Application of oral RNA Interference in Agricultural Pest Control

朱培雯 纪小小 苏祥彬

2023-12-28

Outline

- Overview of oral RNAi in Agricultural Pest Control – 朱培雯
- Mechanisms of oral RNAi in Agricultural Pest Control – 苏祥彬
- Challenges of oral RNAi in Agricultural Pest Control – 纪小小
- Discussion

Overview of oral RNAi in Agricultural Pest Control

朱培雯

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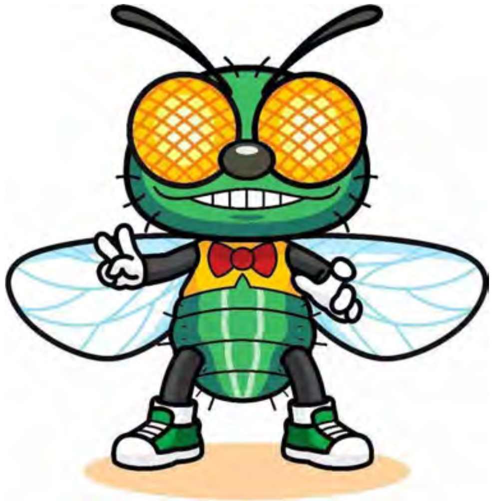
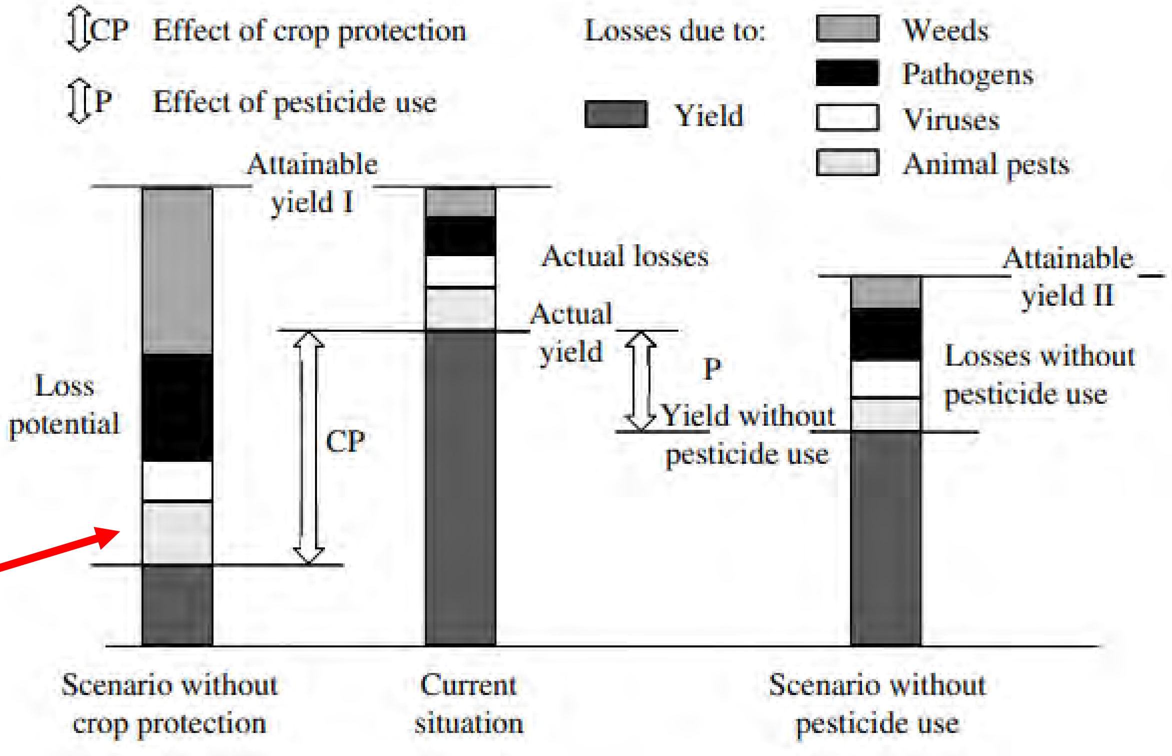
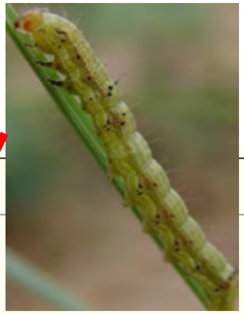
Pests cause crop losses and virus transmission

Common agricultural pests





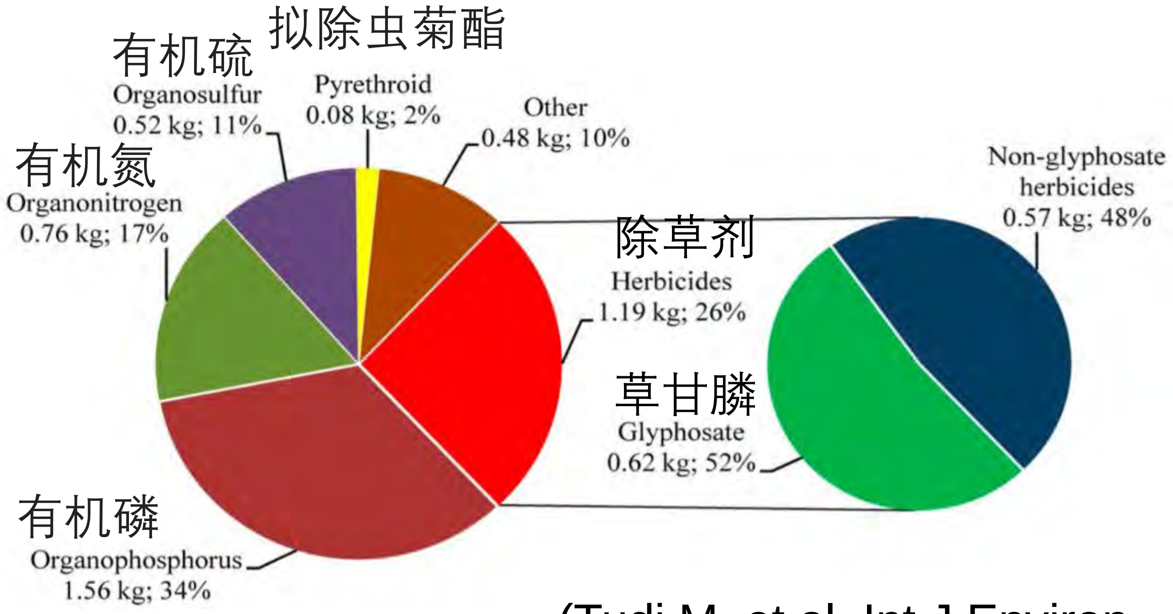
Insect pest	Scientific name	Crop(s)
American bollworm	*Helicoverpa armigera* (Hubner)	Cotton, chickpea, pigeonpea, sunflower, tomato
Whitefly	*Bemisia tabaci* (Gennadius)	Cotton, tobacco
Brown planthopper	*Nilaparvata lugens* (Stal)	Rice
Green leafhopper	*Nephotettix* spp.	Rice
Serpentine leaf miner	*Liriomyza trifolii* (Burgess)	Cotton, tomato, cucurbits, several other vegetables
Fruit fly	*Bactrocera* spp.	Fruits and vegetables
Mealy bugs	Several species	Several field and horticultural crops
Thrips	Several species	Groundnut, cotton, chillies, roses,

(OERKE E-C. The Journal of Agricultural Science, 2006)

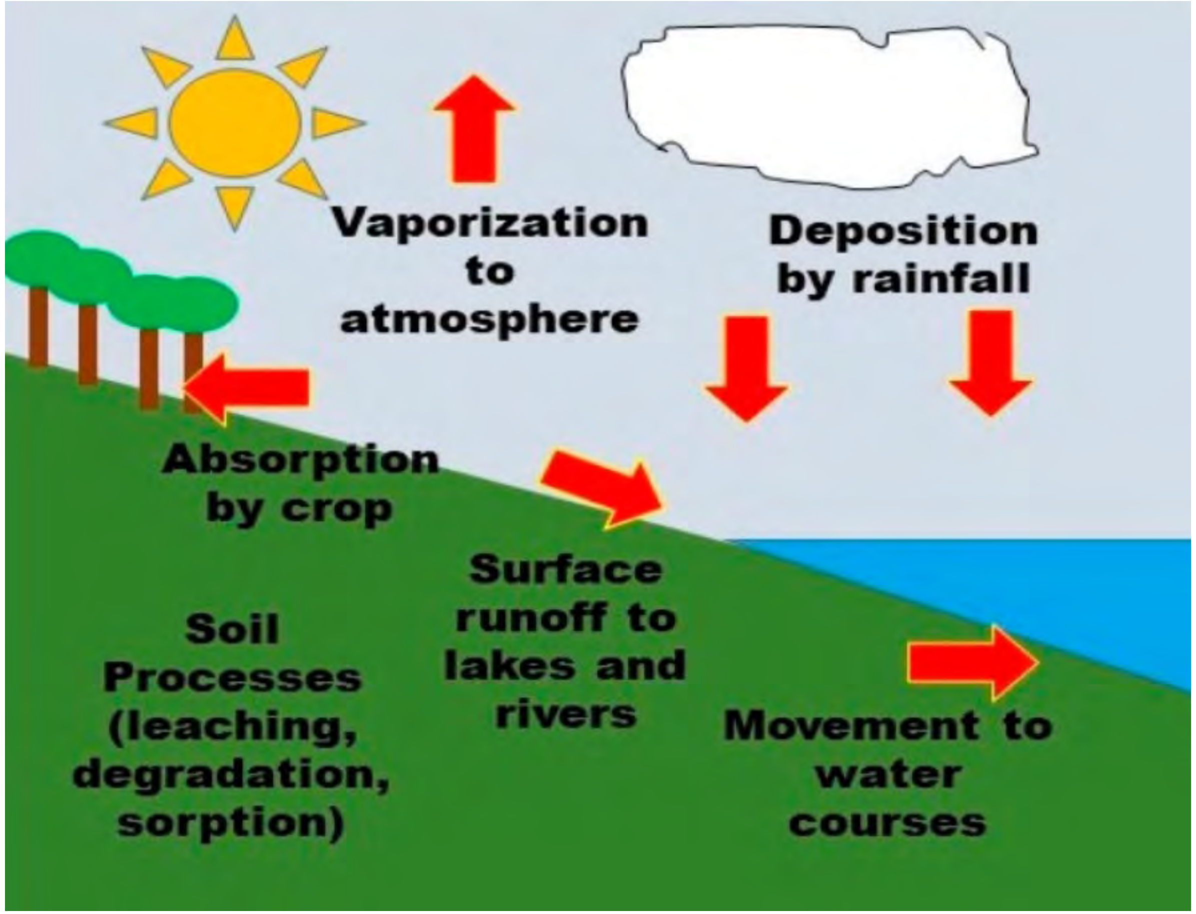
(Mamta B, Rajam MV. Physiol Mol Biol Plants, 2017)

Current pest control strategies and their limitations

① Chemical insecticides



(Tudi M, et al. Int J Environ Res Public Health, 2021)

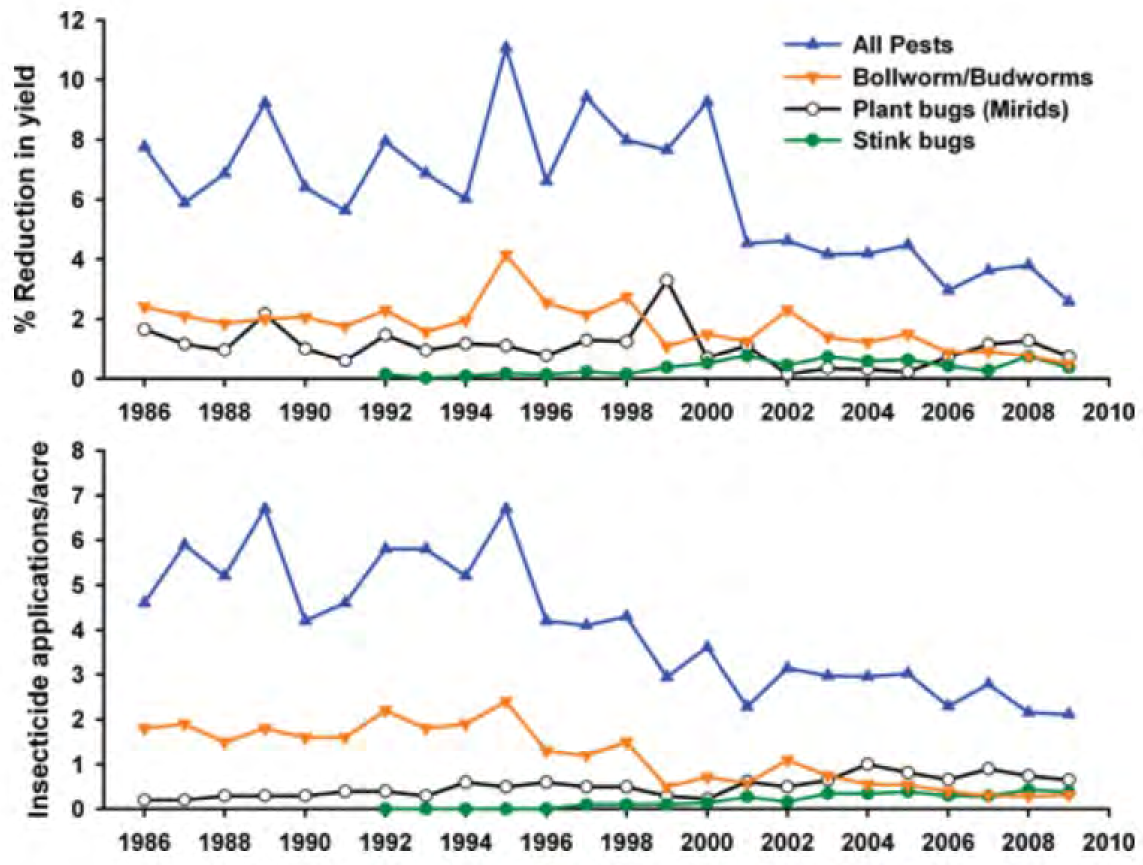


· Organochlorine compounds such as **DDT** have low acute toxicity but show a significant ability to accumulate in tissues and persist in causing long-term damage.

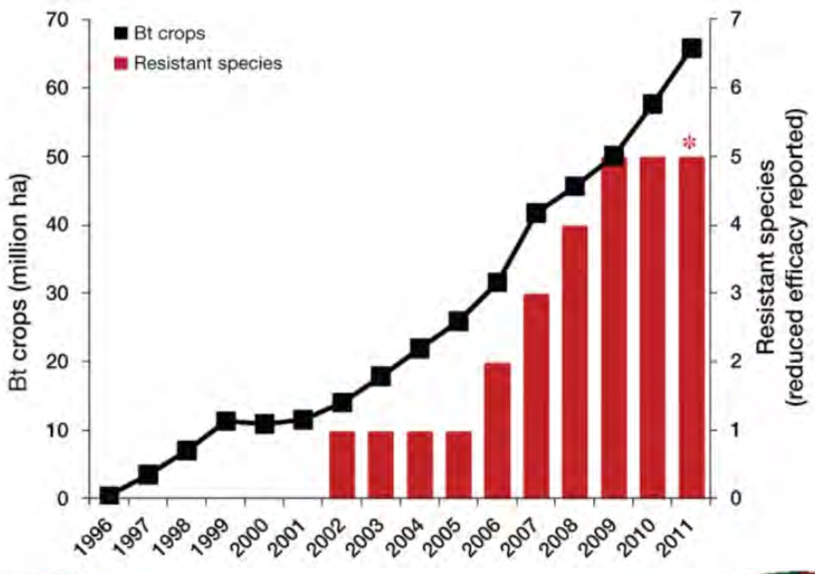
· Organophosphate pesticides are of low persistence, they have appreciable acute toxicity in mammals

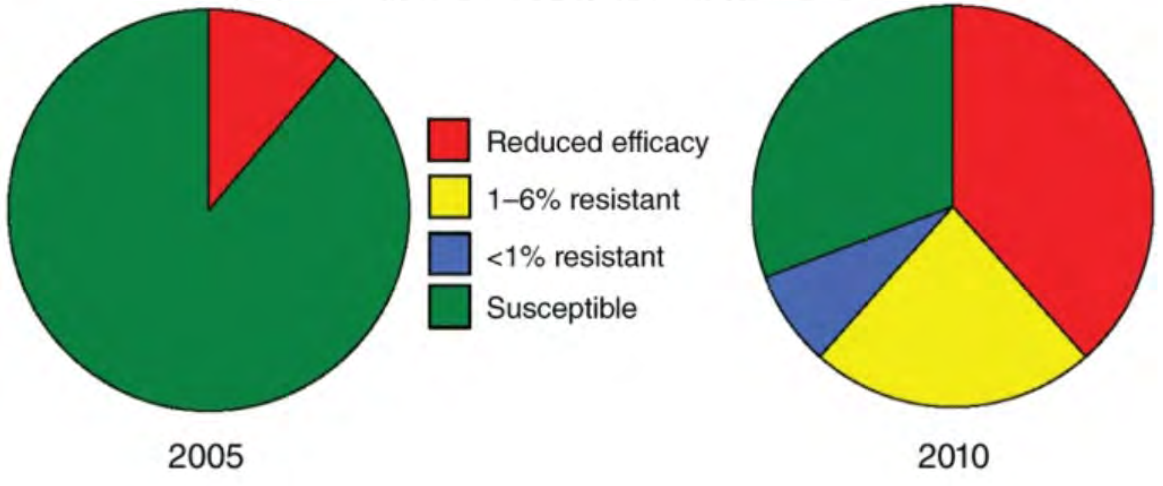
Current pest control strategies and their limitations

② transgenic plants



(Naranjo SE. J Agric Food Chem, 2011)





