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Cross-kingdom RNA trafficking and environmental RNAi — nature's blueprint for modern crop protection strategies

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In plants, small RNA (sRNA)-mediated RNA interference (RNAi) is critical for regulating host immunity against bacteria, fungi, oomycetes, viruses, and pests. Similarly, sRNAs from pathogens and pests also play an important role in modulating their virulence. Strikingly, recent evidence supports that some sRNAs can travel between interacting organisms and induce gene silencing in the counter party, a mechanism termed cross-kingdom RNAi. Exploiting this new knowledge, host-induced gene silencing (HIGS) by transgenic expression of pathogen gene-targeting double-stranded (ds)RNA has the potential to become an important disease-control method. To circumvent transgenic approaches, direct application of dsRNAs or sRNAs (environmental RNAi) onto host plants or post-harvest products leads to silencing of the target microbe/pest gene (referred to spray-induced gene silencing, SIGS) and confers efficient disease control. This review summarizes the current understanding of cross-kingdom RNA trafficking and environmental RNAi and how these findings can be developed into novel effective strategies to fight diseases caused by microbial pathogens and pests.

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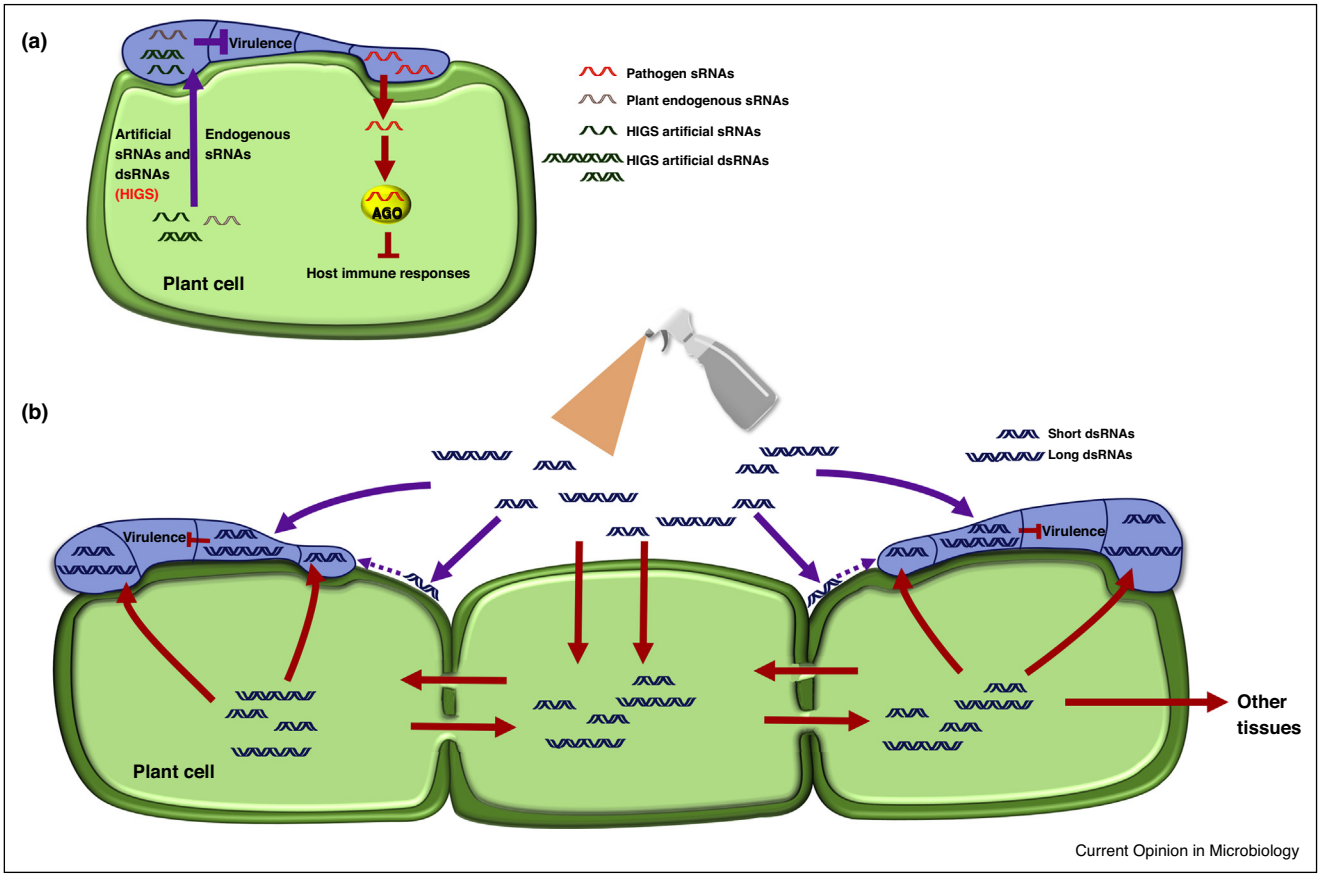
Introduction

