

1 Plant Immune Responses: Aphids Counterattack

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

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14 Like vertebrates and insects, plants resist microbial and fungal infections with an innate
15 immunity response that relies on the detection of invariant pathogen-associated molecular
16 patterns or elicitors, and by subsequent activation of numerous defence genes [1]. Similarly,
17 upon recognition of insect elicitors, plants produce defence proteins and secondary
18 metabolites that are detrimental to the attacker [2]. During evolution, efficient innate
19 immunity has imposed a strong selection pressure on plant pathogens that has led to the
20 development of exquisite strategies to cope with defences. Indeed, microbes deliver hundreds
21 of effectors into host cells to interfere with almost all steps of the innate immunity response
22 [3]. In contrast, much less information is available about defence suppression by insect
23 herbivores. Components in oral secretions of chewing lepidopteran larvae inhibit defence
24 gene expression, but the nature of the effectors is often unknown and the effect of defence
25 suppression on insect performance not always tested [4-6]. Aphids are hemipteran insects that
26 feed from plant phloem sieve elements by inserting a syringe-like mouthpart, the stylet,
27 between cell layers. During feeding, proteins in aphid saliva are secreted and trigger plant
28 defences, including sieve tube occlusion. A few salivary effectors inhibit defences but the
29 function of these proteins is poorly characterized [7-10]. In this issue of *Current Biology*,
30 Naessens *et al.* [11] describe a novel aphid effector that is homologous to Macrophage
31 Migration Inhibition Factor (MIF), an essential modulator of innate immunity and
32 inflammation in vertebrates.

33 The identification of aphid effectors is not an easy task. Aphid body size ranges from
34 1.5 to 3.5 mm and salivary glands containing putative secreted proteins are ca. 200 µm organs

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

47 Knocking-down *ApMIF* genes by RNAi leads to a poorer survival and fecundity of *A.*
48 *pisum* on its host plant *Vicia faba*. Behavioural studies by electropetrography (EPG)
49 recordings indicate that underexpressing MIF genes impairs phloem feeding. To see if MIFs
50 alters plant defences, the authors transiently express *MpMIFs* in *Nicotiana benthamiana*,
51 which is a good plant model for functional analyses of proteins and a host for *M. persicae*. To
52 trigger plant immune responses they apply cryptogein, an elicitor from the plant pathogen
53 *Phytophthora cryptogea*. When each of the three *MpMIFs* is expressed in leaves, cryptogein-
54 induced programmed cell death, callose accumulation and transcriptional activation of
55 pathogenesis-related genes are strongly suppressed. Localized programmed cell death is
56 known to contain the spread of pathogens, callose is a glucose polymer involved cell wall
57 reinforcement and sieve tube plugging, and pathogenesis-related proteins are thought to have
58 antimicrobial activities. Importantly, only *MpMIF1* can fully restore normal survival and
59 fecundity of *MpMIF*-downregulated (RNAi) aphids when transiently expressed in *N.*
60 *benthamiana*, suggesting a specific role for MIF1 in suppressing plant defences and
61 corroborating the observation that only this family member is present in aphid saliva.

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

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

76 The finding of MIFs in aphids was initially associated with a role in antibacterial and
77 antiparasitoid responses [13]. Curiously, most insect genomes beyond hemipteran species do
78 not contain MIFs [13]. Naessens *et al.* [11] now show that aphids, which are insect parasites
79 that have a long-lasting and close interaction with their host, benefit from secreting MIF1 that
80 suppresses defences. This finding is remarkable as it indicates that animal and plant parasites
81 may share molecular features in infectious processes. However, several open questions
82 remain. A demonstration that MIF inhibits host defences during actual aphid feeding is still
83 lacking, as well as the localization of MIF1 *in planta*. Plants do not have circulating immune
84 cells and, although there are parallels between plant and animal innate immunity, defences
85 compounds and proteins are plant-specific. Although human and parasite MIFs block
86 apoptosis, for instance by inhibiting p53 [17,18], programmed cell death in plants is regulated
87 differently. Thus, how aphid MIF inhibits this process will have to be elucidated. Importantly,
88 there are no reports of immunosuppressive function of vertebrate MIFs but there is evidence
89 that MIF from a parasitic nematode lowers the production of Th2-related interleukins in mice
90 [19]. That aphid MIFs inhibit defence responses in plants is fascinating and underlies a
91 potentially novel mode of action. Finally, it is not known if MIF1 expression in salivary
92 glands is regulated and if secretion is activated in response to feeding stimulants. How
93 secreted MIF1 acquired a distinct role from other MIFs is another important question.

94 Interestingly, a recent analysis of plant genomes identified MIF homologues in algae,
95 mosses, lycophytes, gymnosperms and angiosperms. In the model plant *Arabidopsis thaliana*,
96 three genes encode proteins with ca. 30% identity to human MIF (*AtMDL1*, *AtMDL2*,
97 *AtMDL3*) [20]. Amino acids that are required for the tautomerase activity of human MIF are
98 conserved whereas two cysteine residues that belong to a motif crucial for oxidoreductase
99 activity are not present in *Arabidopsis*. Strikingly, the similar position of an intron in
100 *AtMDL1*, *AtMDL2*, and human MIF suggests the presence of a common ancestor before the
101 split between animal and plant lineages. In addition, homology modelling of *Arabidopsis*
102 MIFs predicts a three-dimensional structure very similar to that of human MIF [20]. These

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

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

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

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

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

