Plant Immune Responses: Aphids Counterattack

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To survive and complete their life cycle, herbivorous insects face the difficult challenge of coping with the arsenal of plant defences. A new study reports that aphids secrete evolutionary conserved cytokines in their saliva to suppress host immune responses.

Like vertebrates and insects, plants resist microbial and fungal infections with an innate immunity response that relies on the detection of invariant pathogen-associated molecular patterns or elicitors, and by subsequent activation of numerous defence genes [1]. Similarly, upon recognition of insect elicitors, plants produce defence proteins and secondary metabolites that are detrimental to the attacker [2]. During evolution, efficient innate immunity has imposed a strong selection pressure on plant pathogens that has led to the development of exquisite strategies to cope with defences. Indeed, microbes deliver hundreds of effectors into host cells to interfere with almost all steps of the innate immunity response [3]. In contrast, much less information is available about defence suppression by insect herbivores. Components in oral secretions of chewing lepidopteran larvae inhibit defence gene expression, but the nature of the effectors is often unknown and the effect of defence suppression on insect performance not always tested [4-6]. Aphids are hemipteran insects that feed from plant phloem sieve elements by inserting a syringe-like mouthpart, the stylet, between cell layers. During feeding, proteins in aphid saliva are secreted and trigger plant defences, including sieve tube occlusion. A few salivary effectors inhibit defences but the function of these proteins is poorly characterized [7-10]. In this issue of *Current Biology*, Naessens *et al.* [11] describe a novel aphid effector that is homologous to Macrophage Migration Inhibition Factor (MIF), an essential modulator of innate immunity and inflammation in vertebrates.

The identification of aphid effectors is not an easy task. Aphid body size ranges from 1.5 to 3.5 mm and salivary glands containing putative secreted proteins are ca. 200 μm organs

that produce only minute amounts of liquid. Initial attempts relied on proteomic studies of salivary extracts obtained from artificial diets but a functional characterization of effectors has been achieved by transgenic expression of cDNAs in plants and analysis of aphid performance [9,10,12]. Naessens and colleagues [11] focus on the role of MIFs from the pea aphid, *Acyrthosiphon pisum*, and from the green peach aphid, *Myzus persicae*. In a previous study, the same group searched the recently available *A. pisum* genome for immune regulators and discovered the presence of five genes coding for MIFs [13]. *Ap*MIFs were expressed in circulating immune cells and upregulated after pathogen and parasitoid infection, implying a role in host immune response [13]. Here, Naessens *et al.* [11] observe that one member of the *Ap*MIF family, *ApMIF1*, and its homologue *MpMIF1* from *M. persicae* are expressed in salivary glands and that the corresponding MIF proteins are secreted during feeding. This intriguing finding prompts the authors to test the role of MIF1 in plant-aphid interaction.

Knocking-down *ApMIF* genes by RNAi leads to a poorer survival and fecundity of *A. pisum* on its host plant *Vicia faba*. Behavioural studies by electropenetrography (EPG) recordings indicate that underexpressing MIF genes impairs phloem feeding. To see if MIFs alters plant defences, the authors transiently express *Mp*MIFs in *Nicotiana benthamiana*, which is a good plant model for functional analyses of proteins and a host for *M. persicae*. To trigger plant immune responses they apply cryptogein, an elicitor from the plant pathogen *Phytophora cryptogea*. When each of the three *Mp*MIFs is expressed in leaves, cryptogein-induced programmed cell death, callose accumulation and transcriptional activation of pathogenesis-related genes are strongly suppressed. Localized programmed cell death is known to contain the spread of pathogens, callose is a glucose polymer involved cell wall reinforcement and sieve tube plugging, and pathogenesis-related proteins are thought to have antimicrobial activities. Importantly, only *Mp*MIF1 can fully restore normal survival and fecundity of *Mp*MIF-downregulated (RNAi) aphids when transiently expressed in *N. benthamiana*, suggesting a specific role for MIF1 in suppressing plant defences and corroborating the observation that only this family member is present in aphid saliva.

The study by Naessens and colleagues [11] adds a novel member to the (short) list of characterized salivary effectors. The surprise comes from the identity of this protein. MIFs are small (ca. 12 kDa) well-known cytokines in vertebrates with important functions in inflammation and innate immunity [14]. Human MIF is expressed in several immunity-related cell types and in tissues exposed to environmental challenges. Among other roles, MIF stimulates the detection of endotoxin-containing bacteria in macrophages, promotes cell proliferation, controls apoptosis, triggers the production of other cytokines, and antagonizes

the immunosuppressive effects of glucocorticoid steroids [14]. At the catalytic level, MIF has both a dopachrome tautomerase and a thiol-protein oxidoreductase activity but the biological substrates are still unknown. Interestingly, MIF homologues are found in animal parasites including nematodes, ticks, *Plasmodium* spp., *Leishmania* spp., *Toxoplasma gondii,* and are secreted [15,16]. Whether parasite MIFs interfere with recognition from their host by inhibiting macrophage migration or modulate inflammation for a successful infection are intriguing hypotheses that need further confirmation [15,16].