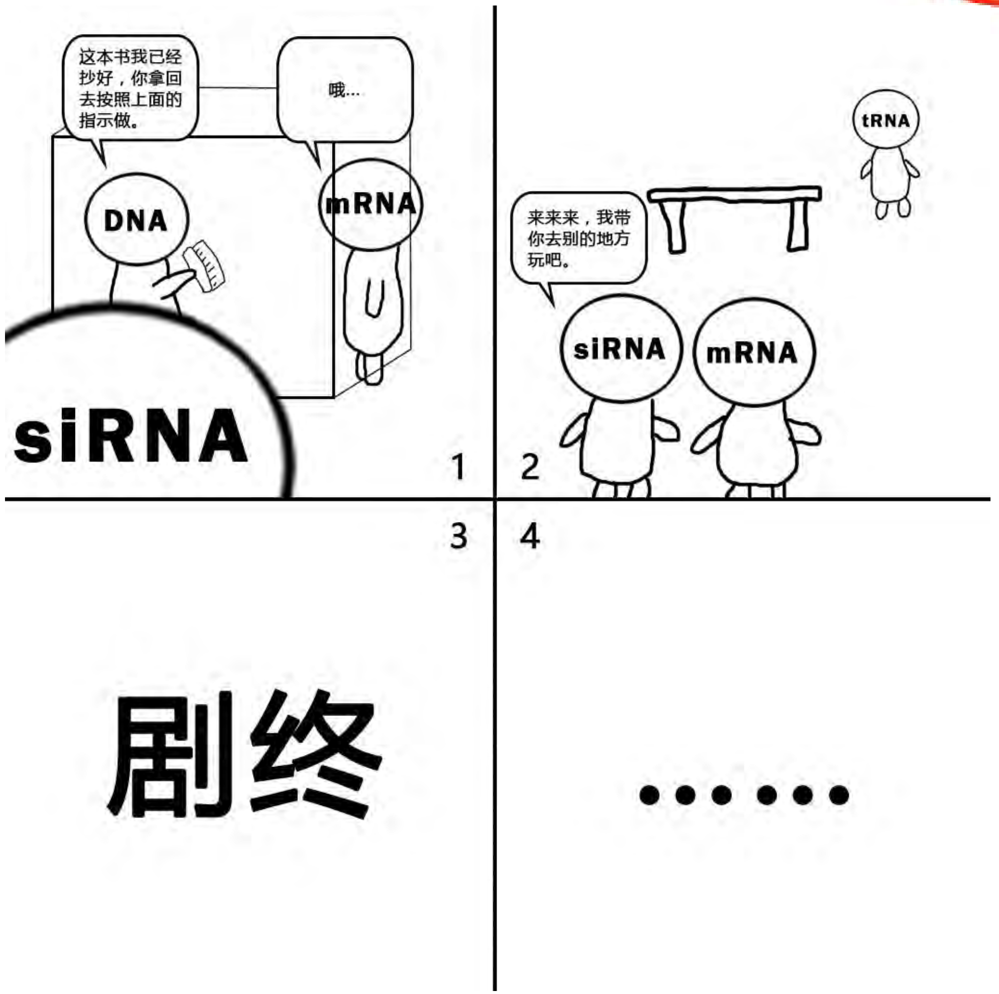
(Tudi M, et al. Int J Environ Res Public Health, 2021)

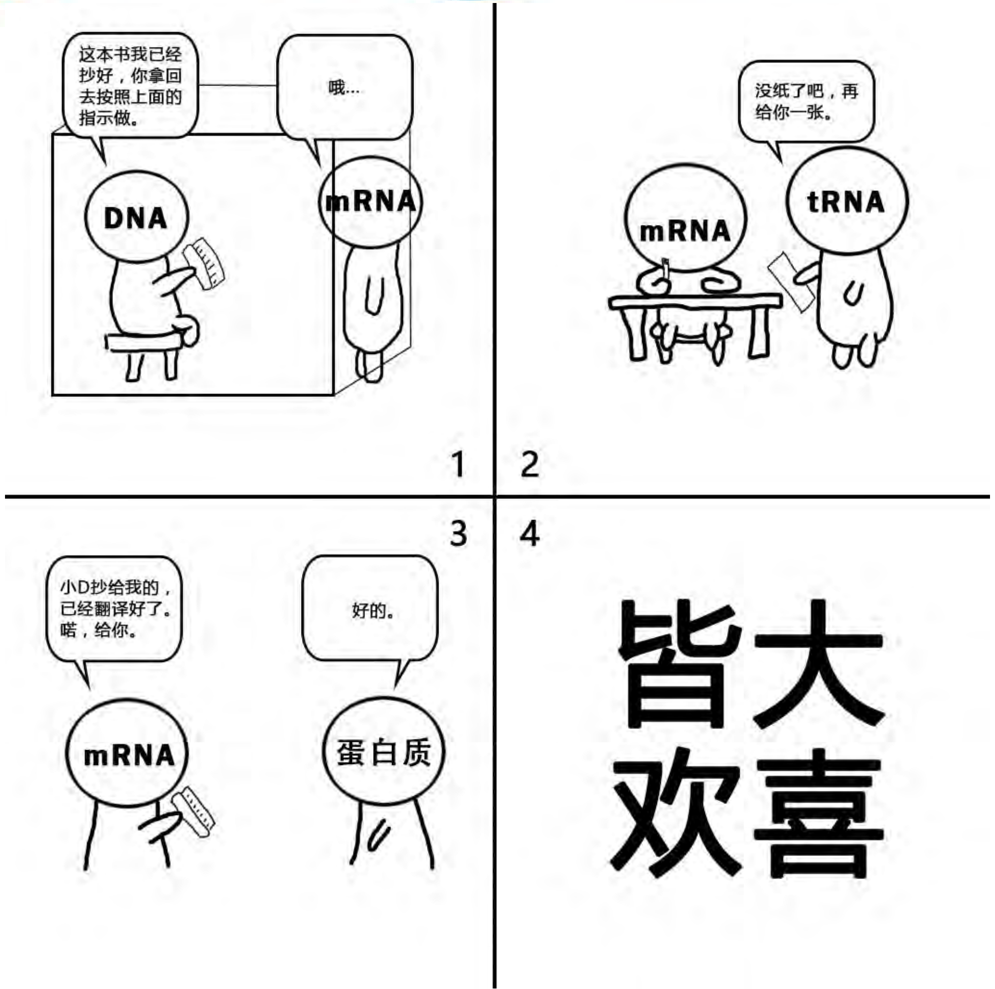
RNAi: next generation pest control strategy

What is it?

How did it come?

How to use it?





The birth of the RNAi revolution

In plants:

The Plant Cell, Vol. 2, 279–289, April 1990 © 1990 American Society of Plant Physiologists

Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes *in trans*

Carolyn Napoli,[1] Christine Lemieux, and Richard Jorgensen[2]
DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, California 94608

RNA interference is already being widely used in basic science as a method to study the function of genes and it may lead to novel therapies in the future.
RNA干扰在基础科学中的广泛应用，使其成为一项基因功能研究的新技术，甚至在未来能成为一种新型的疾病治疗办法。——*瑞典卡罗林斯卡医学院诺贝尔奖评审委员会*

In animals

nature

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nature › letters › article

Letter | Published: 19 February 1998

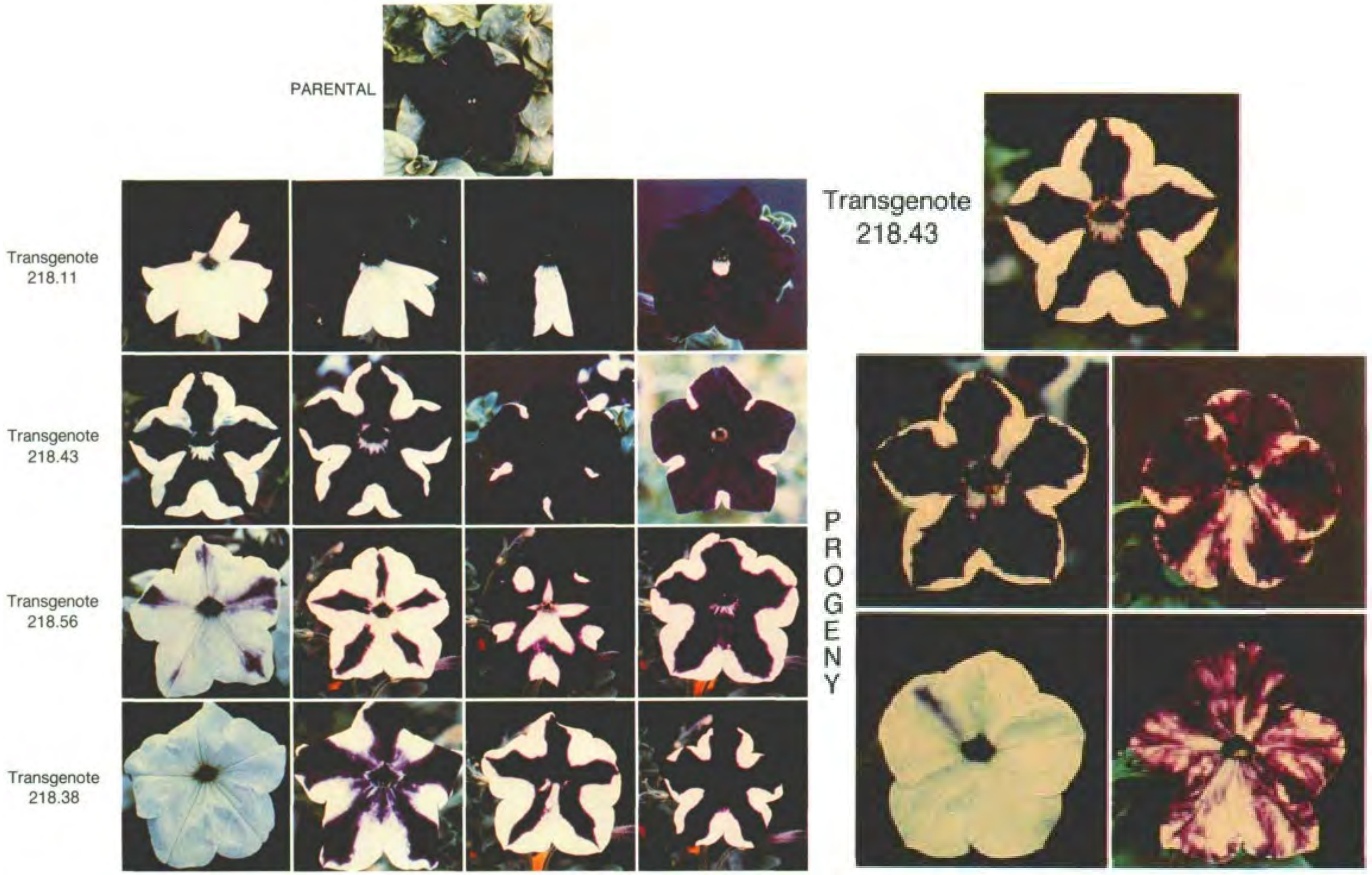
Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

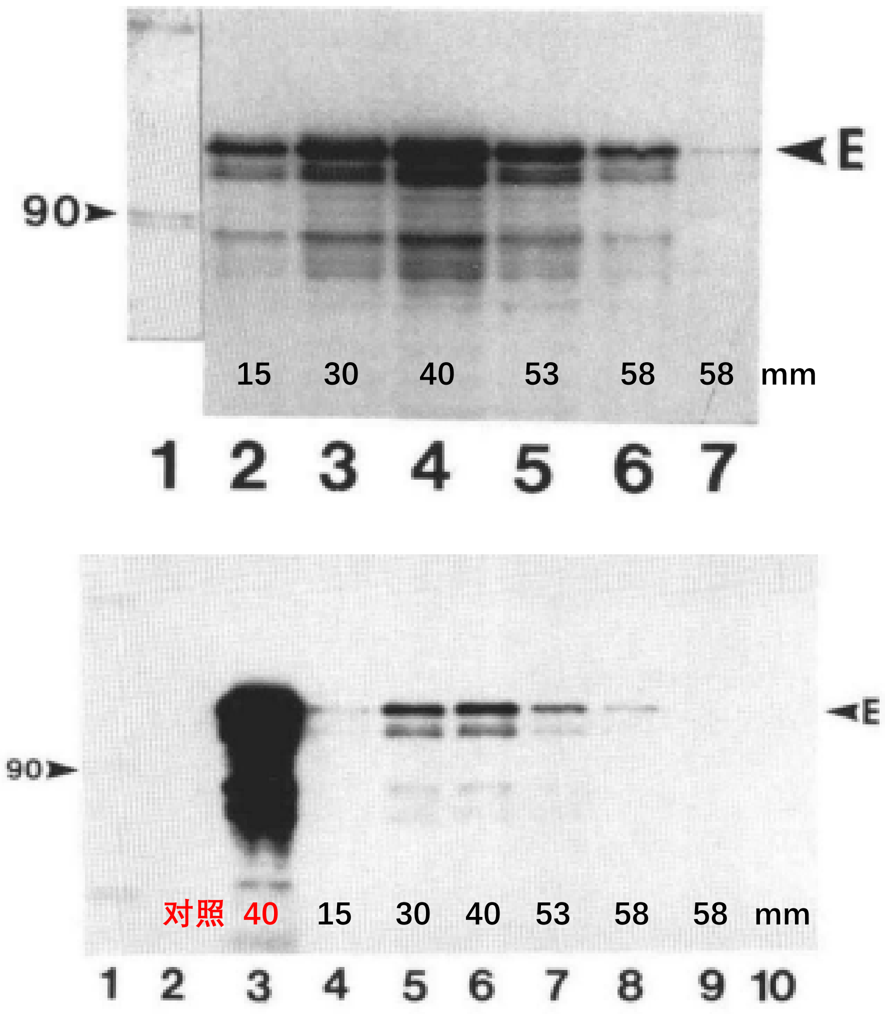
Andrew Fire, SiQun Xu, Mary K. Montgomery, Steven A. Kostas, Samuel E. Driver & Craig C. Mello

Nature **391**, 806–811 (1998) | Cite this article

2006年诺贝尔生理学及医学奖获得者Craig C. Mello（左）和Andrew Fire（右）

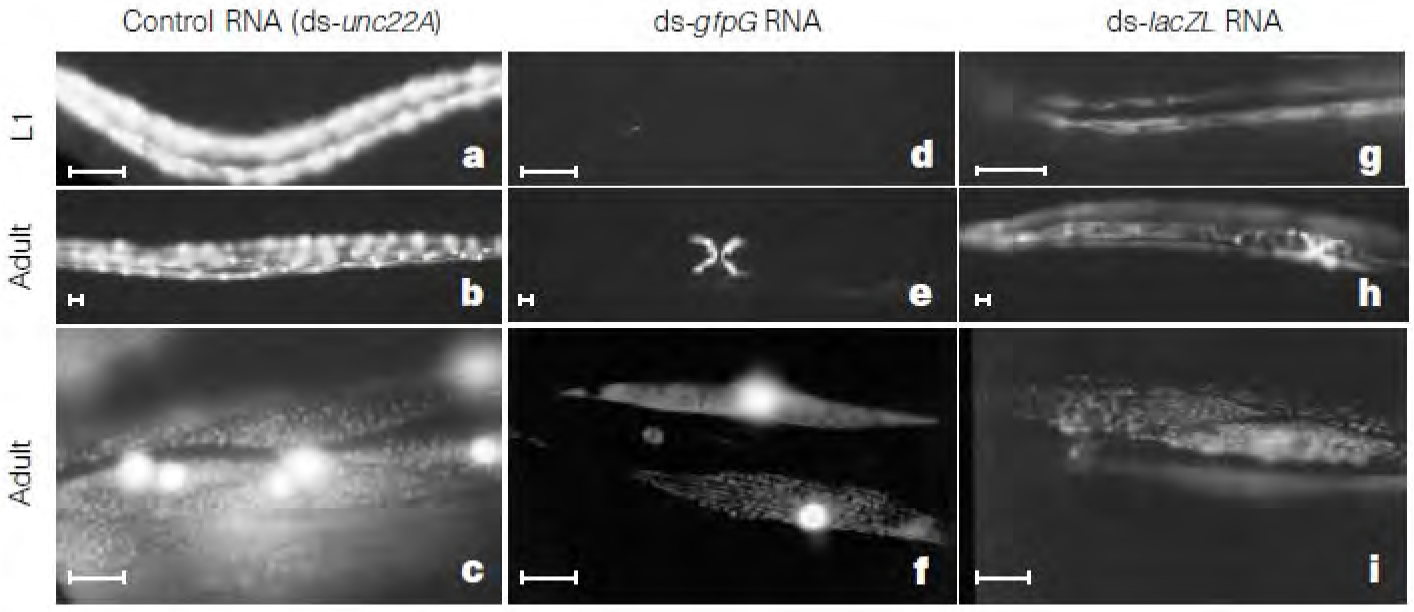
Co-suppression or post transcriptional gene silencing in plants

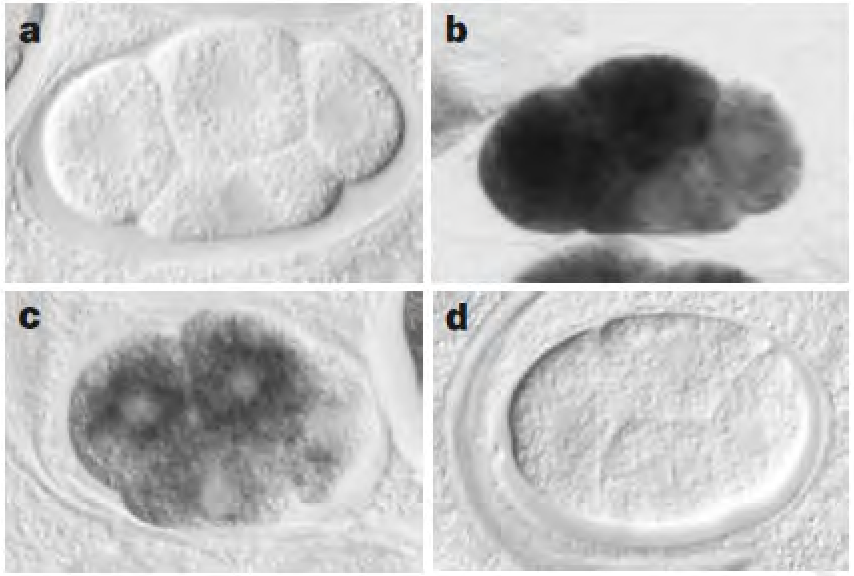




(Carolyn Napoli, et al.
The Plant Cell, 1990)

