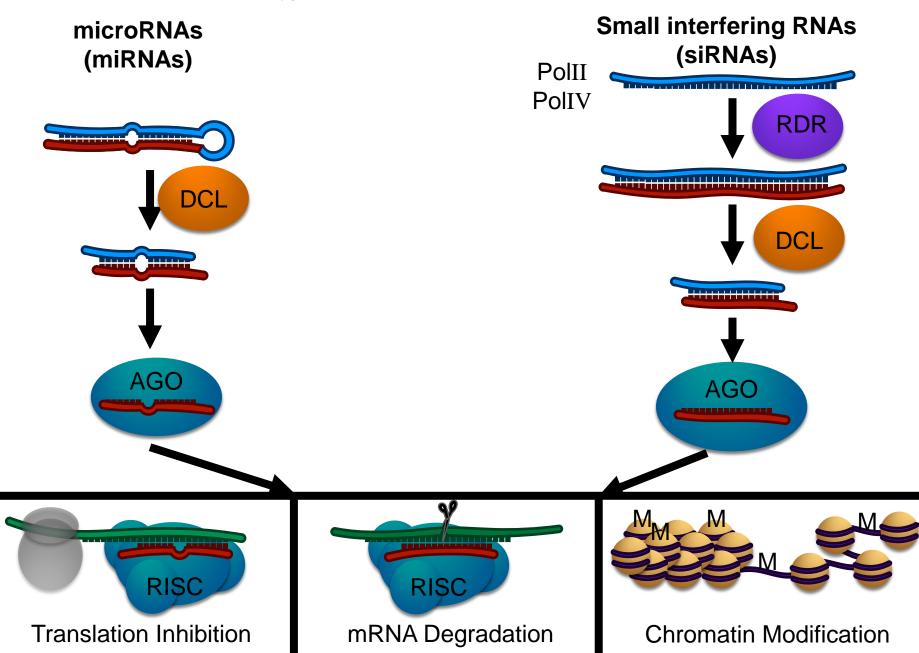
Small RNAs and Cross-Kingdom RNAi in Plant-Microbial Interaction

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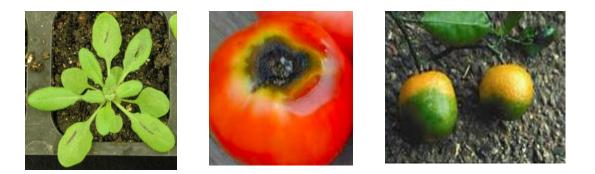
UCREASING UNIVERSITY OF CALIFORNIA

Small RNAs

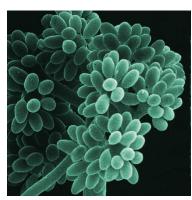


Small RNAs and Cross-Kingdom RNAi in plant-pathogen interactions

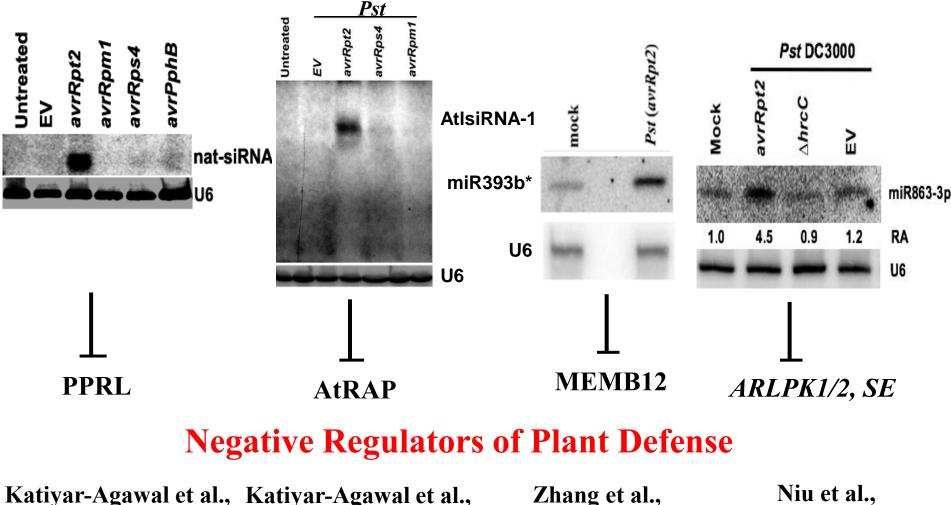
• Host endogenous small RNAs and RNAi machinery