The finding of MIFs in aphids was initially associated with a role in antibacterial and antiparasitoid responses [13]. Curiously, most insect genomes beyond hemipteran species do not contain MIFs [13]. Naessens *et al.* [11] now show that aphids, which are insect parasites that have a long-lasting and close interaction with their host, benefit from secreting MIF1 that suppresses defences. This finding is remarkable as it indicates that animal and plant parasites may share molecular features in infectious processes. However, several open questions remain. A demonstration that MIF inhibits host defences during actual aphid feeding is still lacking, as well as the localization of MIF1 *in planta*. Plants do not have circulating immune cells and, although there are parallels between plant and animal innate immunity, defences compounds and proteins are plant-specific. Although human and parasite MIFs block apoptosis, for instance by inhibiting p53 [17,18], programmed cell death in plants is regulated differently. Thus, how aphid MIF inhibits this process will have to be elucidated. Importantly, there are no reports of immunosuppressive function of vertebrate MIFs but there is evidence that MIF from a parasitic nematode lowers the production of Th2-related interleukins in mice [19]. That aphid MIFs inhibit defence responses in plants is fascinating and underlies a potentially novel mode of action. Finally, it is not known if MIF1 expression in salivary glands is regulated and if secretion is activated in response to feeding stimulants. How secreted MIF1 acquired a distinct role from other MIFs is another important question.

Interestingly, a recent analysis of plant genomes identified MIF homologues in algae, mosses, lycophytes, gymnosperms and angiosperms. In the model plant *Arabidopsis thaliana*, three genes encode proteins with ca. 30% identity to human MIF (*At*MDL1, *At*MDL2, *At*MDL3) [20]. Amino acids that are required for the tautomerase activity of human MIF are conserved whereas two cysteine residues that belong to a motif crucial for oxidoreductase activity are not present in Arabidopsis. Strikingly, the similar position of an intron in *At*MDL1, *At*MDL2, and human MIF suggests the presence of a common ancestor before the split between animal and plant lineages. In addition, homology modelling of Arabidopsis MIFs predicts a three-dimensional structure very similar to that of human MIF [20]. These

findings and the distribution of MIFs in different kingdoms point to shared biochemical activities among these proteins. *AtMDL3* expression is induced upon pathogen challenge or treatment with microbial elicitors and this gene is co-expressed with several genes implicated in plant defence, suggesting a role in plant innate immunity [20]. As suggested by Naessens *et al.* [11], a tantalizing hypothesis is that aphids have evolved a strategy to suppress plant defence by secreting a MIF that interferes with endogenous MIFs (Figure 1). MIF forms a trimer [14] and the occurrence of heterocomplexes can be envisaged. Alternatively, molecular mimicry by secreted MIF1 may inhibit plant MIFs-related processes. Further investigation will be required to test such scenarios but the discovery of this new aphid effector should help to uncover the function of plant and aphid MIFs. These cytokines may participate in a specific part of innate immunity that is conserved across kingdoms.

Aphids feed on many plant species worldwide, including various crops, and have the propensity to transmit plant viruses. They thus constitute pests of agronomical importance. Knowledge on the nature of effectors and mechanisms of defence suppression should help to develop strategies aiming at reducing the negative impact of phloem-feeders on agriculture.

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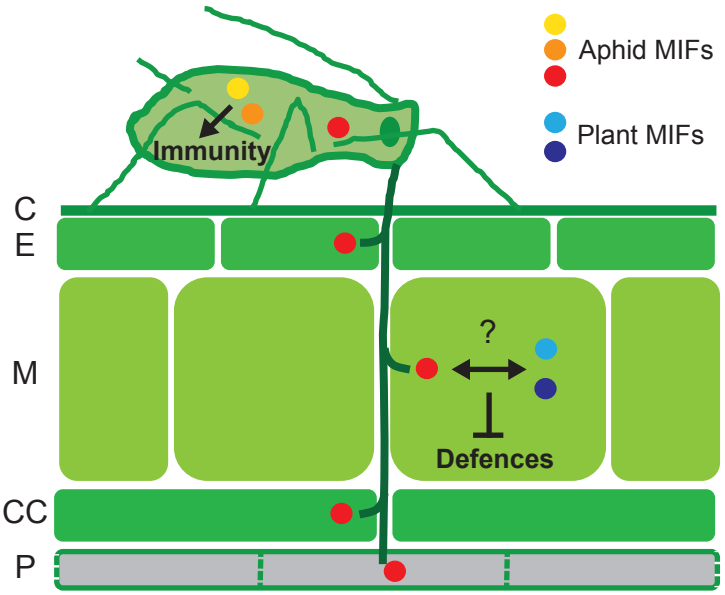
Figure 1. Suppression of plant defences by a novel aphid effector.

When feeding on host plants, aphids penetrate the leaf surface with a specialized mouthpart termed the stylet. After probing cells from different tissue layers, the stylet locates phloem cells from which aphids get nutrients. During this process the plant recognizes aphid-derived salivary components and mounts a defence response. In this issue of *Current Biology*, Naessens and colleagues [11] identify a novel salivary effector from *Acyrthosyphon pisum* (pea aphid) and *Myzus persicae* (green peach aphid). One specific member of the Macrophage Migration Inhibition Factor (MIF) family, MIF1 (red dot), is secreted in saliva from both aphid species and suppresses plant defences. MIFs are conserved modulators of innate immunity found in vertebrates, invertebrates and plants. Non-secreted MIF homologues (orange dots) are postulated to play a role in aphid immunity [13]. The mode of action and cell type where the aphid effector is delivered are unknown. An attractive hypothesis is that

aphid MIF1 interacts or interfere with plant MIFs (blue dots) to inhibit plant defences [13]. The number of MIF homologues depicted here is arbitrary since it varies between aphid and plant species. C, cuticule; E, epidermis; M, mesophyll; CC, companion cells; P, phloem.


Aphid MIFs
Plant MIFs
Immunity
C
E
M
CC
P
?
Defences