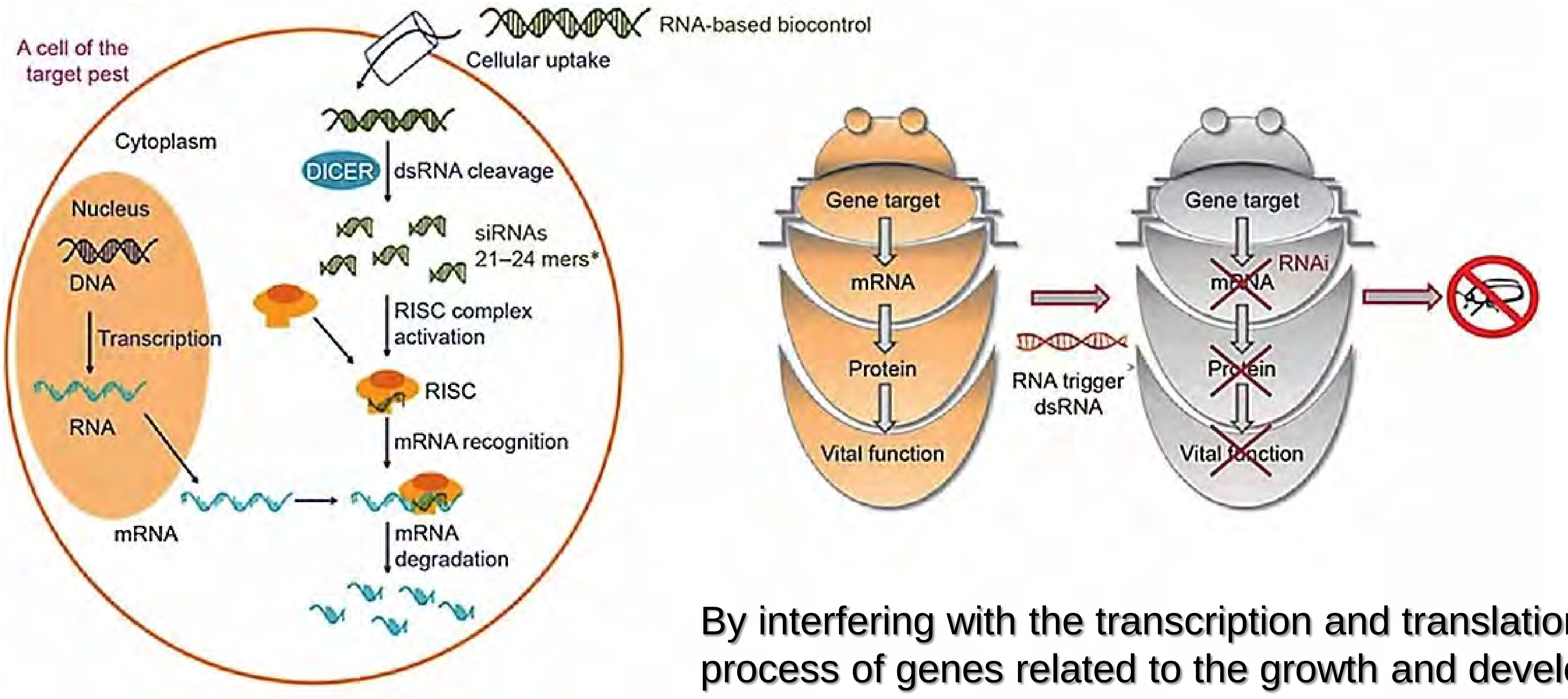
Validation of the Gene Silencing Phenomenon





(Andrew Fire, et al. Nature, 1998)

Types and mechanisms of RNAi



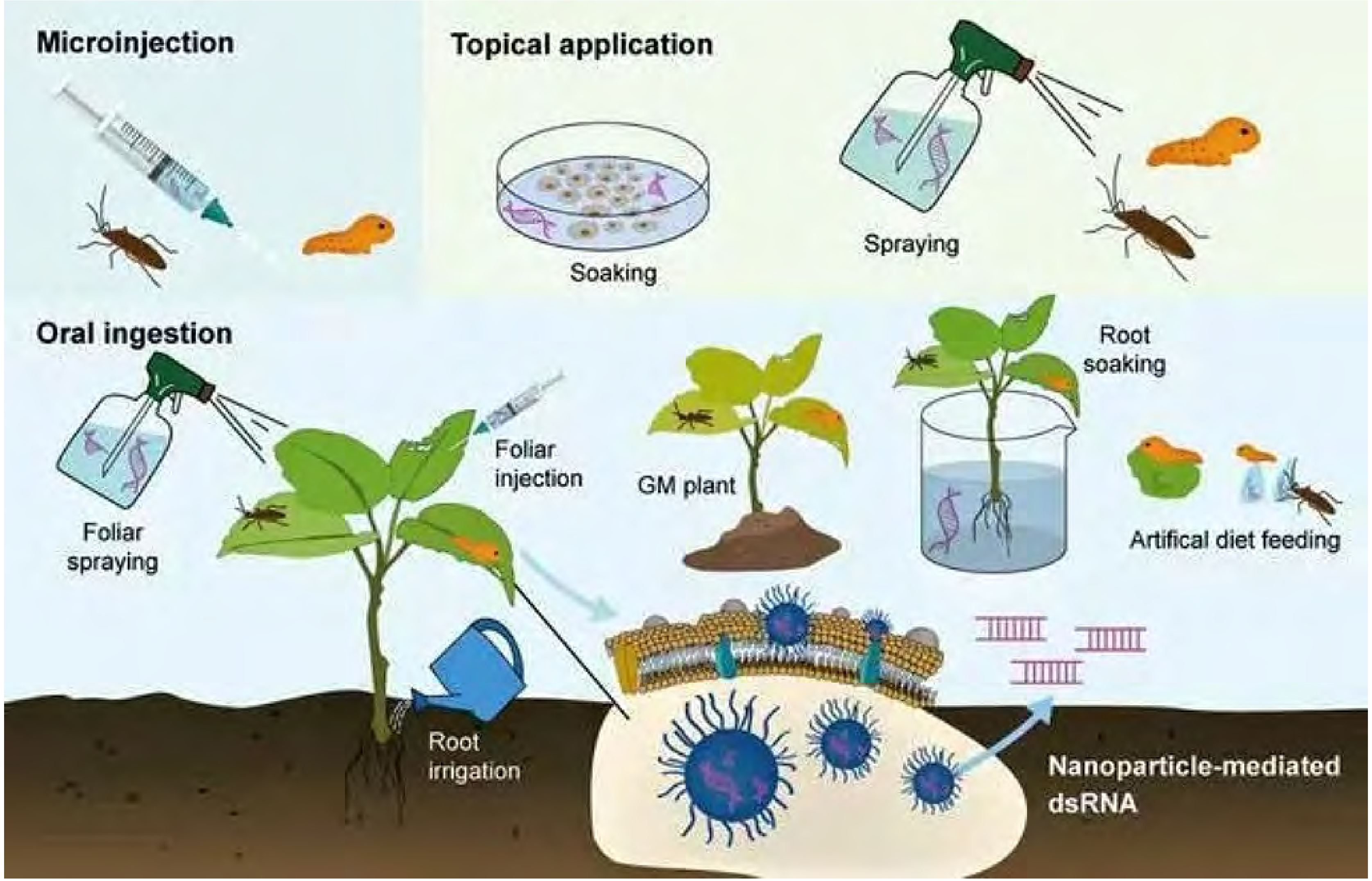
By interfering with the transcription and translation process of genes related to the growth and development of pests, the pest-related genes are silenced, the synthesis of proteins is prevented, and the environmental adaptability or death of pests is reduced, and finally the purpose of pest control is achieved.

The application of RNAi technology in agriculture

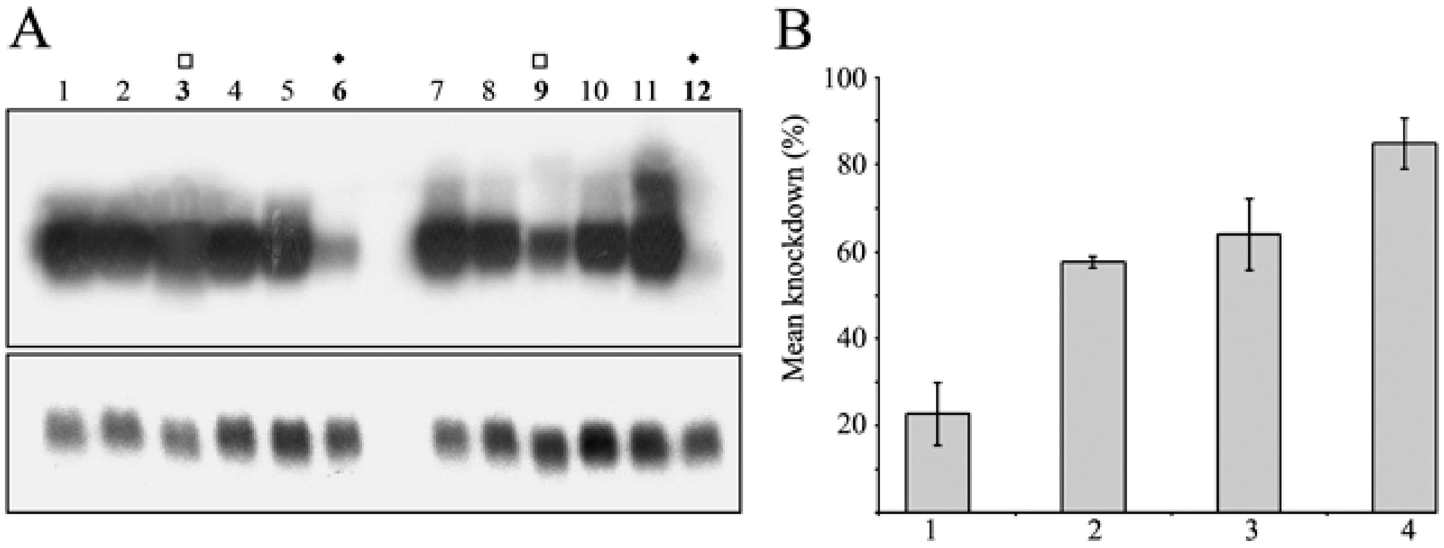
•寄主诱导的基因沉默（host-induced gene silencing，HIGS）

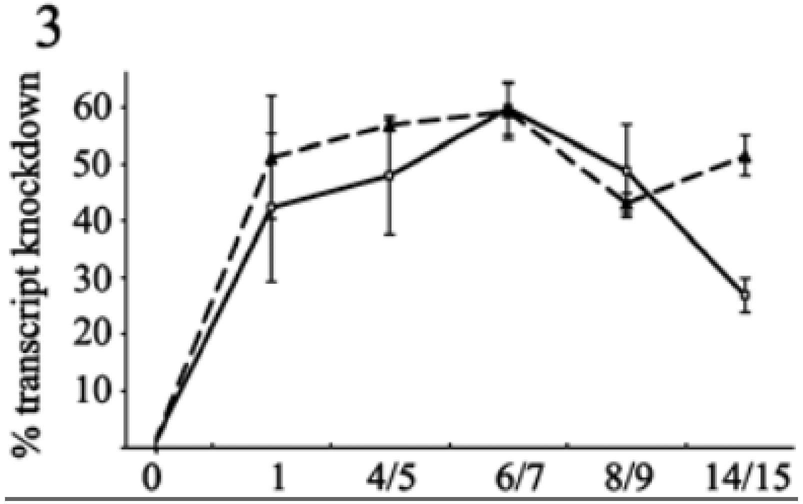
•病毒诱导的基因沉默（virus-induced gene silencing，VIGS）

•外源dsRNA诱导的基因沉默(Exogenous dsRNA-induced gene silencing，EdIGS)



Why do we choose oral RNAi？





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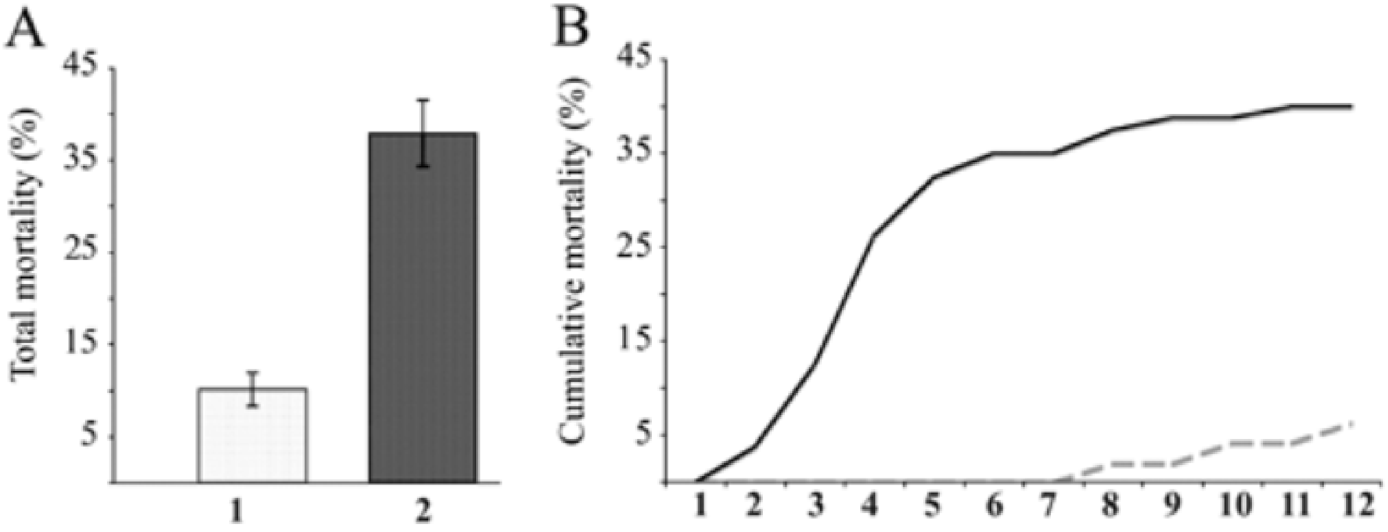


(D. P. Walshe, et al. Insect Molecular Biology, 2009)

A variety of oral delivery strategies

① **direct ingestion**

② **larval soaking** (most likely occurs through ingestion)

③ **nanoparticle-mediated** (uptake with chitosan or other nanoparticles)

④ **microbially-expressed vector systems** (such as bacteria and yeast)

① Direct ingestion

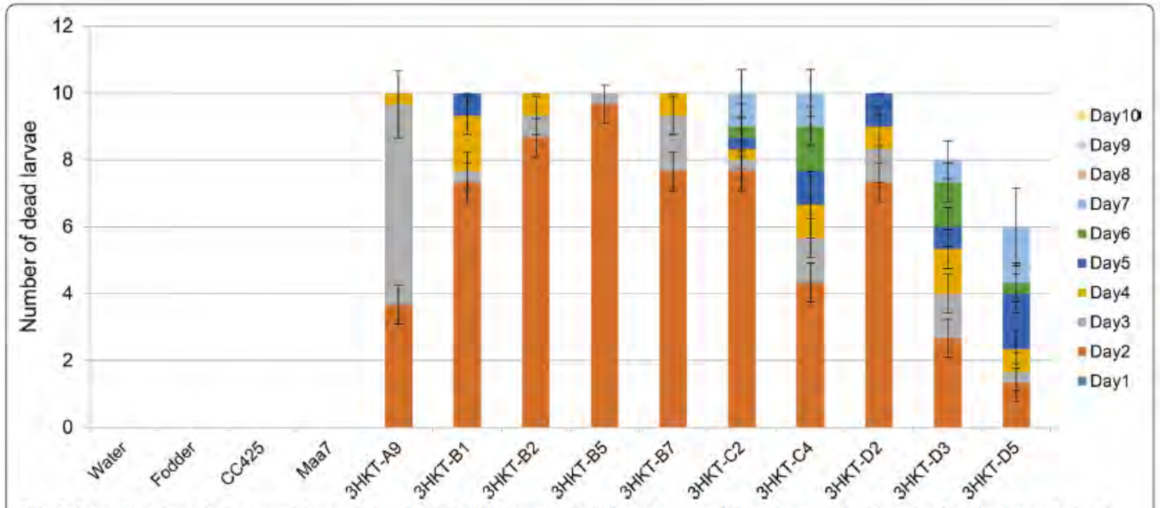
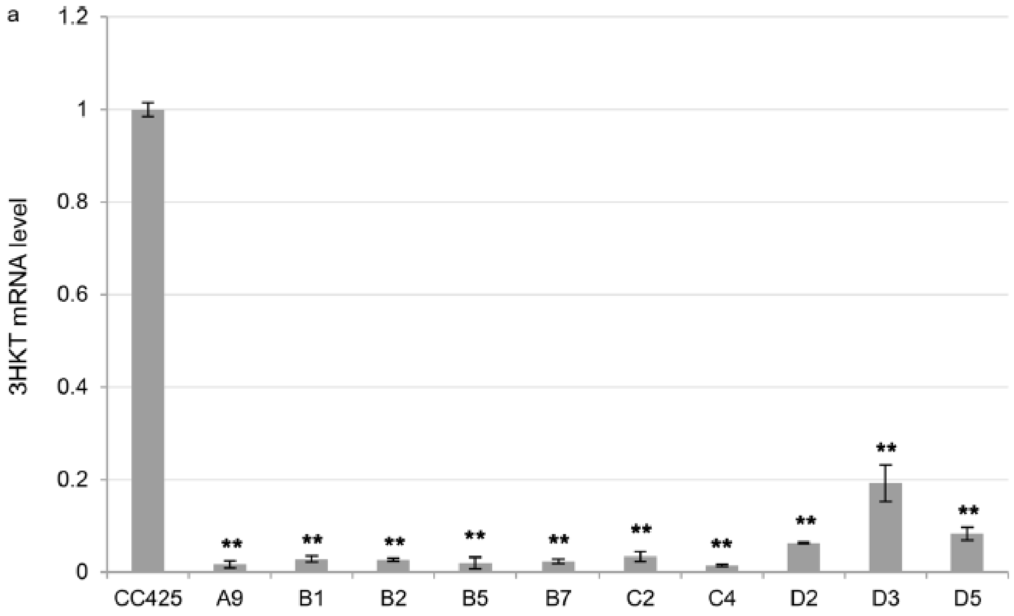
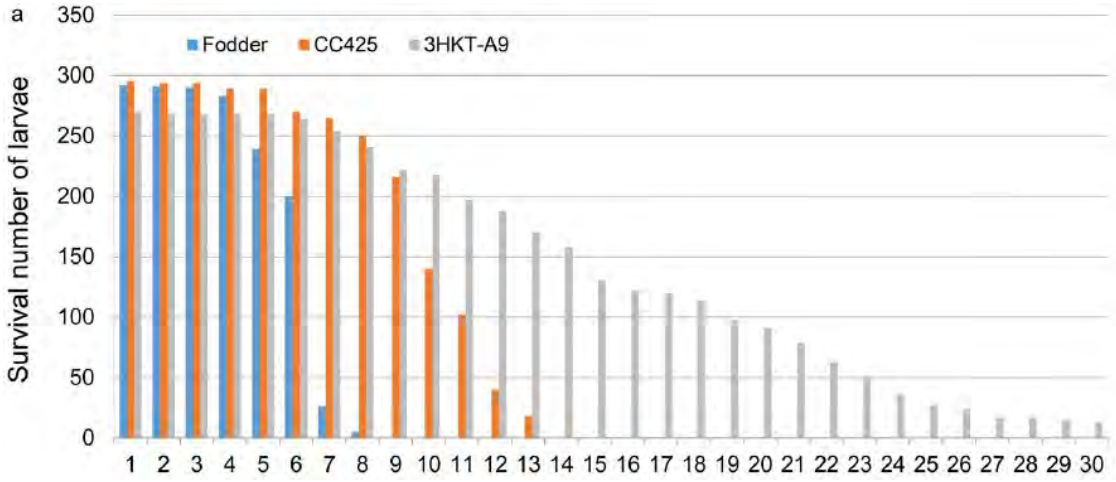
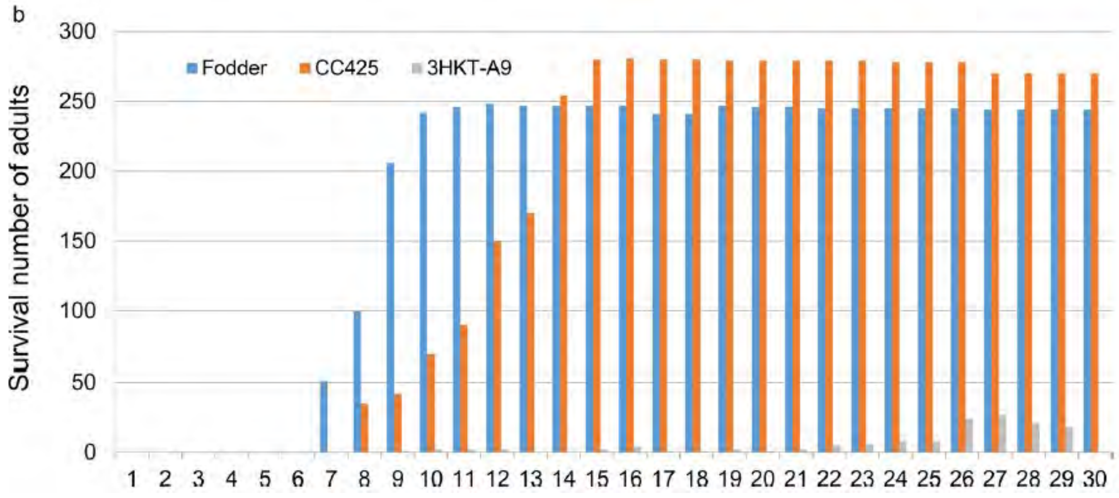