To meet the increasing food and energy demands of a fast-growing population, it will be necessary to roughly double crop yields worldwide over the next 40–50 years. Each year, pathogens and pests destroy 20–40% of attainable crop production globally. The demonstration that eukaryotic pathogens and pests are inhibited by small RNAs (sRNAs) targeting their essential and/or pathogenicity genes has raised the possibility that plants can be protected by a new generation of eco-friendly RNA-based fungicides or insecticides, which are highly specific and can be easily adapted to control multiple diseases simultaneously. The novel strategy employs the recent discoveries that sRNAs can move across the cellular boundaries between hosts and interacting pathogens and pests and induce gene silencing in trans, designated 'cross-kingdom RNA interference (RNAi)' [1••,2,3,4••] and that some pathogens and pests are capable of taking up RNAs from the environment, termed 'environmental RNAi' [5••,6]. These mechanisms enable us to successfully control crop diseases by transgene-mediated cross-kingdom RNAi or spray-induced gene silencing (SIGS) that spraying pathogen gene-targeting dsRNAs and sRNAs on plant surfaces to suppress pathogen virulence [6]. We review here the current understanding and application of cross-kingdom RNA trafficking and environmental RNAi.

Pathogen-derived cross-kingdom sRNAs suppressing host immunity

Pathogen-derived sRNAs can move into host cells to suppress host immunity (Figure 1a). The grey mold fungal pathogen *Botrytis cinerea* (*Bc*) produces sRNA effectors, the majority of which derived from clusters within long-terminal repeat (LTR) retrotransposons in the fungal genome, which can migrate into and down-regulate Arabidopsis and tomato genes involved in immunity [1••]. Some sRNA effectors can target multiple host immunity genes to enhance *Bc* pathogenicity. For example, *Bc*-siR37 suppresses host immunity by targeting at least 15 Arabidopsis genes, including WRKY transcription factors, receptor-like kinases, and cell wall-modifying enzymes [7•]. The *Bc* sRNAs utilize the host RNAi machinery by binding to Arabidopsis ARGONAUTE1 (AGO1) to silence host immunity genes [1••,7•]. Consistent with this finding, *Bc* causes less disease symptoms on the Arabidopsis *ago1-27* mutant compared to wild type plants. In addition, the Dicer (DCL) double mutant strain *Bc-dcl1dcl2* can no longer produce these *Bc*-sRNAs also displays much reduced pathogenicity on various plant species [1••,5••], indicating that sRNA effectors are essential for *Bc's* pathogenicity. Similarly, the *ago1-27* mutant is more resistant to the pathogenic ascomycete *Verticillium dahlia* (*Vd*), which causes *Verticillium* wilt

Figure 1



Cross-kingdom RNA trafficking and spray-induced gene silencing for plant protection against eukaryotic pathogens. **(a)** Cross-kingdom RNA transfer and gene silencing in a plant and an interacting pathogen. Plant pathogens deliver sRNAs into host plant cells, where they suppress host immune responses by hijacking host cell RNAi machinery (red block arrow). Host cells also deliver sRNAs into pathogen cells, either artificial HIGS sRNAs or endogenous sRNAs, to target virulence genes and other essential genes of pathogens (purple block arrow). **(b)** Mechanism of SIGS to counteract pathogen virulence. The sprayed short or long dsRNAs, which target pathogen virulence-related genes, can either translocate directly to the eukaryotic pathogen (purple arrows), via uptake from the plant surface (purple dotted arrows), or indirectly through the host cells (red arrows). These RNAs can also move systemically between cells or to other tissues in the plant, most likely through plasmodesmata and vascular bundles.

disease on many plants [5••]. An RNA immunoprecipitation (RIP) assay showed that *Vd*-sRNAs that have potential host targets are predominantly associated with Arabidopsis AGO1 during infection [5••], suggesting that *Vd* also uses sRNAs to silence host target genes. One of the most destructive pathogens of wheat *Puccinia striiformis* (*Ps*) also delivers sRNAs, such as a novel microRNA-like RNA1 (milR1), into host cells and suppresses wheat *Pathogenesis-related 2* gene in the defense pathway. Silencing of the *Ps* milR1 precursor led to enhanced wheat resistance to the virulent *Ps* isolate [8••]. Cross-kingdom RNA silencing does not necessarily require canonical RNAi machinery in the pathogens or pests. For example, two non-coding RNAs, *OxyS* and *DsrA*, of *Escherichia coli* could enter and affect gene expression and physiology of its host *Caenorhabditis elegans* [9]. Moreover, the protozoan parasite *Trypanosoma cruzi* produces tRNA-derived sRNAs, which contribute to the ability to infect mammalian cells, though *Trypanosoma cruzi* lacks canonical sRNA pathways [10].

Host plant-derived cross-kingdom sRNAs regulate the outcome of microbial attacks

Recent discoveries that animals and plants deliver host sRNAs into interacting microbes to suppress their virulence has created new ideas for practical disease control [5••,6,11,12•,13,14••] (Figure 1a). *Verticillium dahlia* (*Vd*), recovered from infected cotton plants contained 28 miRNAs from cotton, implying that host-derived sRNAs were transmitted into the pathogen during infection [14••]. Two of those cotton miRNAs, miR166 and miR159, target the fungal genes *Ca^{2+}-dependent cysteine protease calpain* (*VdClp-1*) and *Isotrichodermin C-15 hydroxylase* (*VdHiC-15*), respectively. Consistent with host-mediated