• Pathogen-derived small RNAs and Cross-Kingdom RNAi



Pathogen-induced host small RNAs regulate immunity by suppressing negative regulators of plant defense



Katiyar-Agawal et al.,Zhang et al.,PNAS 2006Genes & Dev 2007Molecular Cell 2011

Niu et al., Nature Comm 2016

Fungal small RNAs in host pathogen interaction

Fungal Pathogen

Botrytis cinerea



Gray mold disease

- Super aggressive Infect more than 200 species including almost all the fruits and vegetables
- Has its own sRNA machinery

Plant Hosts

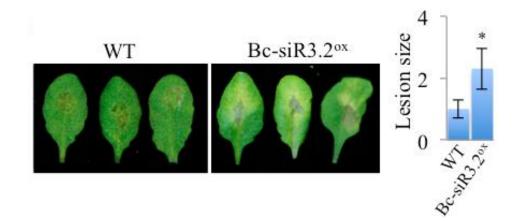


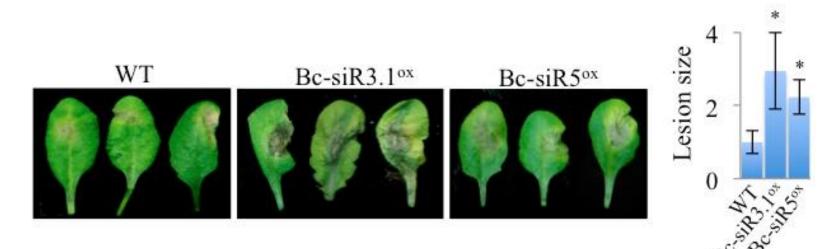
Some *Bc*-sRNAs have predicted targets in both Arabidopsis and tomato

ID		lized read			Alignment	Score	Predicted target gene
ID	Arabidopsis	S.lycopersicun	1 B.cinerea		2	Score	Fredicied target gene
	1,033	5,573	252	Bc-siR3.2	3`UGGAUGUUCUAGGUGUUACAU :	3.0	At MPK2
Bc-siR3				target Bc-siR3.2	5 `AUCAAGAAGAUCCACAAUGUG 3 `UGGAUGUUCUAGGUGUUACAU	4.5	At MPK1
				target Bc-siR3.2	1:1 : 5`AUCAAGAAGAUUCACAAUGUU 3`UGGAUGUUCUAGGUGUUACAU	2.5	01.5.1
				target	5 AUCUAGAAGAUCCAAAAUGUA	3.5	Sl F-box
				Bc-siR3.2	3 GUGGAUGUUCUAGGUGUUACA	4.5	SI MPKKK4
				target	5 CAUUUAAAAGAUCCACCAUGU		
				Bc-siR3.1	3 CGGGUGGAUGUUCUAGGUGUU	2.5	At Aminotransferase-like
				target Bc-siR3.1	5 `AUCCACAUACAAGAUCCACAA 3 `CGGGUGGAUGUUCUAGGUGUU : :	4.0	At Microspore-specific
				target Bc-siR3.1	5 'GUCCCCUUACAACAUCCACAA 3 'CGGGUGGAUGUUCUAGGUGUU	4.5	At PRXIIF
				target	5 GCCUAGCUACAAGAGCCACAU		
				Bc-siR3.1	3 CGGGUGGAUGUUCUAGGUGUU	4.25	SI Autophagy ATG2-like
				target Bc-siR3.1	5 `AUCCACUUUCAAGAUCCACAG 3 `CGGGUGGAUGUUCUAGGUGUU	4.5	SI Vacuolar protein-sorting
				target	5 `ACCCACCUGCAACAUCCACGA		
				Bc-siR5	3`UUCAUAUGUAAGGCUCAGUUU	4.0	At Unknown
Bc-siR5	1,708	3,741	889	target Bc-siR5	5`UAGGAAACUUUCCGAGUCAAA 3`UUCAUAUGUAAGGCUCAGUUU : : : :	4.0	At Clathrin, heavy-chain
				target Bc-siR5	5 GAGUUUGCAUUCCGGGUCGAA 3 UUCAUAUGUAAGGCUCAGUUU :: : ::	4.25	At Cell wall-associated kinase
				target Bc-siR5	5`GGGUAUACAUUCCGGGUCAGG 3`UUCAUAUGUAAGGCUCAGUUU :	4.5	At MADS transcription factor
				target Bc-siR5	5 GAAUUUACAAUCCGAGUCAAA 3 UUCAUAUGUAAGGCUCAGUUU	4.0	SI TOM34
				target Bc-siR5	5 CAGUAUAGAUUCCGUGUCAAA		
				BC-S1R5 target	3`UUCAUAUGUAAGGCUCAGUUU : : 5`AGGUAGACAUUCUGAGGCAAA	4.5	SI Pentatricopeptide