Fig. 1 Mortality rate of *Aedes aegypti* larvae fed with 3HKT RNAi transgenic *Chlamydomonas*. Water: larvae fed with water; Fodder: larvae fed with fodder; CC425: larvae fed with non-transgenic *C. reinharditii* strain CC425; Maa7: larvae fed with empty plasmid Maa7IR/XIR transgenic *C. reinharditii* strain; 3HKT-A9 to 3HKT-D5: larvae fed with Maa7IR/3HKTIR transgenic *C. reinharditii* strains 3HKT-A9 to 3HKT-D5. Note: each treated or control group contained 10 *Aedes* larvae. Experiments were repeated thrice, and average values are reported. Duration: 10 days

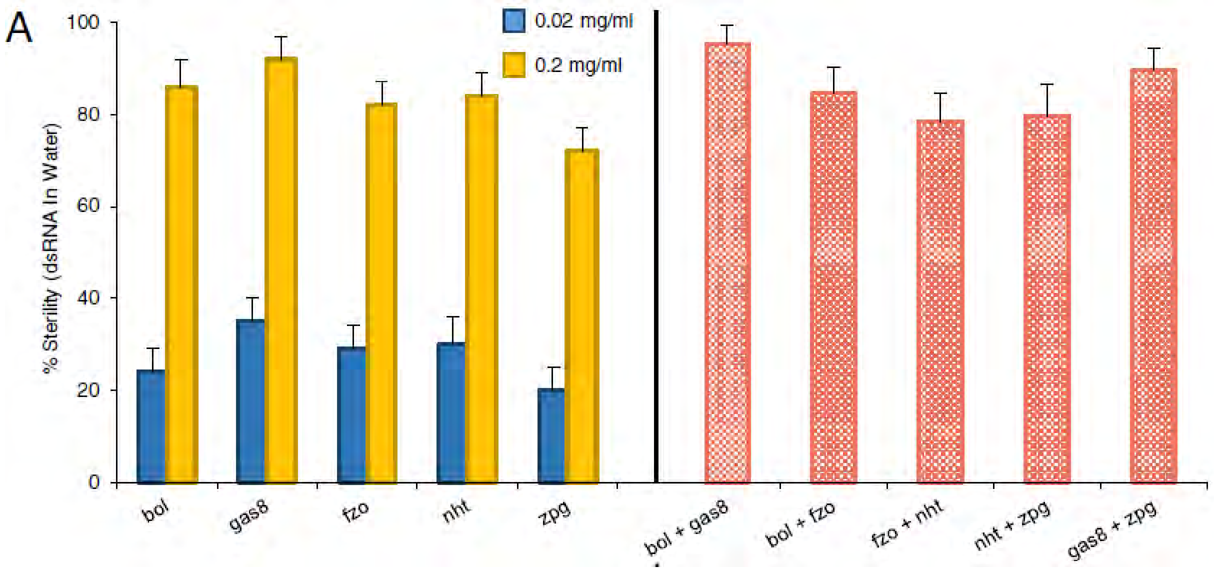


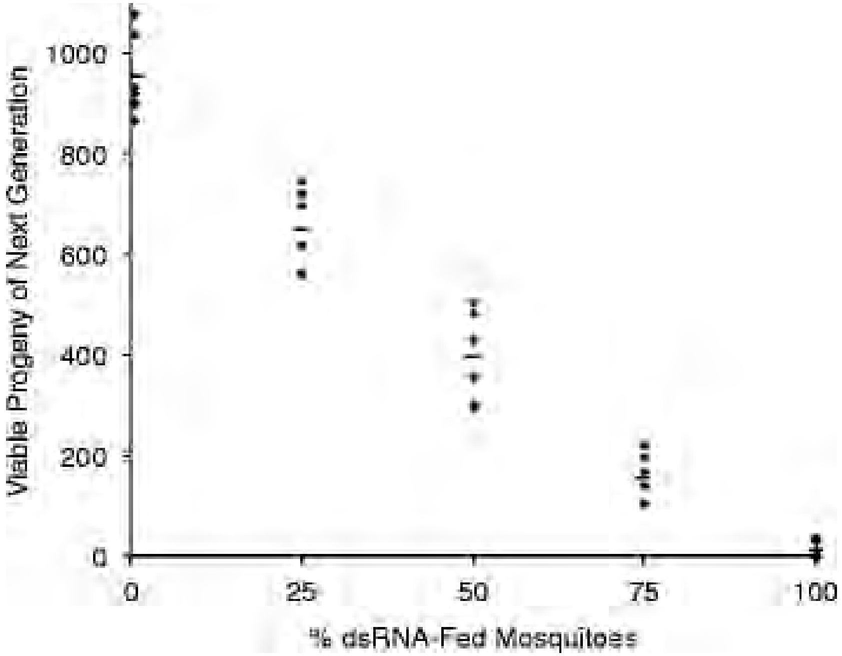




(Xiaowen Fei, et al. Parasit Vectors, 2021)

② Larval soaking

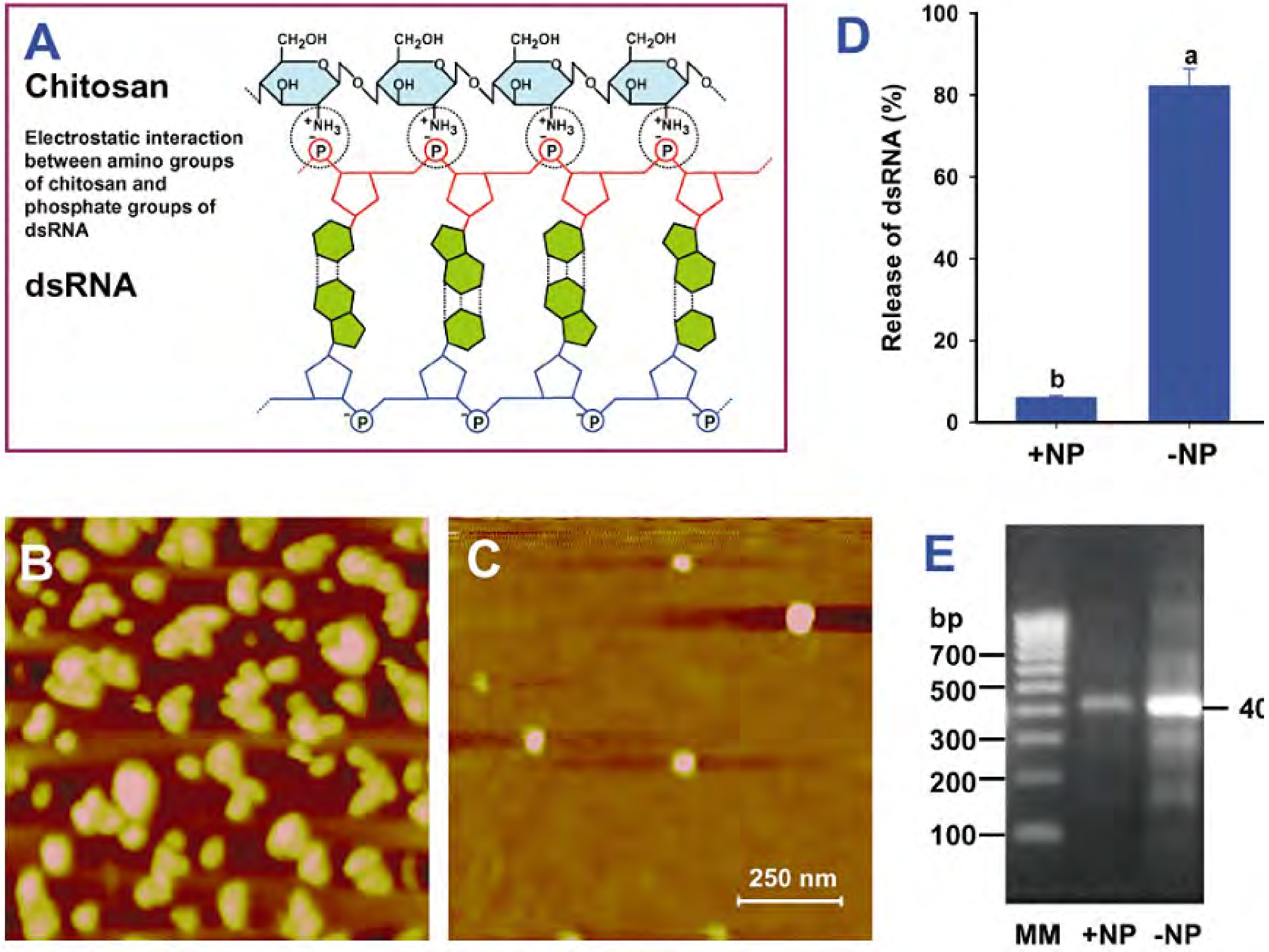


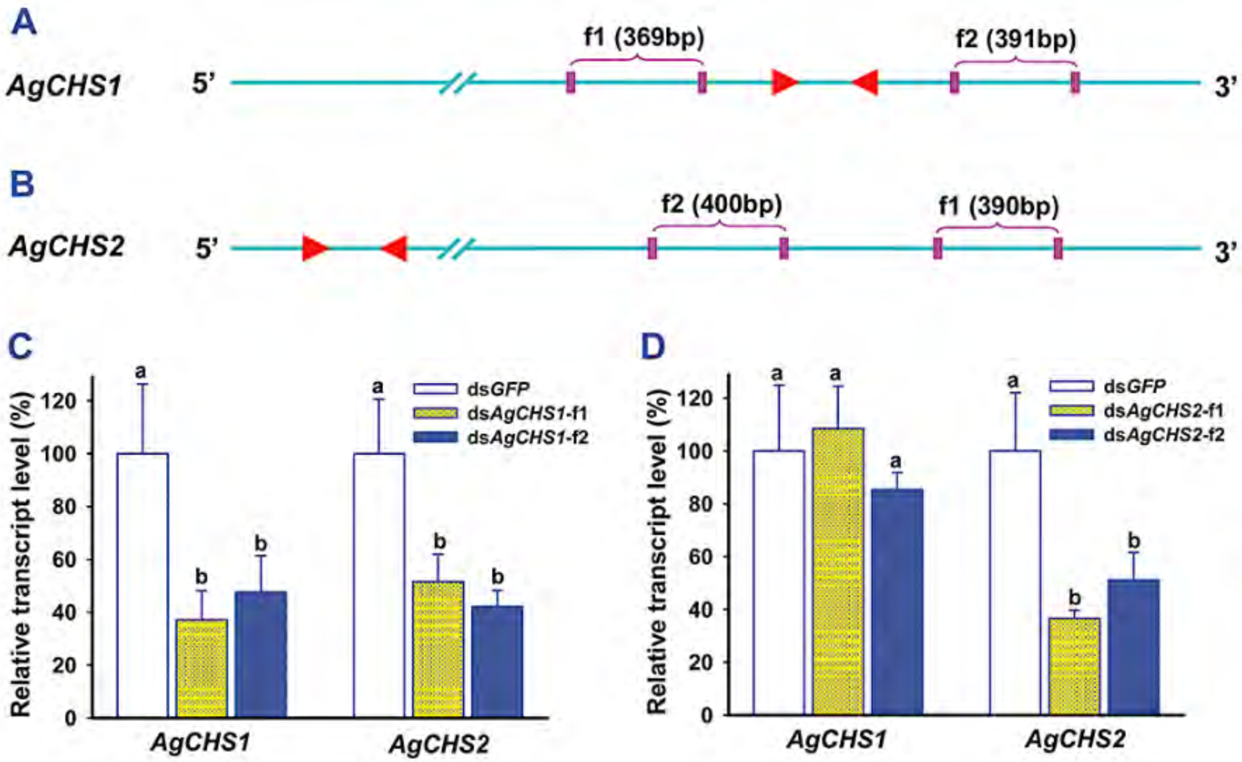


dsRNA	dsRNA delivery	# Larvae treated[3]	Females/males that developed	# Females that blood fed	# Females that produced progeny[4]
gus	Daily soakings[1]	420	207/200	162	139
dsx[F]	Daily soakings[1]	440	6/242	0	0

(Steve Whyard, et al. Parasit Vectors, 2015)

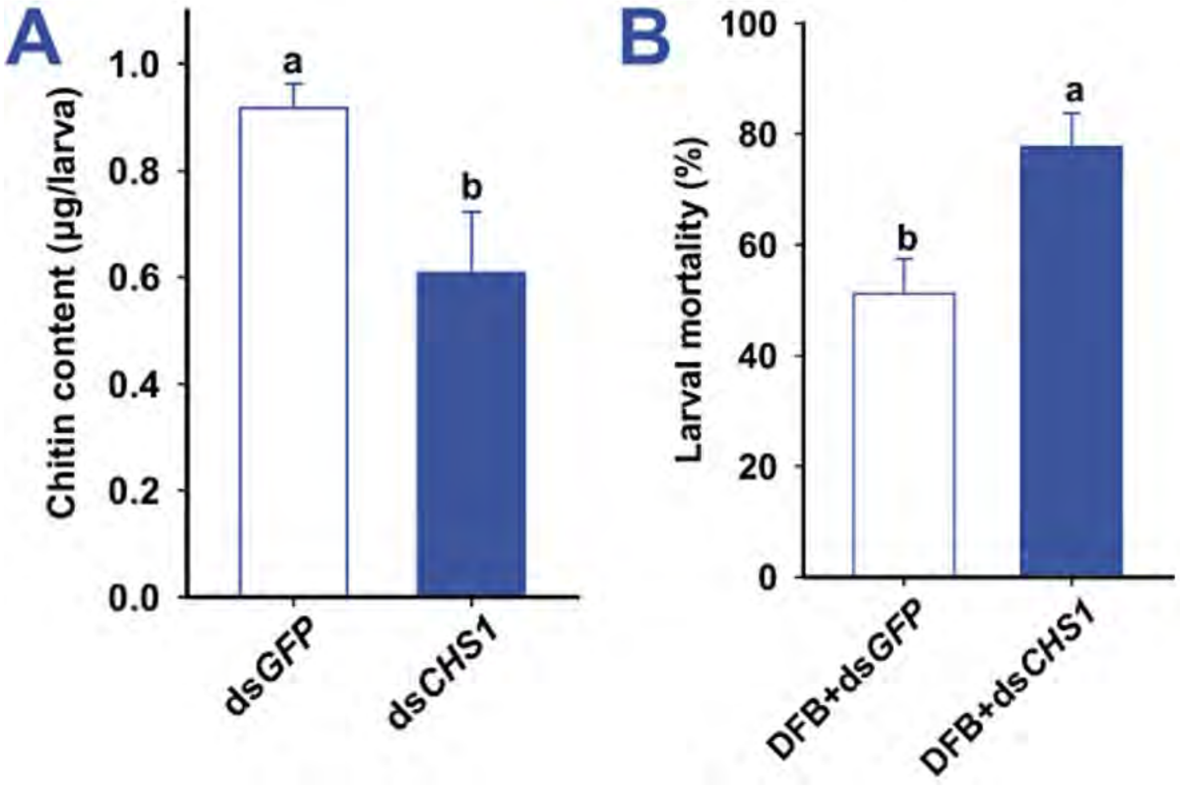
③ Nanoparticle-mediated

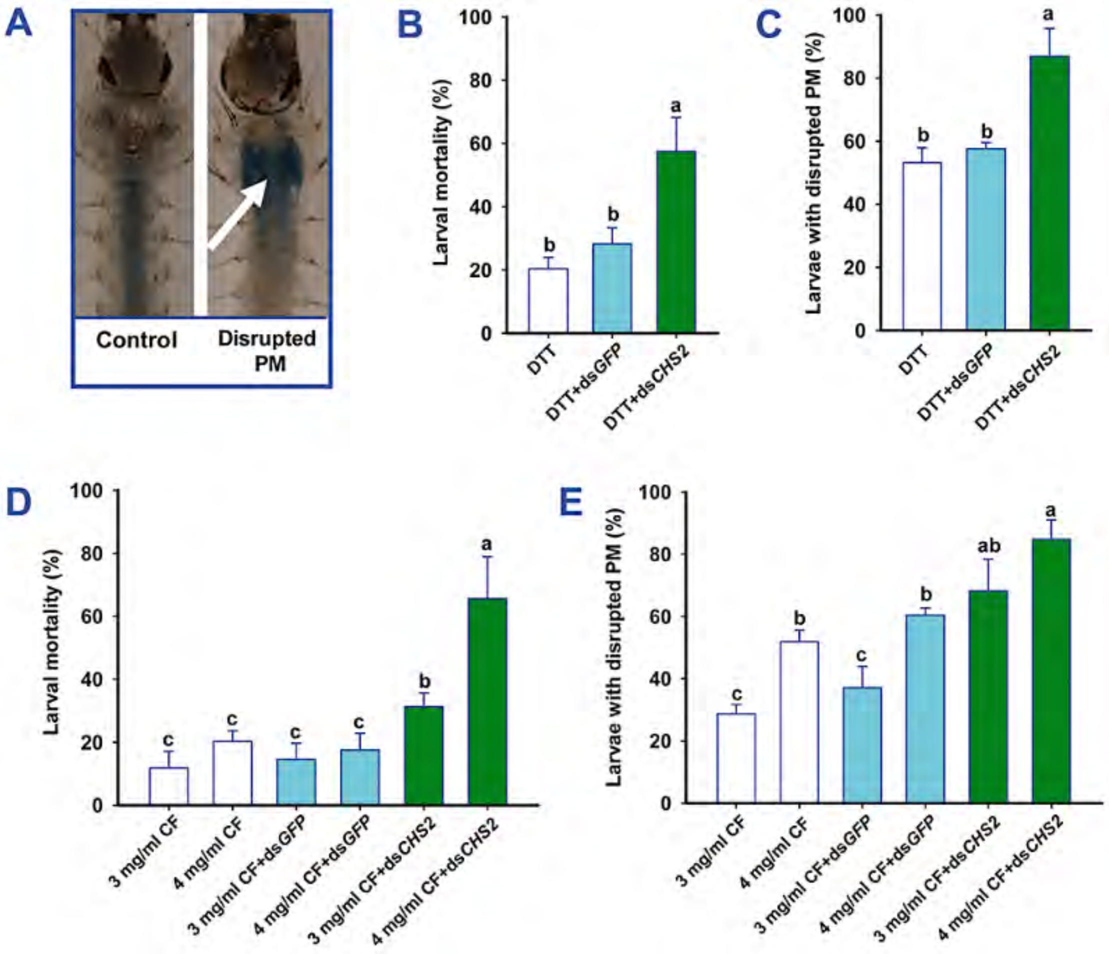




(X. Zhang, et al. Insect Molecular Biology, 2010)

