepts of effector biology obtained from angiosperm-based pathosystems and project this knowledge onto earlier diverging land plant lineages to explore the idea that effectors target evolutionarily conserved plant proteins and processes (Figure 1).

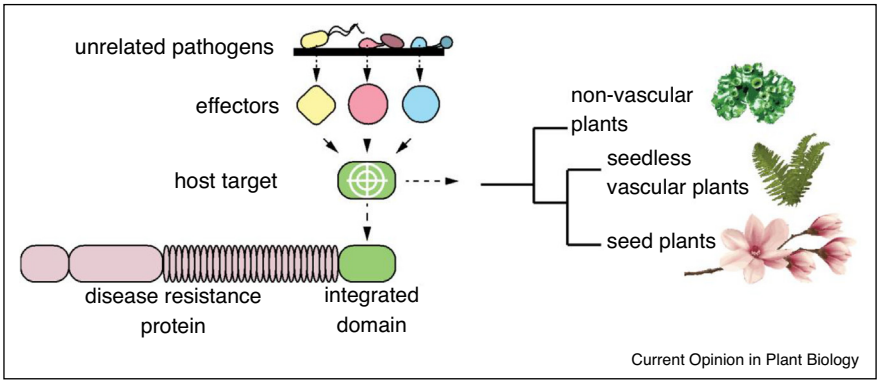
Back to basics: key concepts in effector biology

Pathogen effector molecules are translocated into host tissues and cells, where they target important macromolecules (proteins, cell wall components, nucleic acids, and so on) involved in normal cellular functions and/or immunity [10,11,12•]. Effectors are generally catalogued into two distinct groups; those acting in the extracellular spaces of host tissues (apoplastic) or those acting within host cells (cytoplasmic). Apoplastic effectors are secreted via general eukaryotic secretion systems in oomycetes/fungi [13,14•] or via the type II secretion system (T2SS) of bacterial pathogens [15]. These molecules are typically involved in the enzymatic degradation of plant cell walls, immune evasion, or the suppression of host proteolytic activity [11,16]. Bacterial pathogens such as *Pseudomonas syringae* and *Xanthomonas* spp. inject cytoplasmic effectors directly into plant cells using a specialized type III secretion system (T3SS) and are hence termed 'type III effectors' [12•,15]. In comparison, our understanding of how the cytoplasmic effectors of eukaryotic filamentous microbes are delivered into plant cells remains unclear, however it is generally believed that certain effector families (i.e. RXLR and CRN/crinkler) enter and act within host cells [10,11,17]. These molecules are likely delivered through specialized hyphal structures that invaginate plant cells (haustoria), or perhaps are endocytosed from the apoplast [18,19]. Cytoplasmic effectors have been extensively studied in angiosperms, revealing a suite of virulence strategies wherein effectors access various cellular compartments (nucleus, chloroplast, cytoplasm, and so on) to disrupt the activity of host proteins involved in transcriptional regulation, secretion, metabolism, programmed cell death, and hormone signalling [10,11,12•,20].

Unrelated effector molecules may converge on similar networks of host proteins

Investigating phytopathogen effector function is typically implemented in a formulaic manner, where individual effectors displaying activity in plant cells (i.e. disease promotion/immune suppression) are used as baits to

Figure 1



A conceptualized diagram highlighting the idea that host proteins targeted by unrelated pathogens and the integrated domains of disease resistance (R) proteins are present across the green plant lineage.

identify host targets in yeast 2-hybrid or immunoprecipitation-mass spectrometry (IP-MS) screens (discussed in [17]). This is then followed by more detailed analyses to validate candidate interactors and determine how the effector acts to facilitate disease progression. While highly effective, this approach provides only a snapshot of a given host–microbe interaction, as phytopathogens can deliver anywhere from 30 to 80 type III effectors (bacterial pathogens) or in some cases over 200 cytoplasmic effectors (fungi/oomycetes). It was therefore crucial that a more exhaustive screen for effector targets be conducted. Seminal studies carried out by Mukhtar *et al.* [21••] and Wessling *et al.* [22••] describe high-throughput yeast 2-hybrid screens and network analysis of candidate oomycete (*Hyaloperonospora arabidopsidis*), fungal (*Golovinomyces orontii*), and bacterial (*P. syringae*) effectors in the model angiosperm *Arabidopsis thaliana*. Together, these works revealed a core hub of plant proteins that are likely targeted by unrelated phytopathogens, which suggests that effectors may converge onto these hubs to promote microbial fitness *in planta* (Figure 1). While experimental evidence confirming interactions/modulation of homologous plant targets with individual phytopathogen effectors is lacking on a large scale *in vivo*, limited evidence supports the possibility of such a scenario. For example, the immune-regulator SGT1 associates with the *Ustilago maydis* (fungus) effector SEE1 in maize and with the *Xanthomonas campestris* (bacterial) AvrBsT effector in pepper [23–25]. Moreover, the TCP14 transcription factor, which was found to interact with multiple effectors in the yeast 2-hybrid screening described in Ref. [22••], was also shown to interact with the *Phytophthora capsici* (oomycete) CRN12-997 effector in tomato and the *P. syringae* (bacterial) effector HopBB1 in Arabidopsis [26,27]. Whether such convergent targeting holds true over a diverse range of plant–pathogen interactions remains to be determined. If so, it would imply that effectors manipulate conserved host proteins to promote disease progression in land plants (Box 1).

Box 1 Are effectors deployed during interactions with early diverging land plants?

Our current understanding of plant–microbe interactions is heavily skewed toward angiosperm models, with comparatively less known about how microbes interact with early diverging land plant lineages. This is especially true for plant–pathogen interactions, which are underrepresented compared to interactions between early diverging land plants and symbiotic microbes [44,59]. Evidence for the direct action of effector molecules in early diverging lineages is lacking, yet several lines of evidence suggest that phytopathogens use effectors to manipulate these plants. For example, several plant lineages (mosses, liverworts, ferns) are amenable to *Agrobacterium*-mediated transformation, which requires the successful delivery of transfer (T)-DNA by effector molecules that travel together through the bacterial type IV secretion system [15,60–62]. Moreover, putative apoplastic effectors and toxins from the necrotrophic bacterial pathogen *Pectobacterium caratovorum* were shown to induce cell death in the moss *P. patens*. To our knowledge, the action of cytoplasmic effectors in moss has not yet been described, however, hemi-biotrophic pathogens that deploy effectors in angiosperms (*Phytophthora, Colletotrichum*) successfully colonize moss [63,64•] and likely do so using effectors. Moreover, we recently reported that the hemi-biotrophic oomycete pathogen *P. palmivora* establishes digit and branched intracellular haustoria-like structures in *M. polymorpha* liverwort cells, which was associated with the upregulation of apoplastic and cytoplasmic (predominantly RXLR) effector molecules [65••]. Together, these studies hint at the importance of phytopathogen effectors in manipulating diverse land plant lineages. Future efforts to understand the extent to which effectors of broad-host range pathogens modulate liverwort, angiosperm, and perhaps even lycophyte/fern susceptibility will be of particular importance in exploring the conservation/convergence of effector–host relationships.