73 Bc-sRNAs that present in both Arabidopsis- and tomato-infected libraries have predicted host targets in both hosts. (using stringent target prediction criteria)
12 out of 73 sRNAs have at least 2 targets belong to the same gene family, such as MPKs, NLRs, F-box proteins, WD-40, RLKs, etc. Weiberg et al., Science 342, 118 (2013)

Transgenic plants expressing *Bc*-sRNAs display enhanced disease susceptibility to *B. cinerea*

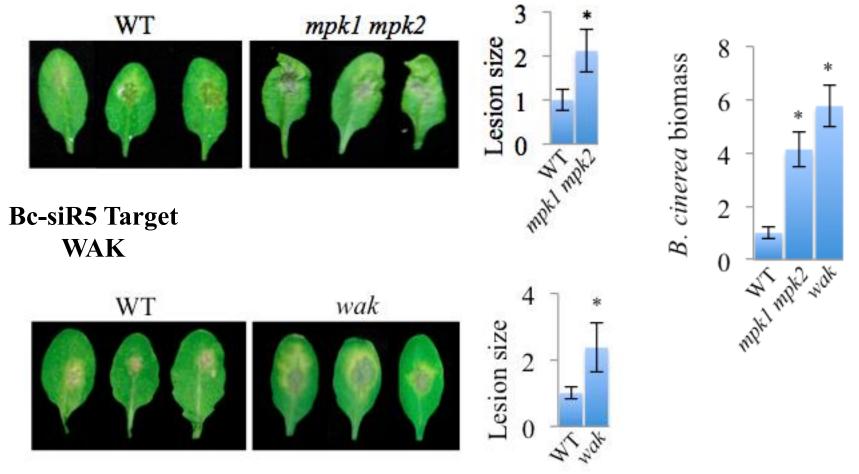




Weiberg et al., Science 342, 118 (2013)

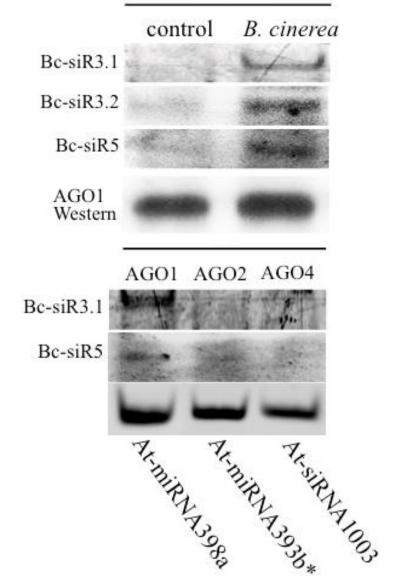
Arabidopsis target genes of *Bc*-sRNAs are important for host defense against *Botrytis*