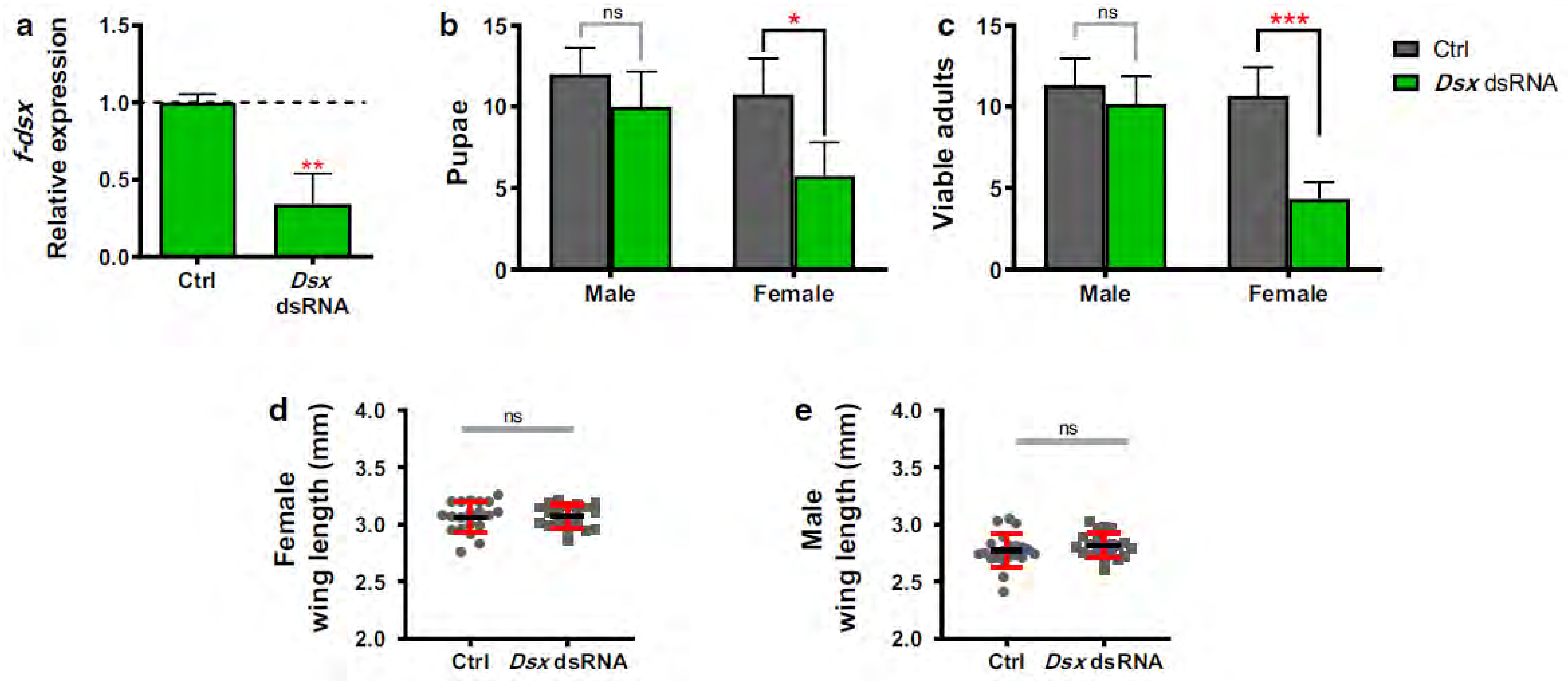
③ Nanoparticle-mediated





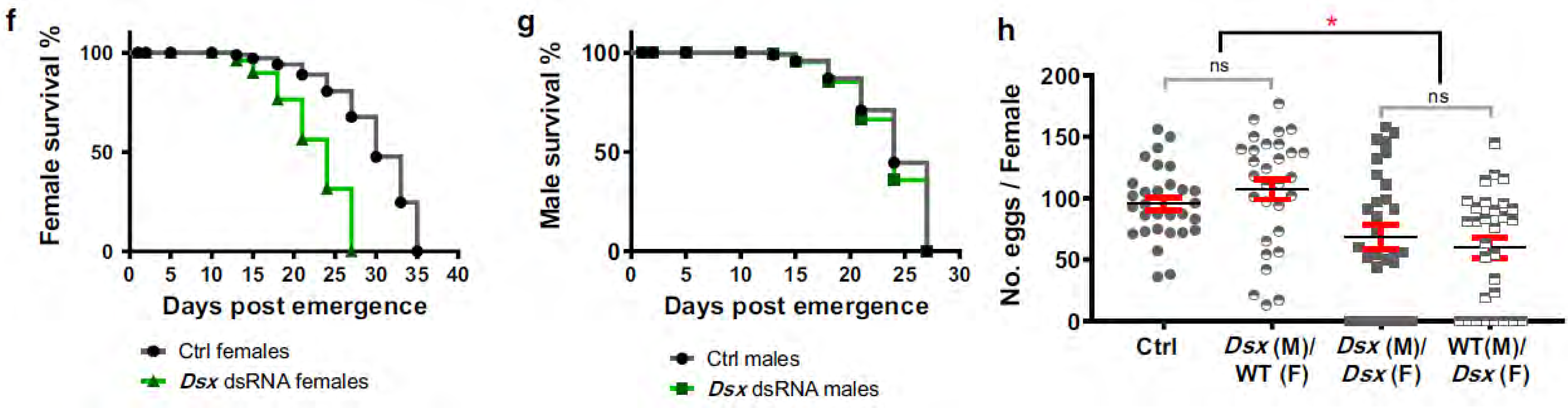
(X. Zhang, et al. Insect Molecular Biology, 2010)

④ Microbially-expressed vector systems



(Mabel L. Taracena, et al.
Parasites Vectors, 2019)

④ Microbially-expressed vector systems



(Mabel L. Taracena, et al. Parasites Vectors, 2019)

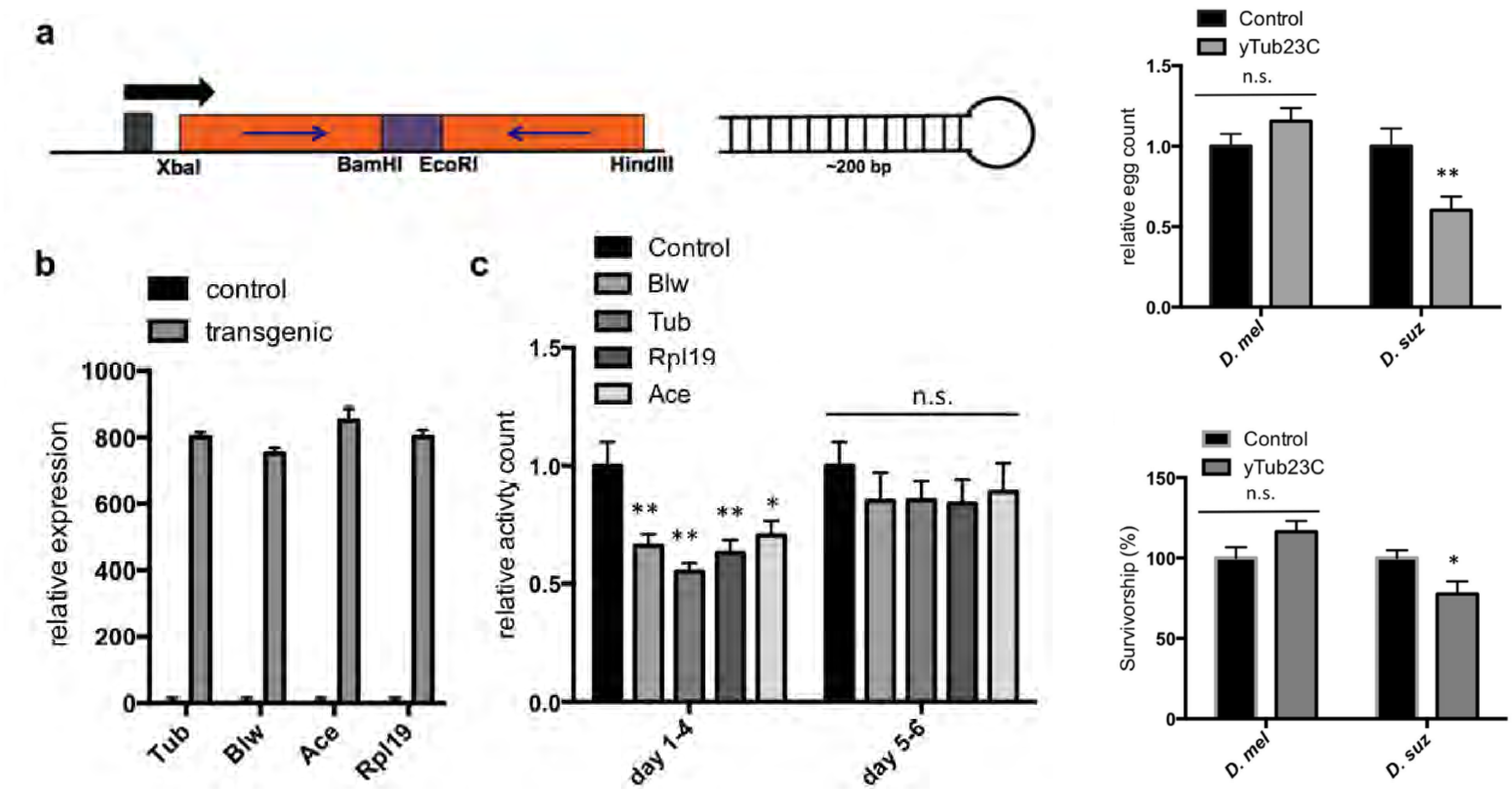
The selection of efficient microbial dsRNA production systems

① **Escherichia coli dsRNA Expression Systems**

② **Saccharomyces cerevisiae dsRNA Expression Systems**

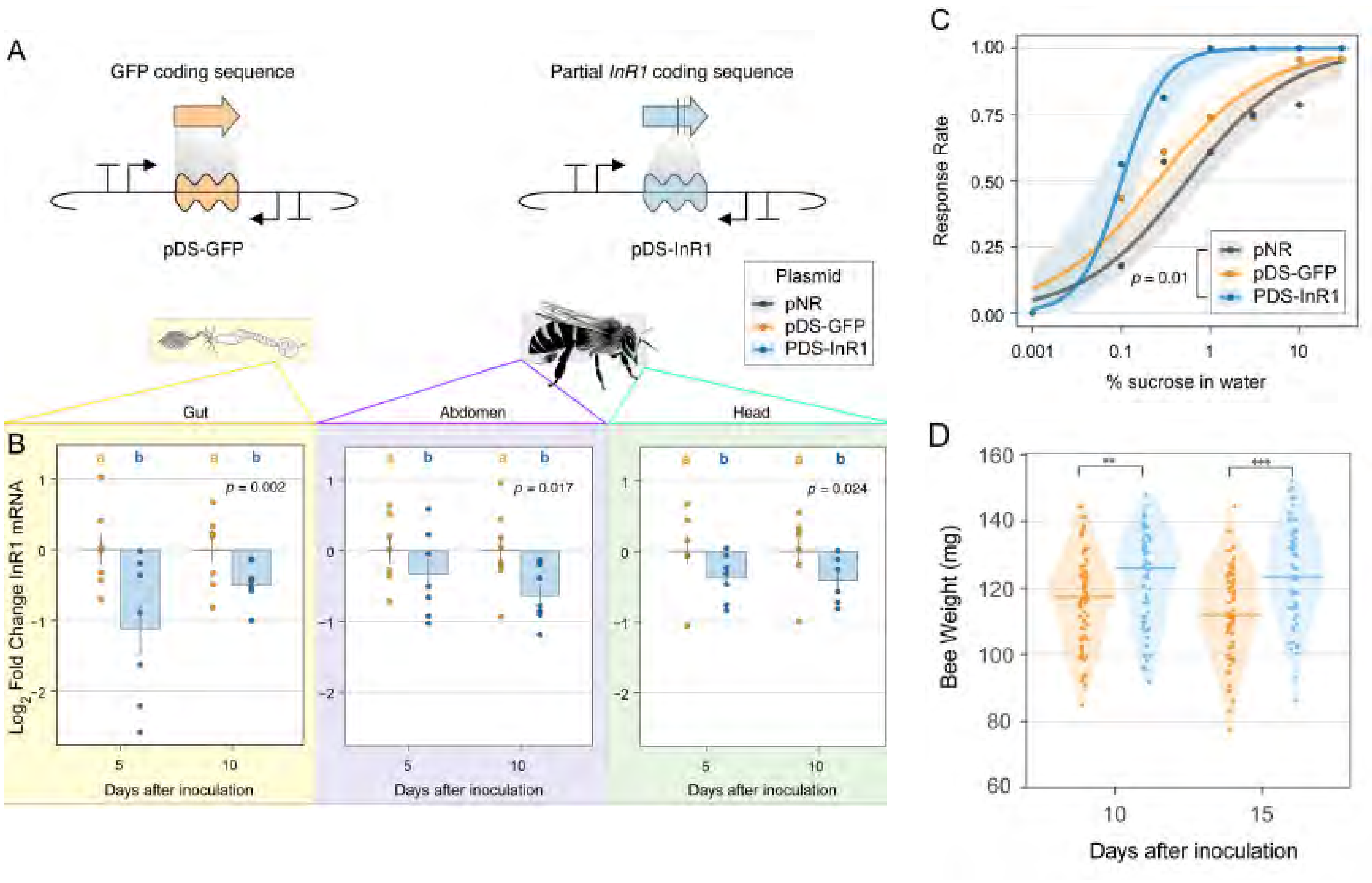
③ **Insect-Symbiotic Bacteria dsRNA Expression Systems**

② Saccharomyces cerevisiae dsRNA Expression Systems



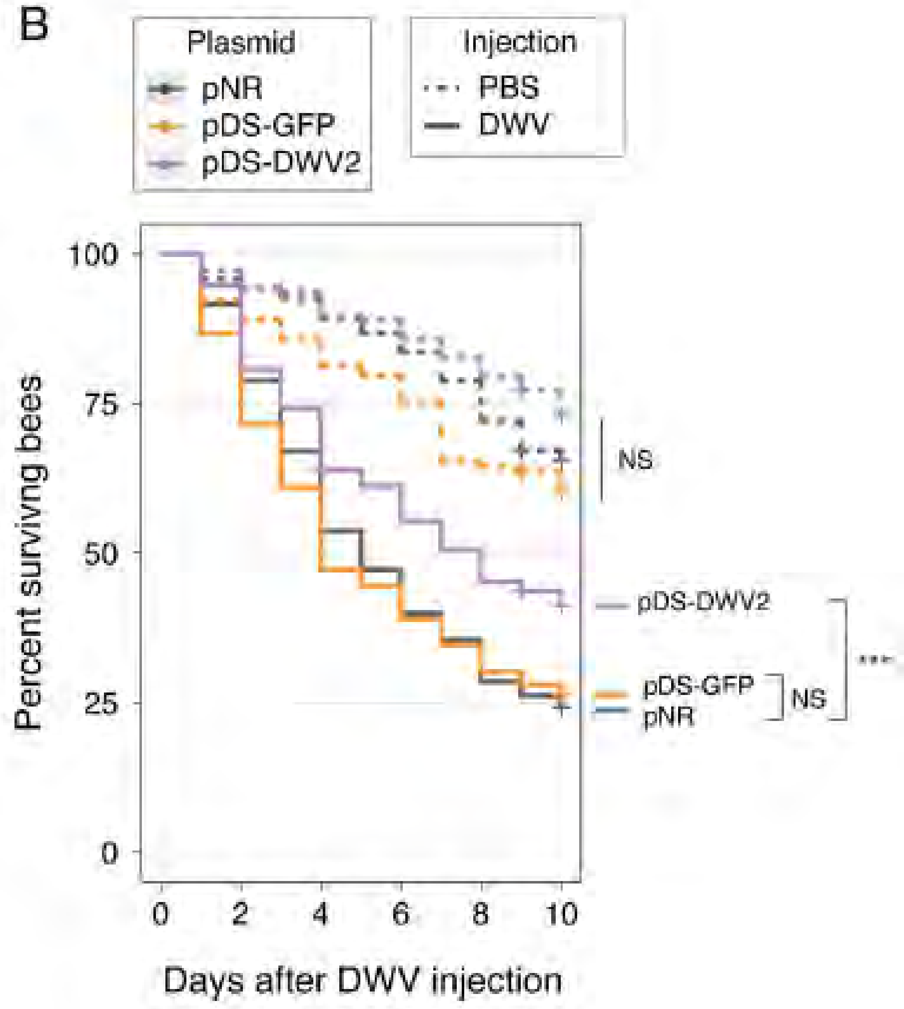
(Katherine A. Murphy, et al. Sci Rep, 2016)

