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BACTERIAL PATHOGENICITY

Phytoplasma converts plants into zombies

The life cycle of the bacterial plant pathogen *Phytoplasma* spp. involves both a plant host and a sap-feeding insect vector (such as a leafhopper), which ingests infected phloem and then transmits the bacteria to healthy plants. The Aster Yellows *Phytoplasma* strain Witches' Broom (AY-WB) causes morphological changes in host plants, such as the abnormal conversion of flowers into leaf-like structures (known as phyllody), greening of petals (known as virescence) and altered growth patterns, which leads to a witches' broom-like appearance. Following infection, AY-WB releases effector proteins, including secreted AY-WB protein 54 (Sap54), which has been shown to cause phyllody; however, the mechanisms that are involved and the consequences of phyllody for the bacterium have been unclear. MacLean *et al.* now report that Sap54-dependent degradation of transcription factors involved in flowering promotes phyllody and attraction of the vector to infected plants, thereby aiding propagation of the bacteria.

Using yeast two-hybrid screens and co-immunoprecipitation assays, the authors show that, in transgenic *Arabidopsis thaliana* cell lines that express Sap54 and in AY-WB-infected plants, Sap54 interacts with several members of the type-II MADS domain transcription factor (MTF) family, including SEPALLATA3 (SEP3) and APETALA1 (AP1). MTFs have a key role in flowering, as they regulate the establishment of a floral meristem and the specification of floral organs. Interestingly, instead of binding to the conserved MADS domain of MTFs, Sap54 was shown to bind to the keratin domain (K-domain) in AP1; this domain is absent in insect MTFs, which suggests that Sap54 might have evolved to selectively target plant MTFs. The authors observed a Sap54-dependent decrease in the levels of SEP3 in infected *A. thaliana* cell lines compared with uninfected cell lines. This finding suggested that Sap54 might function as a protease that directly degrades MTFs; however, the addition of protease inhibitors did not change MTF degradation. By contrast, treatment of infected plants with an inhibitor of the host ubiquitin–proteasome system (UPS) stabilized MTF protein levels, which suggests that Sap54 exploits this pathway to promote the degradation of host proteins. In agreement with this, yeast two-hybrid and co-immunoprecipitation experiments showed that Sap54 interacts with two isoforms of the ubiquitin-binding protein RADIATION SENSITIVE23 (RAD23), RAD23C and RAD23D, which are shuttle proteins that target ubiquitylated proteins to the proteasome for degradation.

Further experiments revealed that AY-WB-infected RAD23-mutant plants grow flowers that resemble those of healthy plants and that SEP3 is stabilized in these mutants. Together, these results suggest that Sap54 induces phyllody by targeting MTFs that are involved in flower development for degradation in a RAD23-dependent manner.

So, how does conversion to phyllody benefit AY-WB? The authors proposed that leaf-like structures might be more attractive than flowers to the insect vector, which would then facilitate the transmission of the bacteria to healthy hosts. To assess this hypothesis, the authors examined whether the aster leafhopper *Macrosteles quadrilineatus* showed a preference for different *A. thaliana* plants, including the wild type and several mutant plants. Female *M. quadrilineatus* were found to preferentially oviposit on plants that overexpress Sap54 rather than on wild-type plants; however, there was no preference for *ap1*-mutant plants (which produce green flowers) over wild-type plants. This suggests that the presence of green flowers alone does not attract the leafhopper and that Sap54-dependent degradation of multiple MTFs may be required.

In summary, this study identifies AY-WB Sap54 as a novel virulence factor that suppresses floral development by promoting the RAD23-dependent degradation of plant MTFs. This causes infected plants to abandon the production of seed-producing flowers; instead, these plants produce leaf-like flowers and are more attractive to the insect vector. Thus, *Phytoplasma* spp. convert their hosts into sterile 'zombie' plants, which facilitates vector-borne transmission and ultimately aids bacterial propagation.

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> "Sap54 … suppresses floral development by promoting the RAD23-dependent degradation of plant MTFs"

ORIGINAL RESEARCH PAPER MacLean, A. M. *et al.* Phytoplasma effector SAP54 hijacks plant reproduction by degrading MADS-box proteins and promotes insect colonization in a RAD23-dependent manner. *PLoS Biol.* http://dx.doi.org/10.1371/journal.pbio.1001835 (2014)