Bc-siR3.2 Targets MPK1 and MPK2



B. cinerea siRNAs hijack Arabidopsis AGO1 during infection to silence host genes

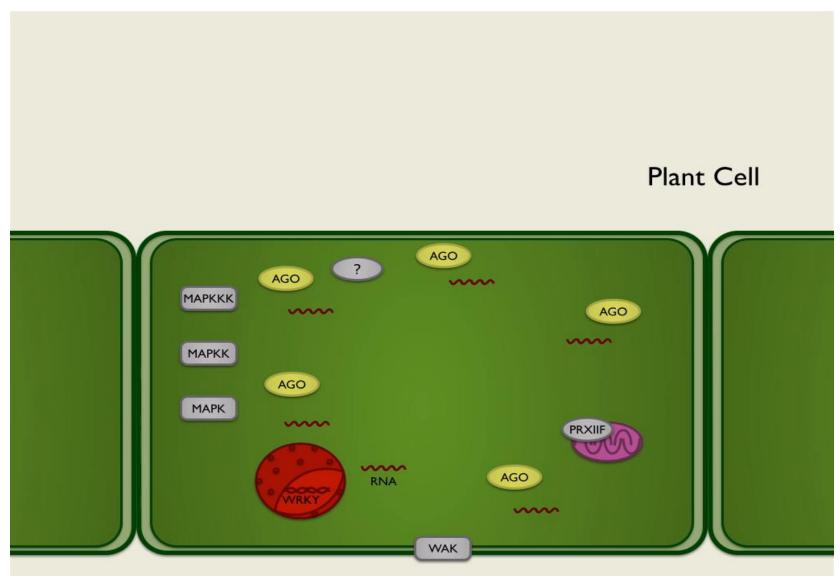
AGO1 IP



Bc-siR3.1 5`UUGUGGAUCUUGUAGGUGGGC Bc-siR3.2 5`UACAUUGUGGAUCUUGUAGGU Bc-siR5 5`UUUGACUCGGAAUGUAUACUU

> AGO-IP sRNA Analysis

Naturally Occurring Cross-Kingdom RNAi (as an Advanced Virulence Mechanism)



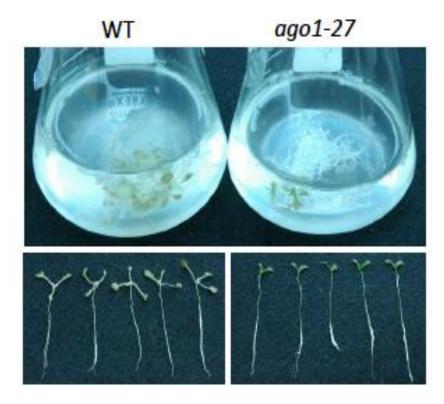
Is such cross-kingdom RNAi virulence mechanism present in other eukaryotic pathogens?

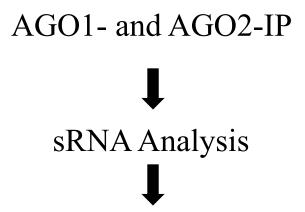
Another fungal plant pathogen, *Verticllium dahliae*, also depends on AGO1 function for its pathogenicity.

J Exp Bot. 2009;60(2):591-602. doi: 10.1093/jxb/ern306. Epub 2008 Dec 19.

RNA silencing is required for Arabidopsis defence against Verticillium wilt disease.

Ellendorff U¹, Fradin EF, de Jonge R, Thomma BP.





We found more than 135 Vd-sRNAs have At Targets in AGO1-IP fraction, vs only 15 in AGO2-IP fraction

(using 100 rpm and 10 fold enrichment as cutoff)

Similar Findings in Animal Systems

Nat Commun. 2014 Nov 25;5:5488. doi: 10.1038/ncomms6488.

Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity.

Buck AH¹, Coakley G¹, Simbari F¹, McSorley HJ¹, Quintana JF¹, Le Bihan T², Kumar S³, Abreu-Goodger C⁴, Lear M¹, Harcus Y¹, Ceroni A⁵, Babayan SA⁶, Blaxter M⁷, Ivens A⁶, Maizels RM¹.