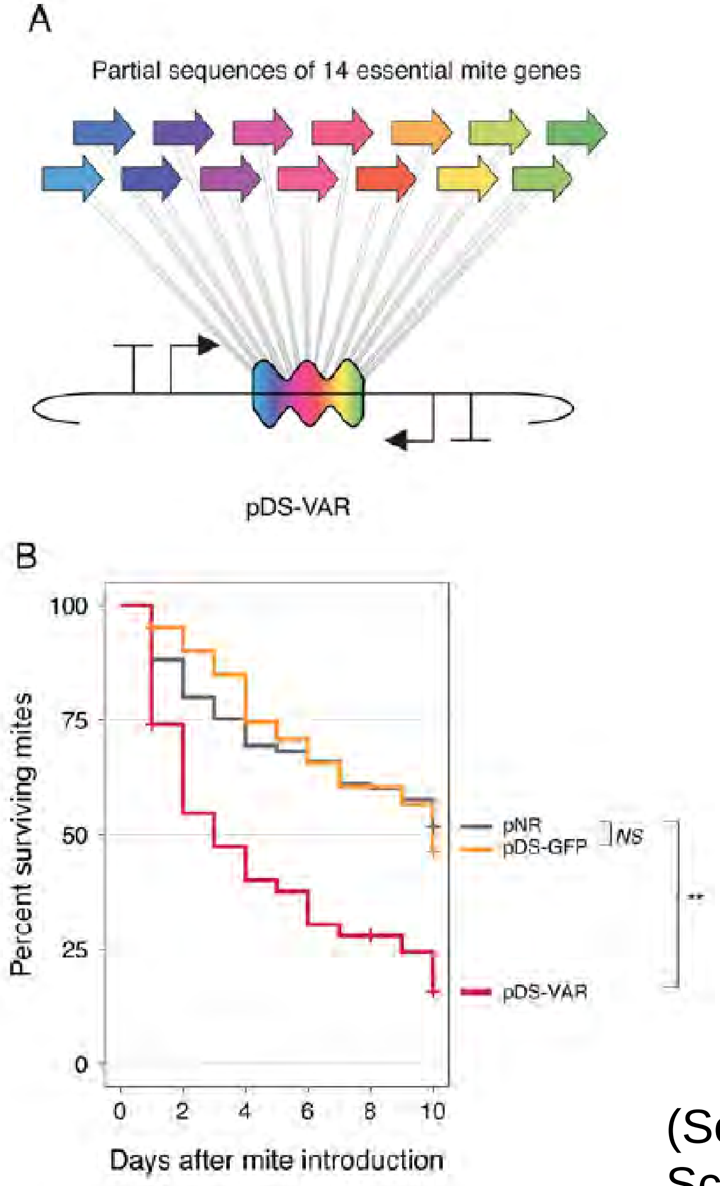
③ Insect-Symbiotic Bacteria dsRNA Expression Systems



(Sean P. Leonard, et al. Science, 2020)

③ Insect-Symbiotic Bacteria dsRNA Expression Systems





(Sean P. Leonard, et al. Science, 2020)

Summary

① The RNAi mechanism was first described in *Caenorhabditis elegans*, and Fire and Mello received the 2006 Nobel Prize in Physiology or Medicine.

② Efficient dsRNA delivery systems are critical for assessing the effects of gene silencing, including but not limited to spraying, soaking, microinjection, and feeding.

③ Four oral delivery strategies are (1) direct ingestion (2) larval soaking (3) nanoparticle-mediated (4) microbially-expressed vector systems

<1>*Escherichia coli* dsRNA Expression Systems

<2>*Saccharomyces cerevisiae* dsRNA Expression Systems

<3>Insect-Symbiotic Bacteria dsRNA Expression Systems

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The research and application of RNAi technology in pest control in recent years

http://www.jsppa.com.cn/news/yanfa/8394.html

昆虫目 Insect Order	昆虫名称 Insect name	靶标基因 Targeted gene	递送方式 Delivery approach	效应 Effect
Lepidoptera		*CYP9A10*	喂食 Feed	提高 α-氯氰菊酯对甜菜夜蛾幼虫的致死率[52] Increase mortality of α-cypermethrin to the larval of *S. exigua*[52]
	二化螟 *Chilo suppressalis*	NADH dehydrogenase，*G3PDH*，*MSL3*	喂食 Feed	幼虫表现出高死亡率和抑制生长[45] Larvae exhibited high mortalities and suppressed growth[45]
鳞翅目 Lepidoptera	棉铃虫 *Helicoverpa armigera*	Juvenile hormone acid methyltransferase (*JHAMT*)，prothoracicotropic hormone (*PTTH*)，pheromone biosynthesis-activating peptide (*PBAN*)，molt regulating transcription factor (*HHR3*)，activated protein 4，eclosion hormone precursor (*EH*)	喂食 Feed	60%~90% 的死亡率，幼虫体重减轻，表型畸形和化蛹延迟[53] Mortality rangs from 60% to 90%, reduce larval weight, phenotypic deformities and delay pupation[53]
		Calcineurin (*CaN*)	喂食 Feed	影响棉铃虫的发育，同时增强了 *Cry2Ab* 对害虫的毒性[54] Affect the development of the cotton bollworm, but also enhance the toxicity of *Cry2Ab* to the pest[54]
		Broad-Complex (*Br-C*)	喂食 Feed	导致幼虫发育畸形和死亡，同时降低中肠和表皮几丁质含量[55] Cause larval developmental deformity and mortality, at the same time decreased chitin contents in the midgut and epidermis[55]
双翅目 Diptera	桔小实蝇 *Bactrocera dorsalis*	Argonaute-1 (*Ago-1*)	喂食 Feed	延缓卵巢发育[65] Delay ovarian development[65]
缨翅目 Thysanoptera	西花蓟马 *Frankliniella occidentalis*	Toll-like receptor 6，apolipophorin，coatomer protein subunit epsilon	喂食 Feed	摄入后导致死亡[67] Cause death after ingestion[67]
	烟蓟马 *Thrips tabaci*	*SNF7*，*AQP*	喂食 Feed	喂食 *SNF7* 或 *AQP* 双链 RNA 导致 62% 和 72% 的死亡率[32] Feed ds*SNF7* or ds*AQP* also caused 62 and 72% mortality[32]

Thanks！

Part II

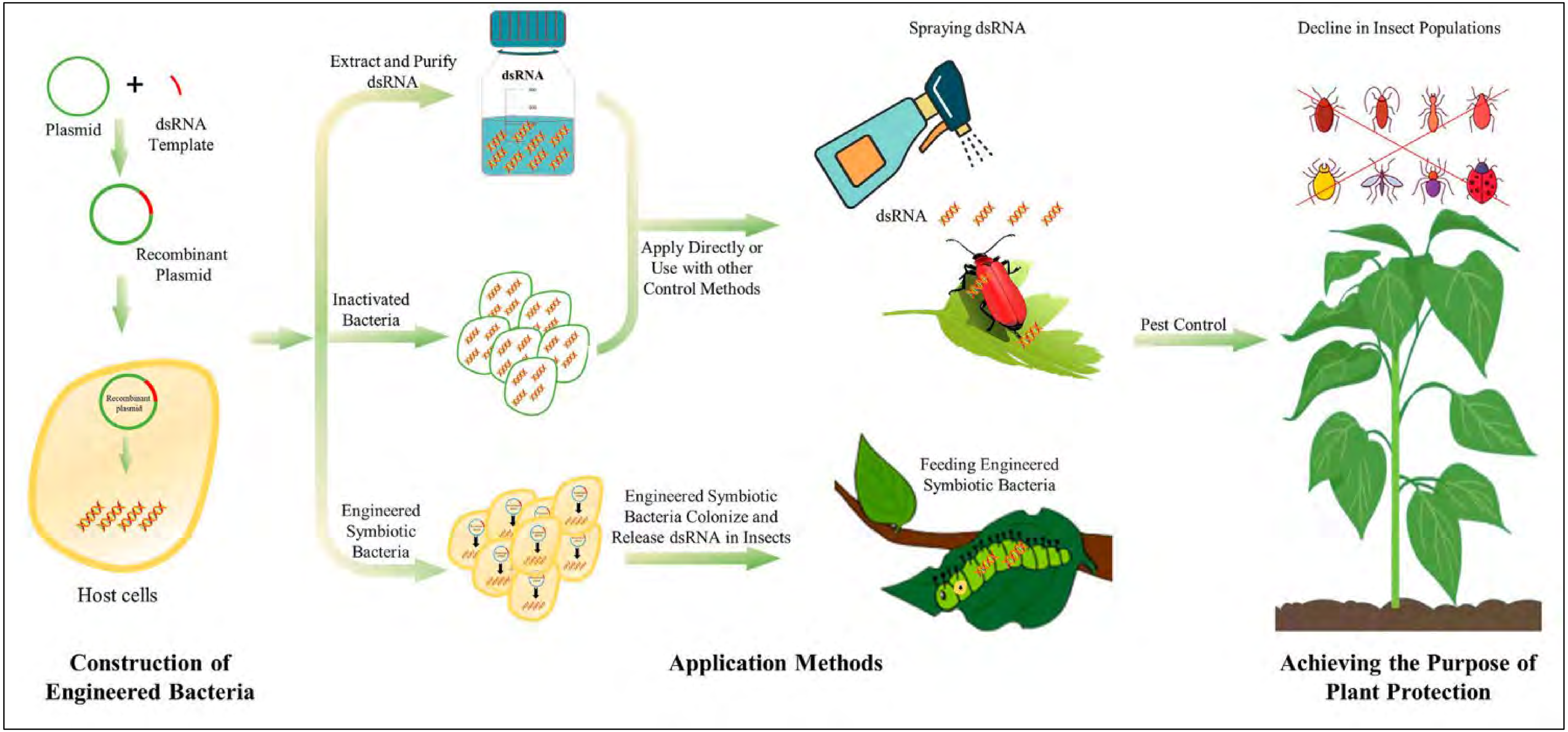
Mechanisms of oral RNAi in Agricultural Pest Control

苏祥彬

20231228

Interfering RNA —?→ insects

The process and use method of microbial dsRNA production system



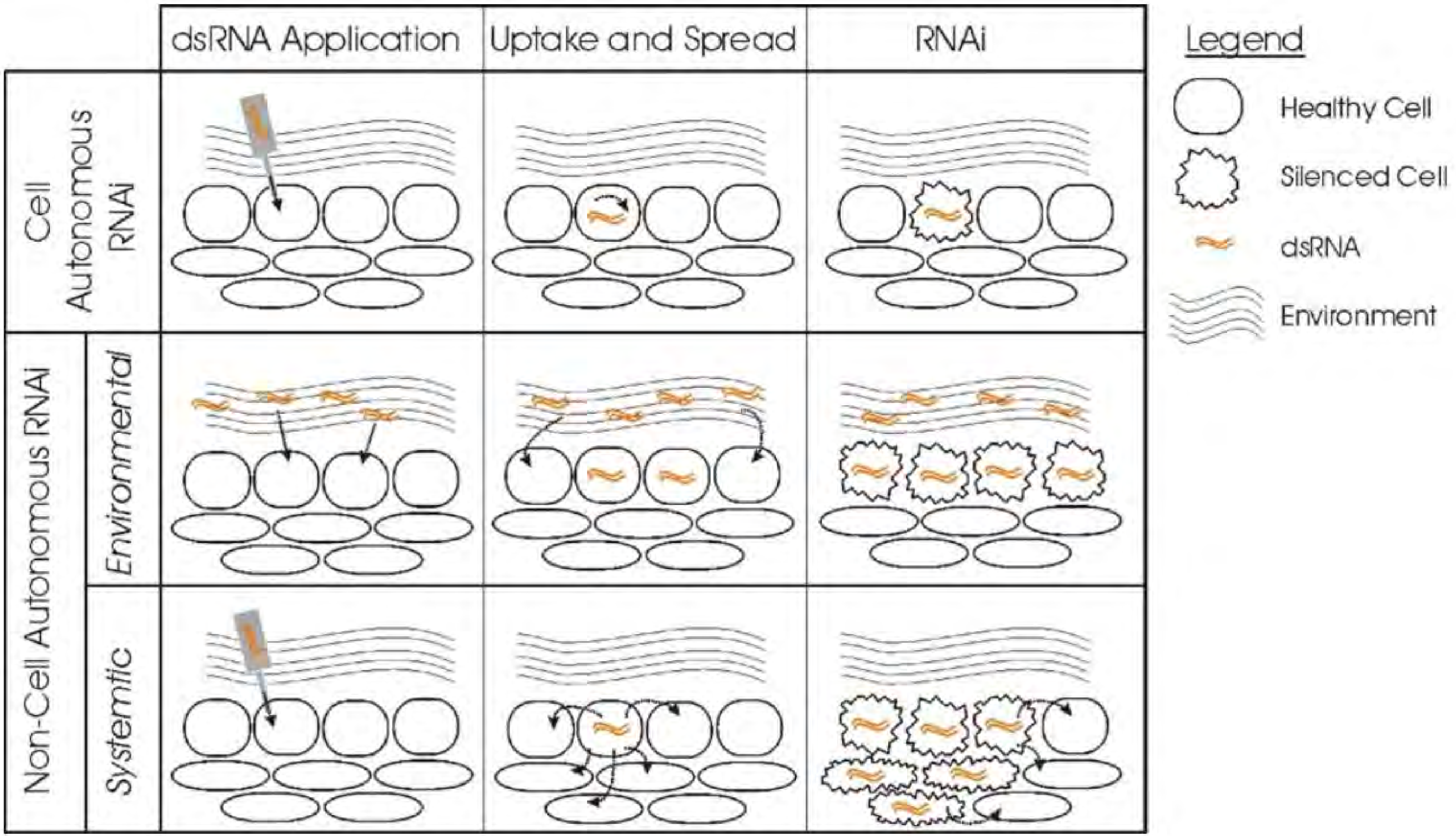
Ruobing Guan et al,. 2021

Questions

- 如何有针对性的杀灭害虫?
- 如何让害虫摄入dsRNA?
- dsRNA如何进入害虫细胞并在细胞间传递的?
- dsRNA如何在细胞内发挥作用并杀灭害虫的?

Interfering RNA —?→ insects

A schematic overview of the different types of RNAi



Hanneke Huvenne, Guy Smagghe,. 2010

Non-cell autonomous RNAi

Part 1 Environmental RNAi

Delivery of interfering RNA to insects

- ➢ Basic Delivery Methods
- ➢ Cationic Liposome–Assisted Delivery
- ➢ Nanoparticle-Enabled Delivery
- ➢ Symbiont-Mediated Delivery
- ➢ Plant-Mediated Delivery

Delivery of interfering RNA to insects

- Basic Delivery Methods
- Plant-Mediated Delivery

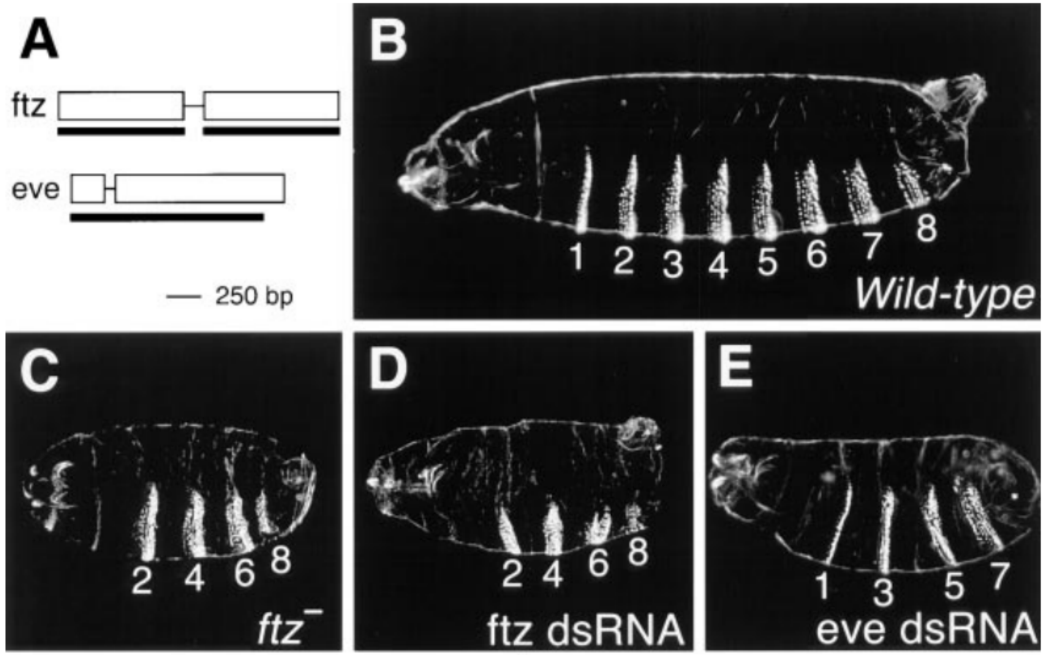
Microinjection

A small amount of dsRNA in an appropriate solution is directly injected into an insect embryo or insect body.

› Cell. 1998 Dec 23;95(7):1017-26. doi: 10.1016/s0092-8674(00)81725-0.

Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway

J R Kennerdell [1], R W Carthew



Oral delivery

Another basic delivery method is to feed insects a diet containing synthetic or microorganism-expressed dsRNA.

Two main strategies

- dsRNAs can be expressed in bacteria
  Escherichia coli（大肠杆菌）
  Saccharomyces cerevisiae（酿酒酵母）
- synthesized in vitro

› Insect Biochem Mol Biol. 2009 Nov;39(11):824-32. doi: 10.1016/j.ibmb.2009.09.007. Epub 2009 Oct 6.