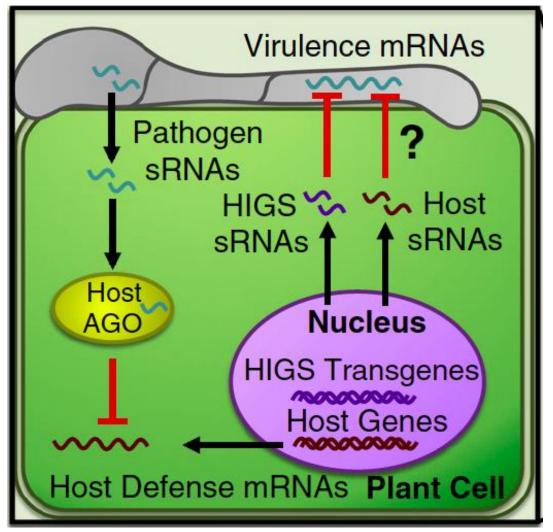
Author information

Table 2 Litomosoides sigmodontis-derived miRNAs foun	d in
mouse serum.	

Name	Mature sequence	Number of reads (infected)
miR-100a	UACCCGUAGCUCCGAAUAUGUGU	479
miR-86	UAAGUGAAUGCUUUGCCACAGUCU	57
Bantam-a	UGAGAUCAUUGUGAAAGCUAUU	45
Bantam-b	UGAGAUCACGUUACAUCCGCCU	45
miR-100b	AACCCGUAGUUUCGAACAUGUGU	40
miR-71	UGAAAGACAUGGGUAGUGAGACG	32
miR-100c	AACCCGUAGAAUUGAAAUCGUGU	22
miR-50-5p	UGAUAUGUCUGAUAUUCUUGGGUU	10
miR-34-5p	UGGCAGUGUGGUUAGCUGGUUGU	8
miR-263/183	AAUGGCACUAGAUGAAUUCACGG	7
Bantam-c	UGAGAUCAUGCCACAUCCGUCU	4
miR-50-3p	CCAGCAUCUCAGACGUAUCGGC	3
miR-153	UUGCAUAGUCACAAAAGUGAUG	3
miR-87-5p	CGCCUGGGACUUCGACUCAACCU	2
miR-2	UAUCACAGCCAGCUUUGAUGU	2
miR-5866	UUACCAUGUUGAUCGAUCUCC	2

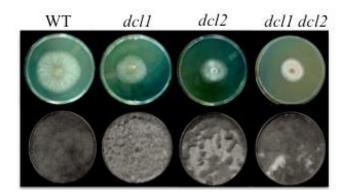
These parasite small RNAs suppress host genes involved in inflammation and immunity, including the receptor for the alarmin IL-33 and a key regulator of mitogen-activated protein kinase (MAPK) signaling

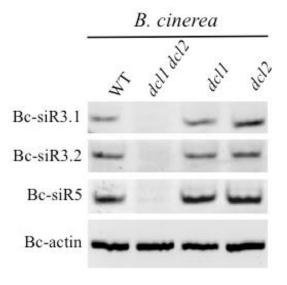
Can we develop an efficient way to control pathogens using small RNA effectors?

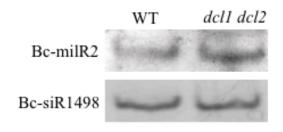


Is cross-kingdom RNAi bidirectional?

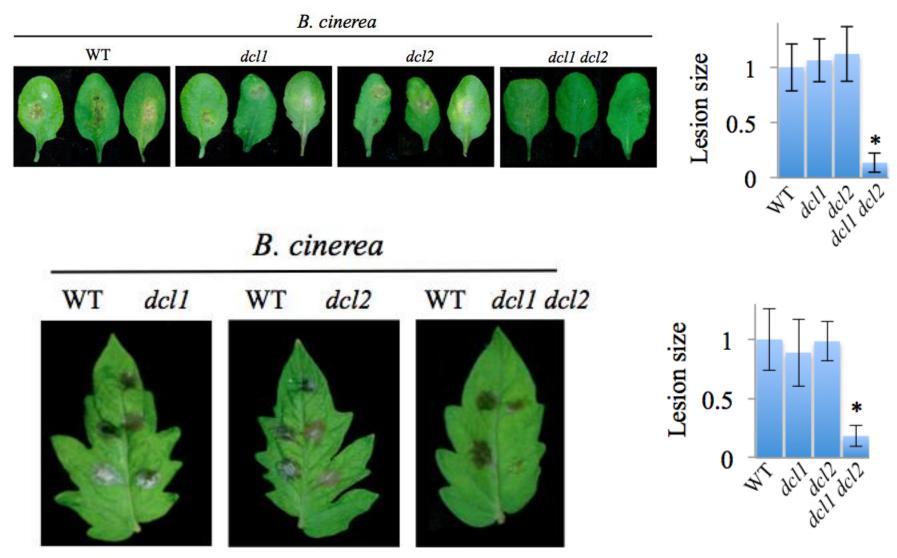
Bc-sRNA effectors are dependent on *B. cinerea* **DCLs**



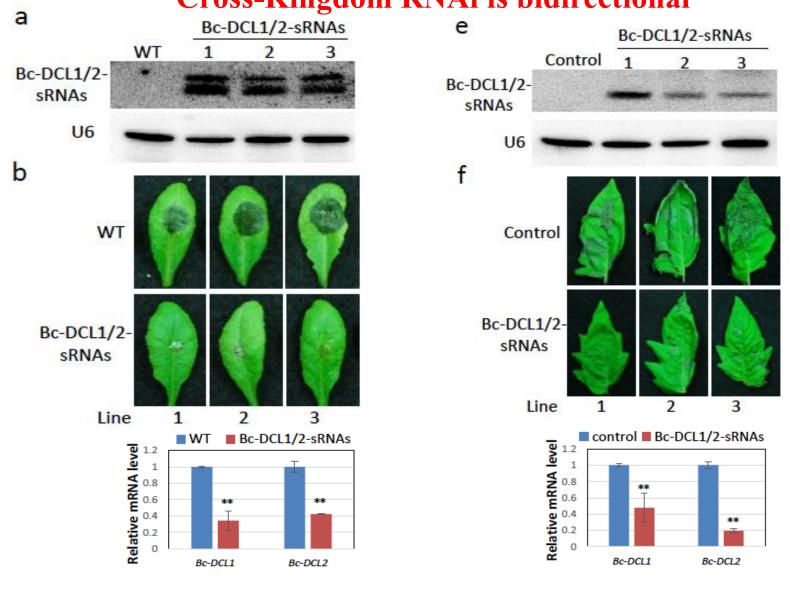




B. cinerea DCLs are essential for its pathogenicity



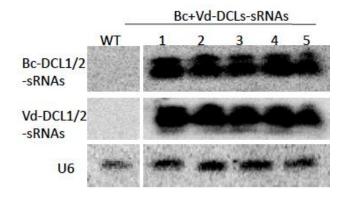
Plants expressing Bc-DCL1/2 targeting sRNAs are more resistance to *B. cinerea* infection Cross-Kingdom RNAi is bidirectional



Cross-Kingdom RNAi-based disease control strategy allows targeting multiple pathogens

WT

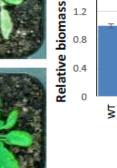
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Verticillium resistance





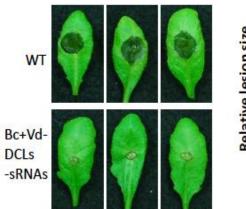


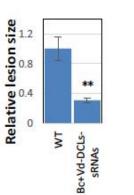
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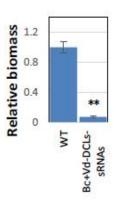
Т