Ingested double-stranded RNAs can act as species-specific insecticides

Steven Whyard [1], Aditi D Singh, Sylvia Wong

Affiliations + expand

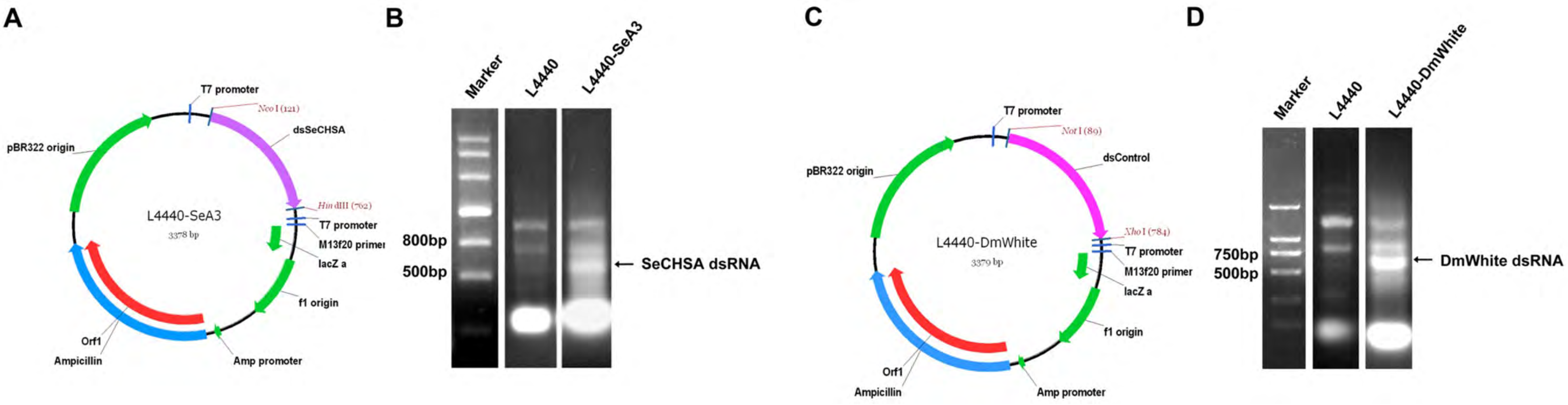
PMID: 19815067 DOI: 10.1016/j.ibmb.2009.09.007

› PLoS One. 2009 Jul 13;4(7):e6225. doi: 10.1371/journal.pone.0006225.

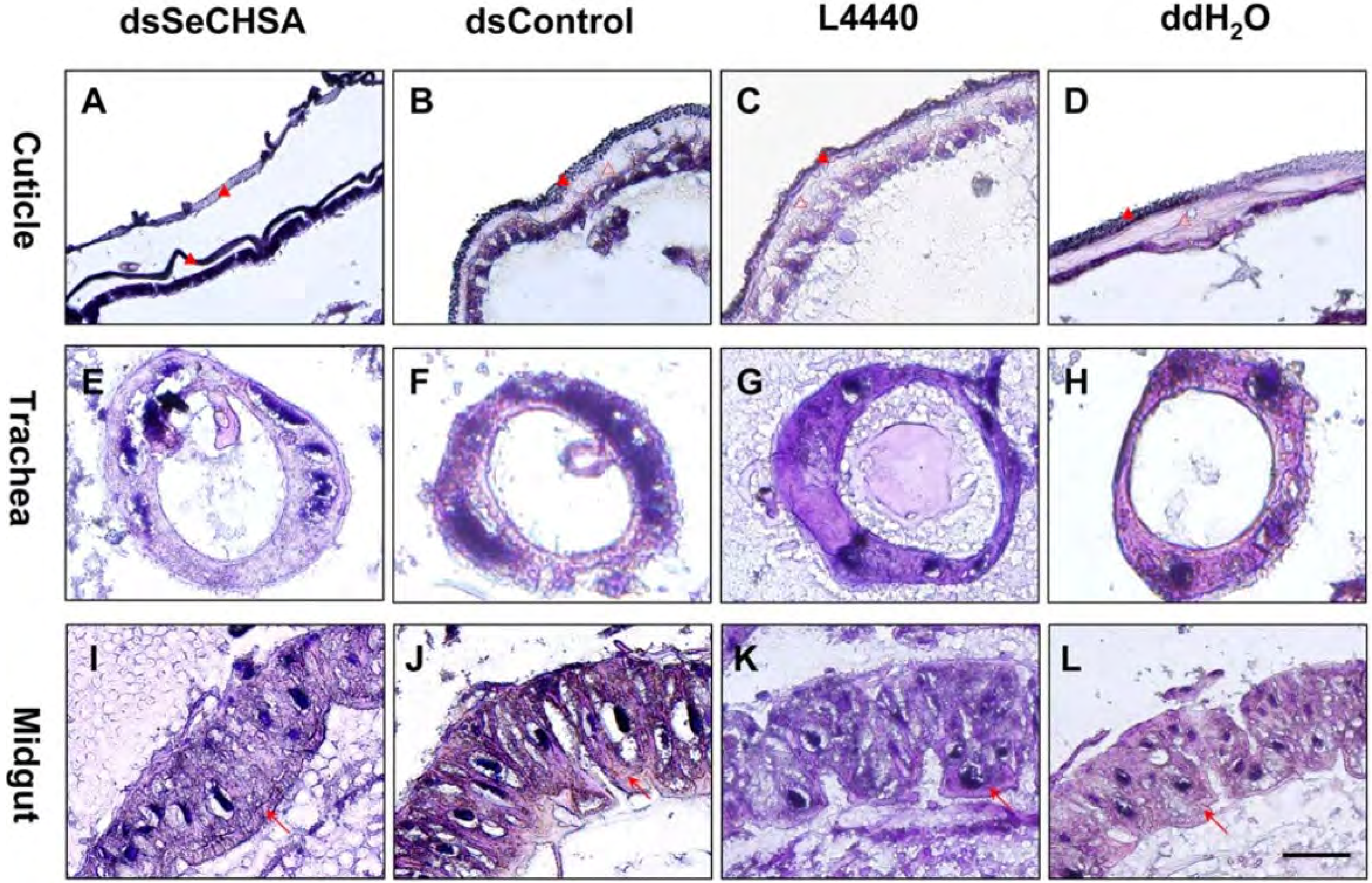
Developmental control of a lepidopteran pest Spodoptera exigua by ingestion of bacteria expressing dsRNA of a non-midgut gene

Honggang Tian [1], Han Peng, Qiong Yao, Hongxin Chen, Qi Xie, Bin Tang, Wenqing Zhang

Schematic diagram of the recombinant plasmids for dsRNA expression and production of dsRNAs



Effects of ingested dsSeCHSA on different tissues of Spodoptera exigua larvae



Delivery of interfering RNA to insects

- Basic Delivery Methods
- Plant-Mediated Delivery

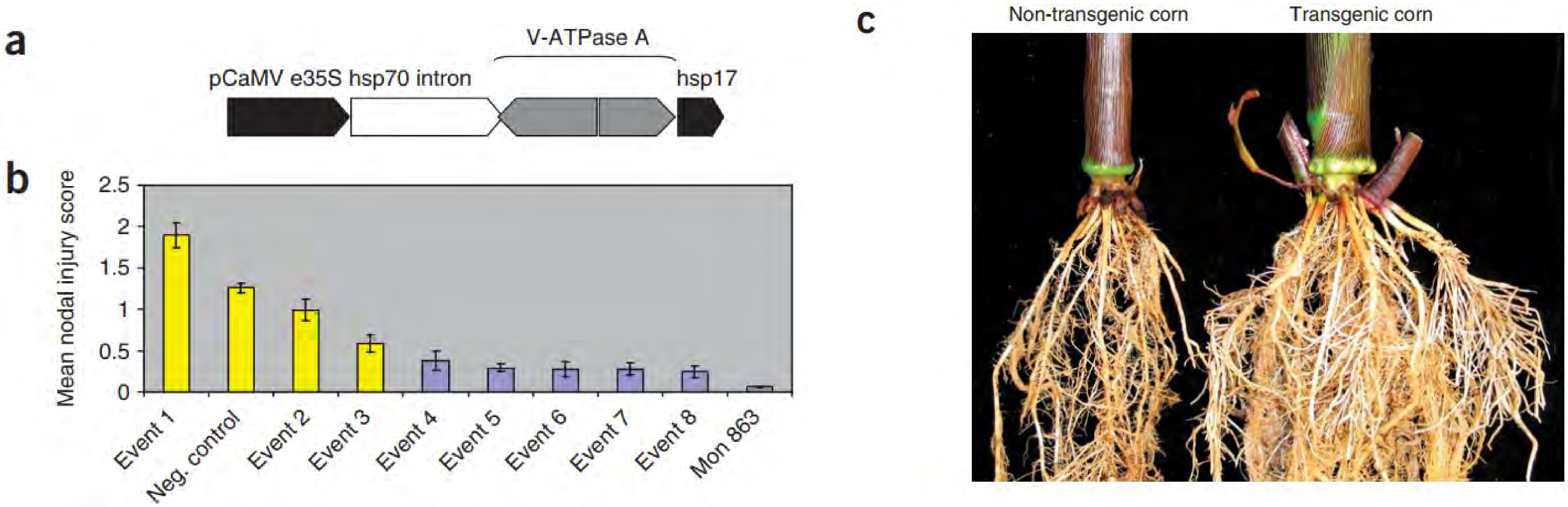
nature > nature biotechnology > letters > article

Letter | Published: 04 November 2007

Control of coleopteran insect pests through RNA interference

James A Baum, Thierry Bogaert, William Clinton, Gregory R Heck, Pascale Feldmann, Oliver Ilagan, Scott Johnson, Geert Plaetinck, Tichafa Munyikwa, Michael Pleau, Ty Vaughn & James Roberts

F1 plants expressing a V-ATPase A dsRNA are protected from WCR feeding damage



Mechanisms of dsRNA uptake

- The transmembrane Sid-1 channel protein-mediated pathway
- The endocytosis-mediated uptake mechanism

Mechanisms of dsRNA uptake

- The transmembrane Sid-1 channel protein-mediated pathway
- The endocytosis-mediated uptake mechanism

SID-1 is a multispan transmembrane protein

> Science. 2003 Sep 12;301(5639):1545-7. doi: 10.1126/science.1087117.

Transport of dsRNA into cells by the transmembrane protein SID-1

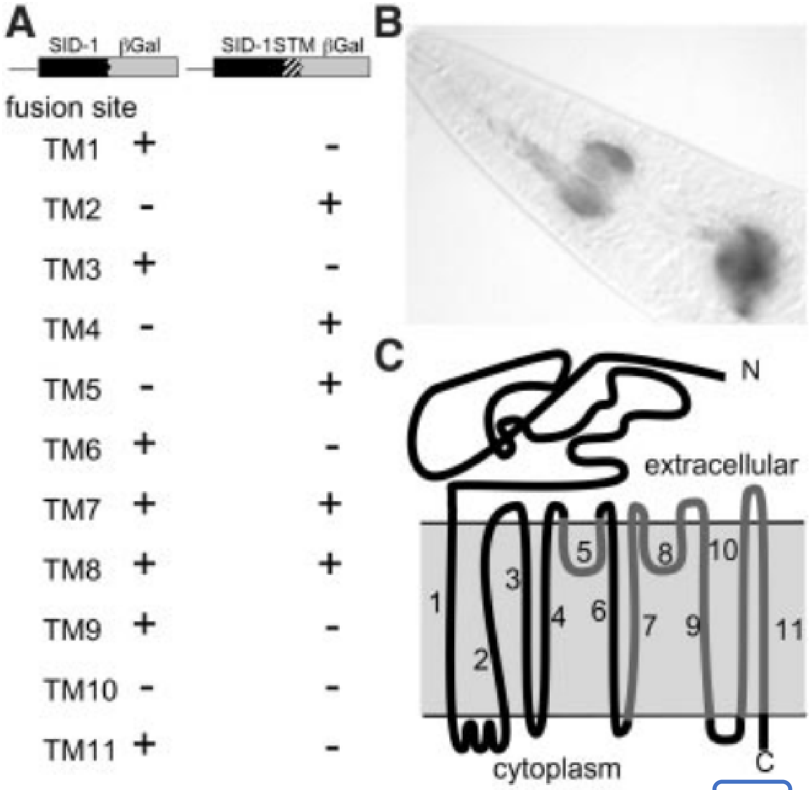
Evan H Feinberg [1], Craig P Hunter

Affiliations + expand

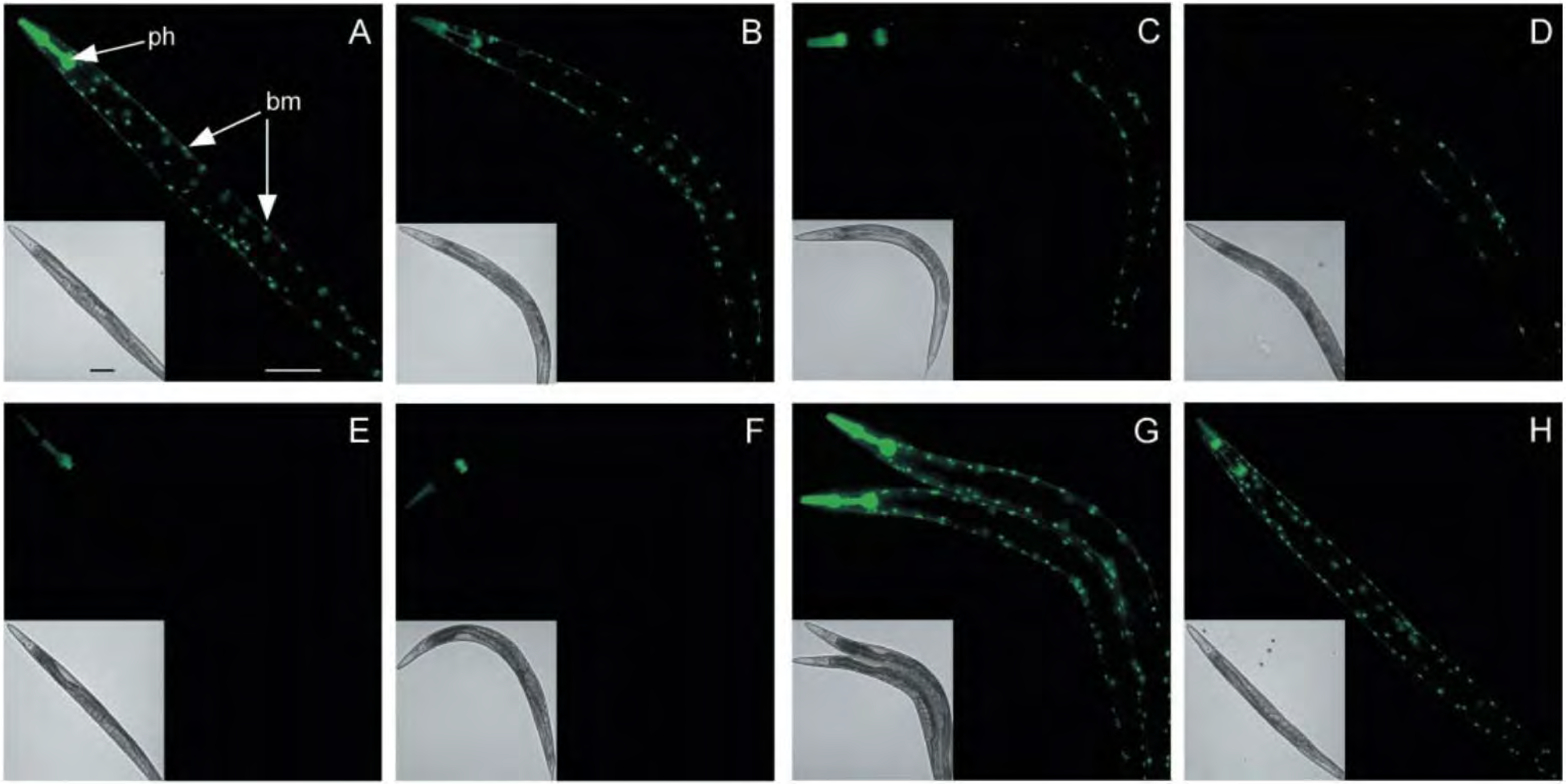
PMID: 12970568 DOI: 10.1126/science.1087117