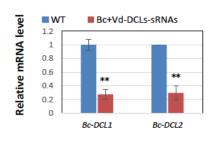
Bc+Vd-DCLssRNAs

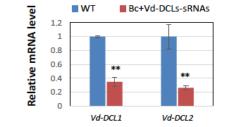
Botrytis resistance













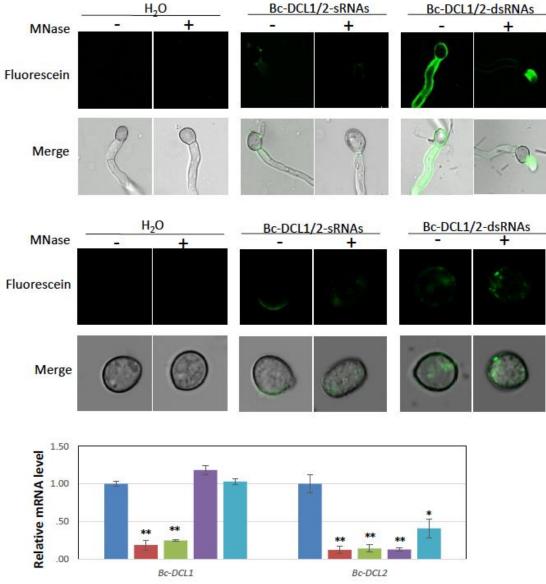


Translocation of Sickle Cell Erythrocyte MicroRNAs into *Plasmodium falciparum* Inhibits Parasite Translation and Contributes to Malaria Resistance

Gregory LaMonte,^{1,2,10,11} Nisha Philip,^{1,2,11,12} Joseph Reardon,² Joshua R. Lacsina,^{3,9} William Majoros,¹ Lesley Chapman,^{1,2} Courtney D. Thornburg,⁴ Marilyn J. Telen,⁵ Uwe Ohler,^{1,6,7} Christopher V. Nicchitta,^{3,9} Timothy Haystead,⁸ and Jen-Tsan Chi^{1,2,*}

miR451 and let-7i are highly enriched in hemoglobin allele (HbS) heterozygous and homozygous erythrocytes, which contributes to resistance against P. falciparum.

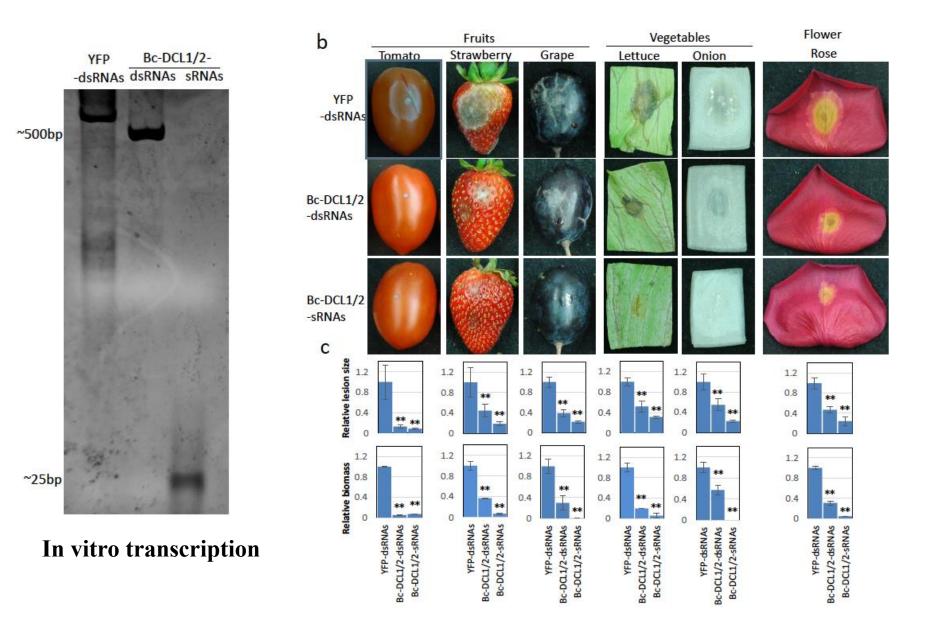
B. cinerea could uptake RNAs from environment



 WATER
 Bc-DCL1/2-sRNAs
 Bc-DCL1/2-dsRNAs

 Bc-DCL2-sRNAs
 Bc-DCL2-dsRNAs

Spraying Bc-DCL1/2 dsRNAs or siRNAs inhibits infection

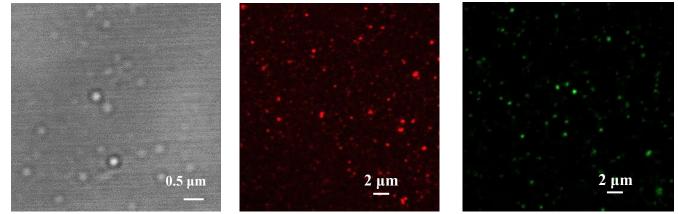


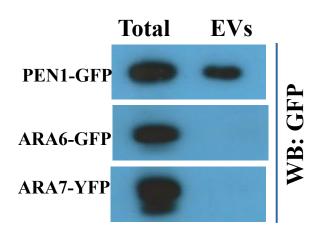
Plant and fungal sRNAs are present in extracellular vesicles

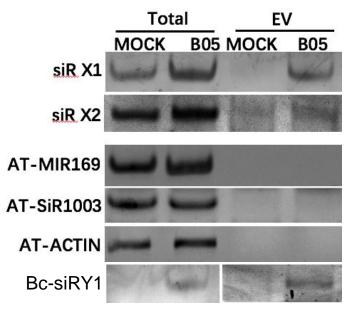
Bright field

FM-64 staining

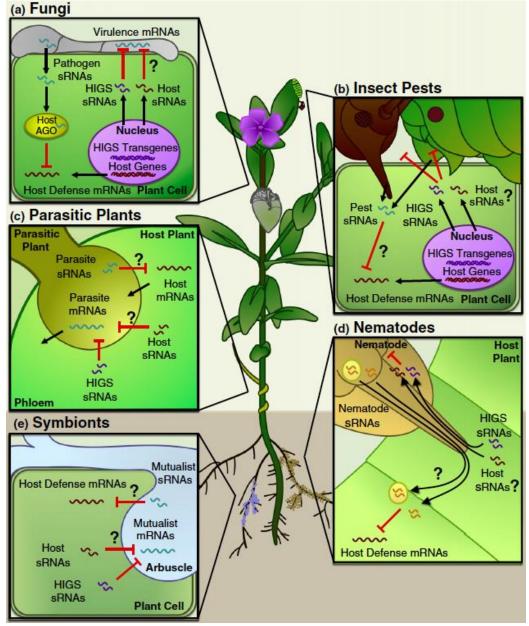
35S:PEN1-GFP





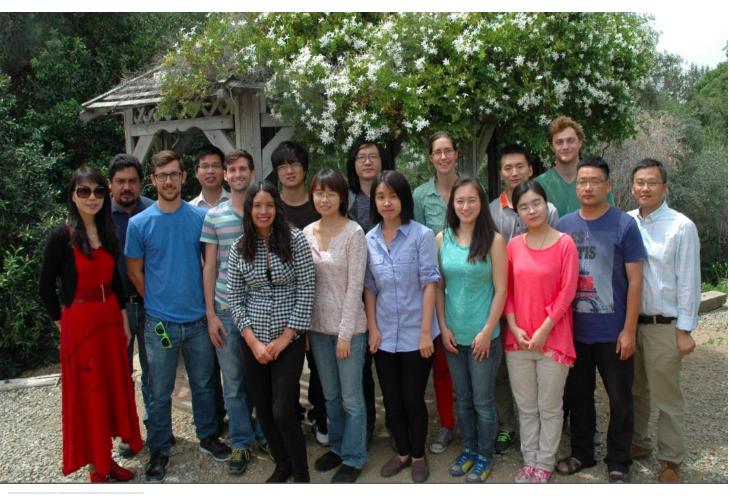


Cross-kingdom RNAi in host-pathogen/pest interaction



Weiberg et al., Curr. Opin. in Biotech. 2015

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