RESEARCH

Mobile RNA and transgenerational epigenetic inheritance

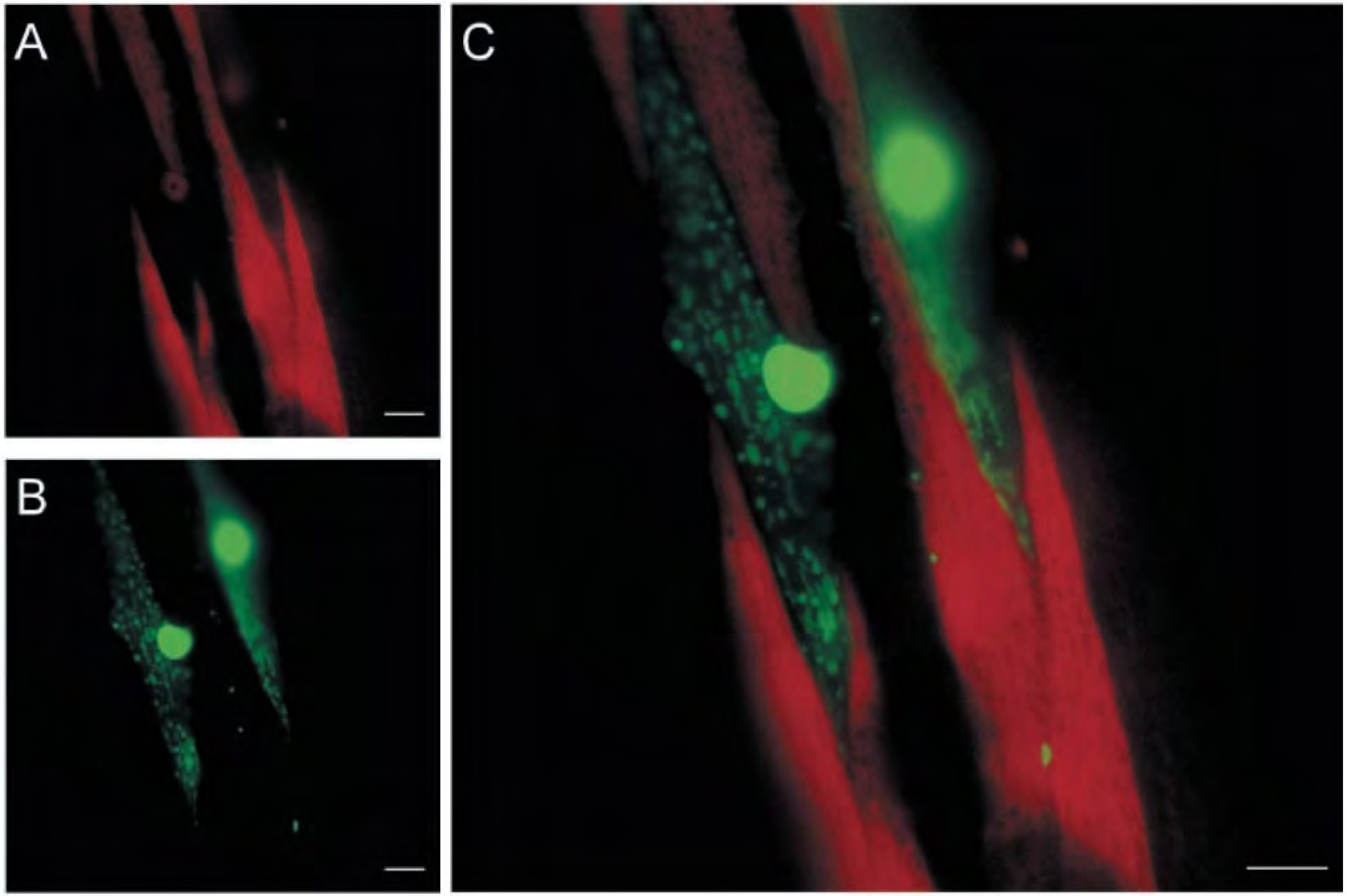


Visualization of systemic RNAi



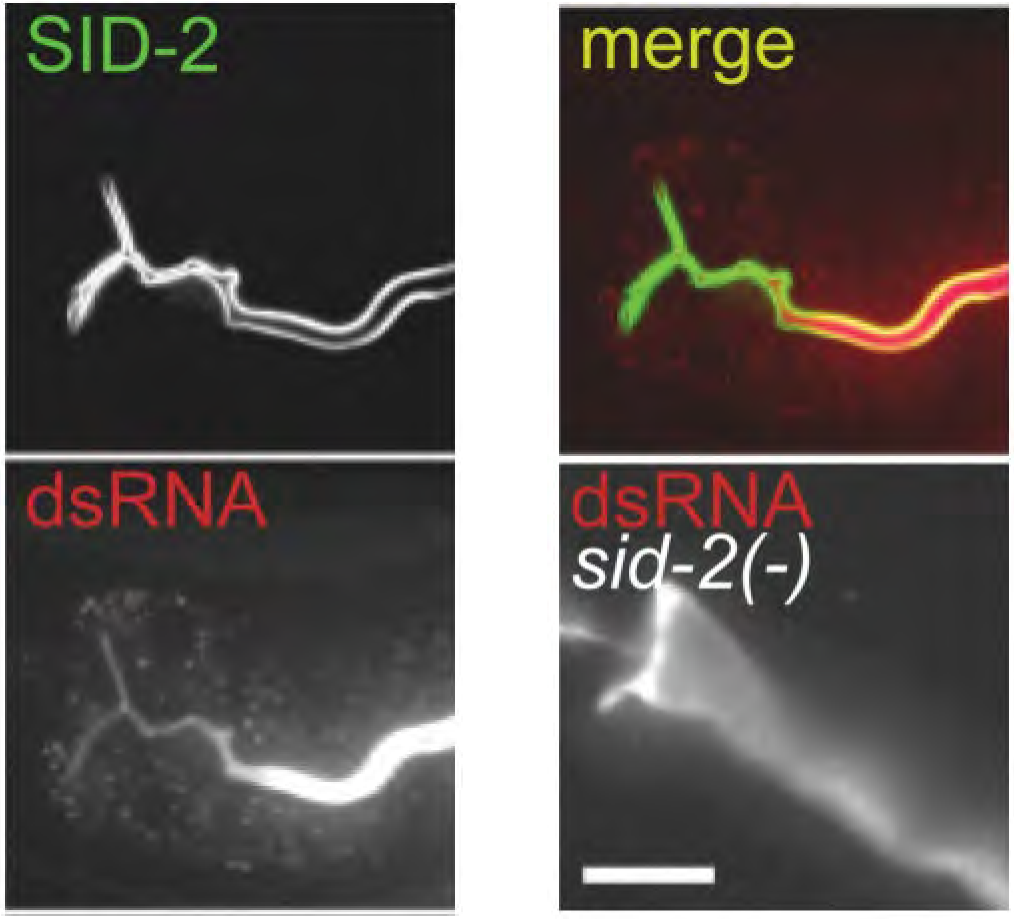
William M. Winston et al., 2002

Mosaic analysis of *sid-1* function



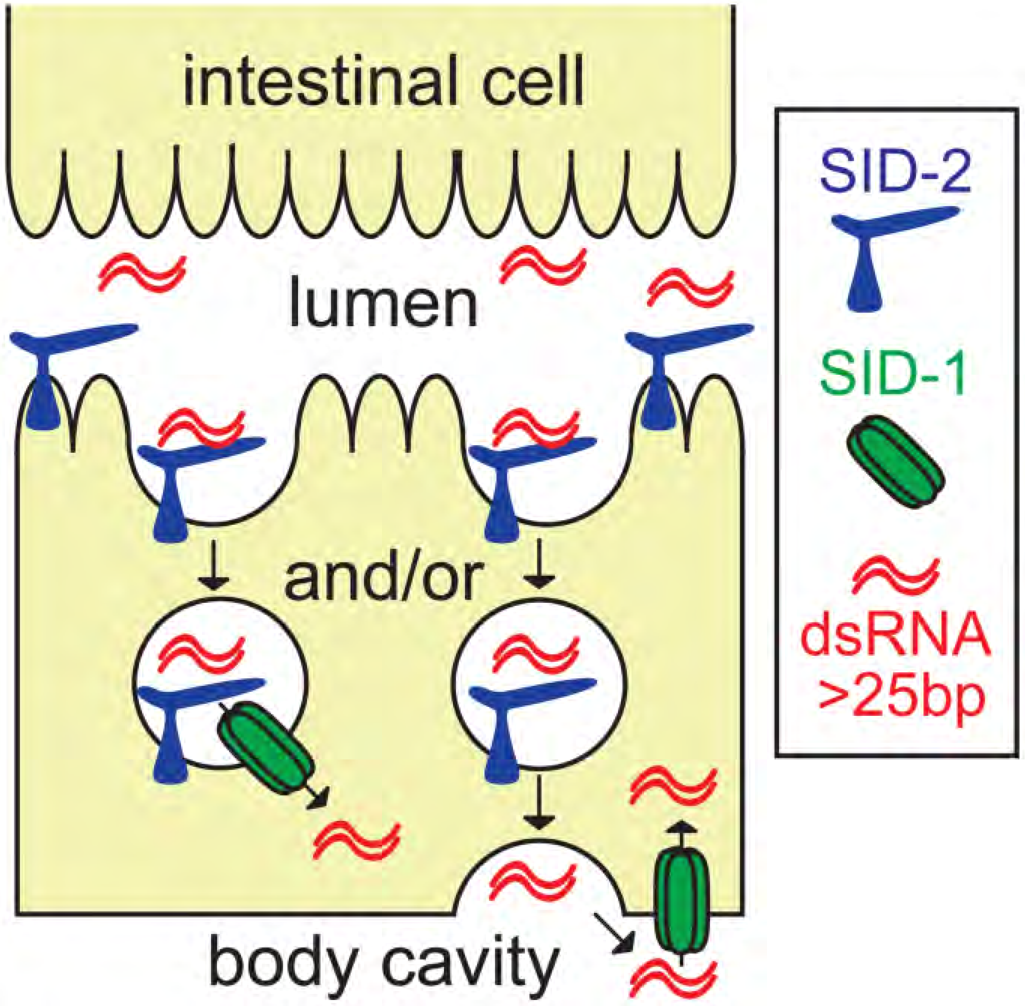
William M. Winston et al., 2002

SID-2 is required to internalize environmental dsRNAs into *C. elegans*



Deborah L McEwan, Alexandra S Weisman, and Craig P Hunter. 2012

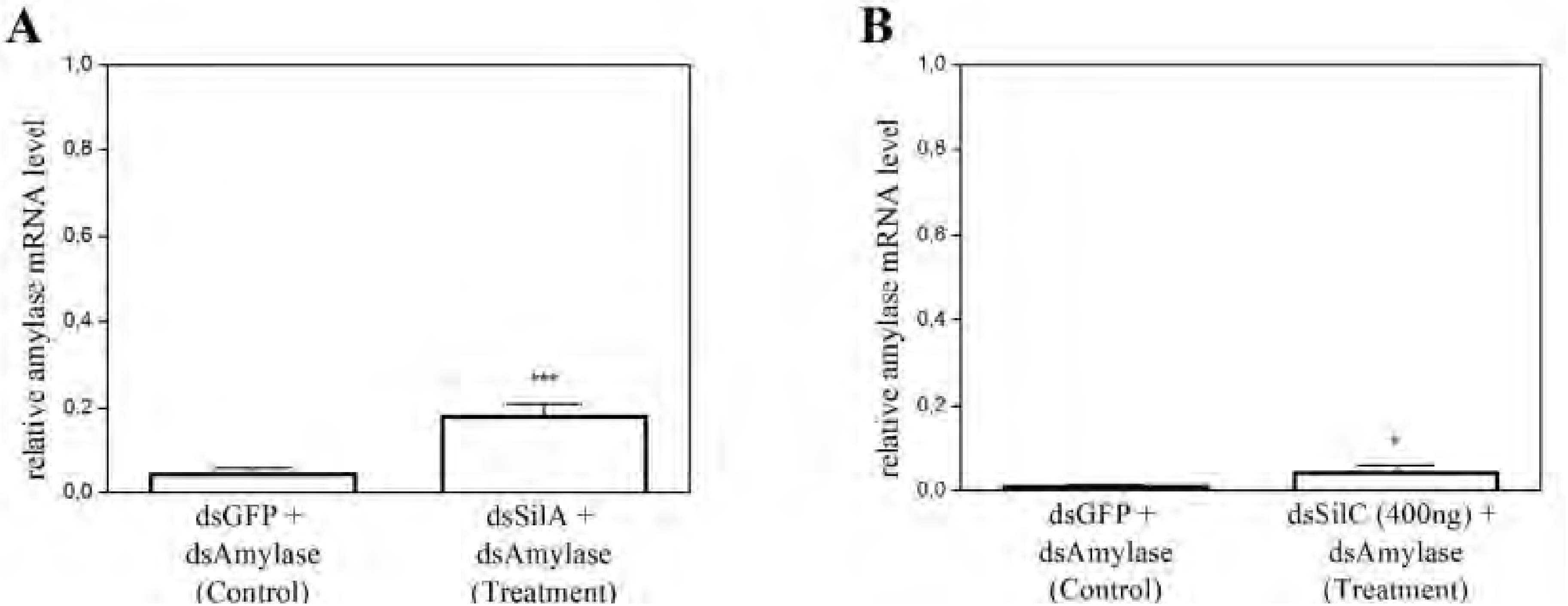
Model of SID-2 and SID-1 coordinated uptake of ingested dsRNA in *C. elegans*



Therefore, environmental RNAi needs cooperation between Sid-1 and Sid-2 proteins

Deborah L McEwan, Alexandra S Weisman, and Craig P Hunter. 2012

The two sid-1-like (sil) genes in the Colorado potato beetle are involved in double-stranded RNA (dsRNA) uptake in the midgut

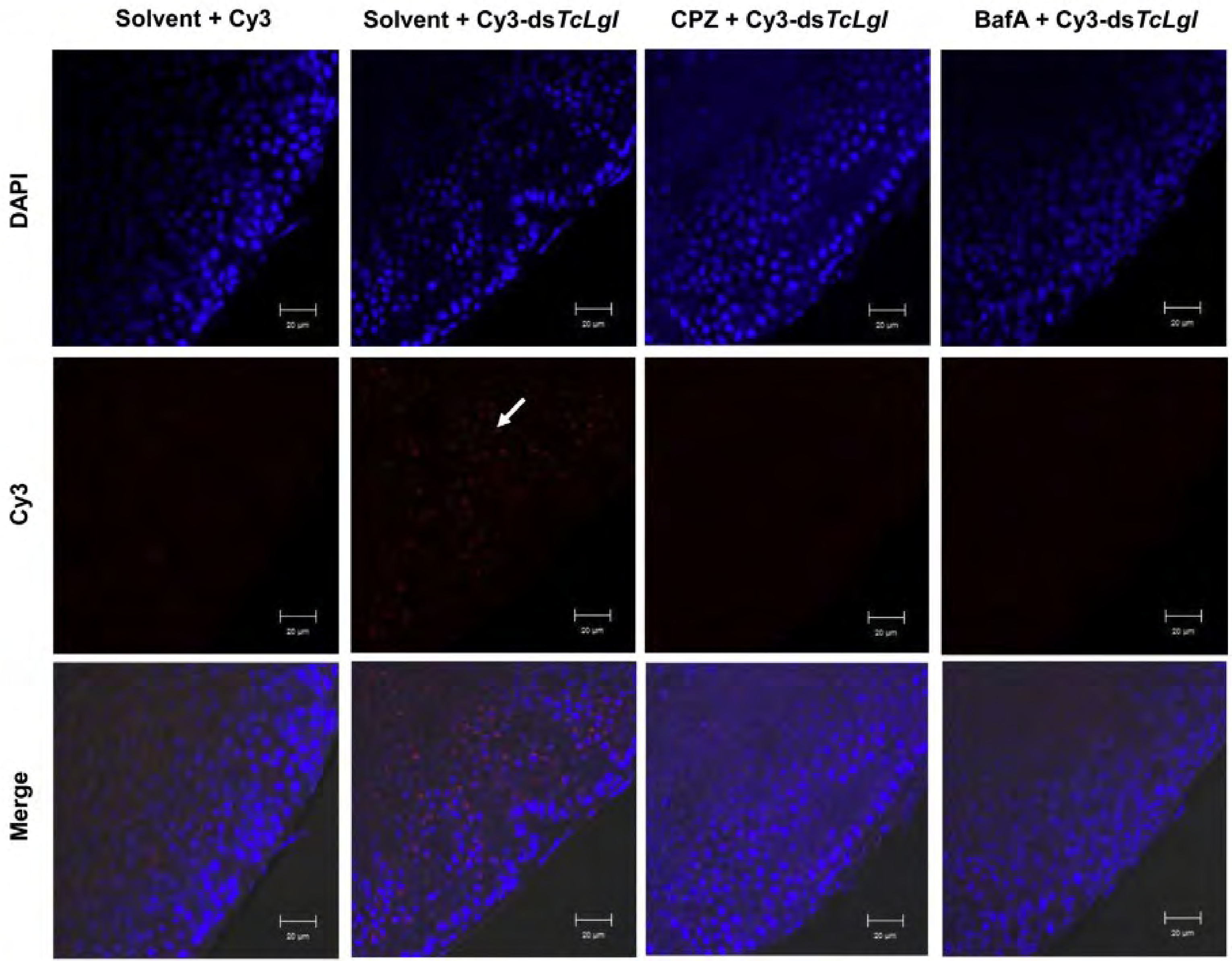


K. Cappelle et al., 2016

Mechanisms of dsRNA uptake

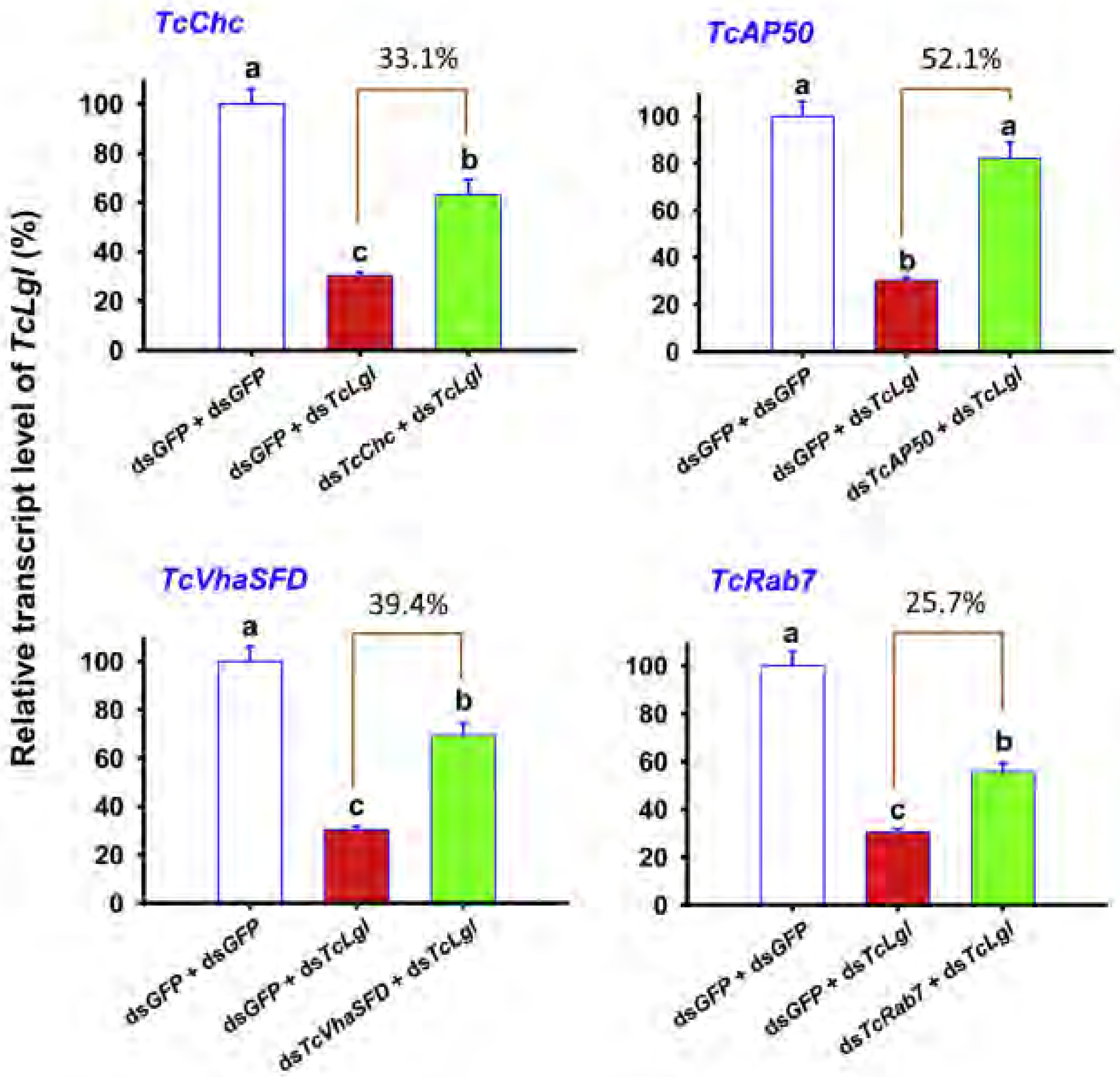
- The transmembrane Sid-1 channel protein-mediated pathway
- The endocytosis-mediated uptake mechanism

Effect of endocytosis inhibitors on dsRNA uptake in larval midgut cells

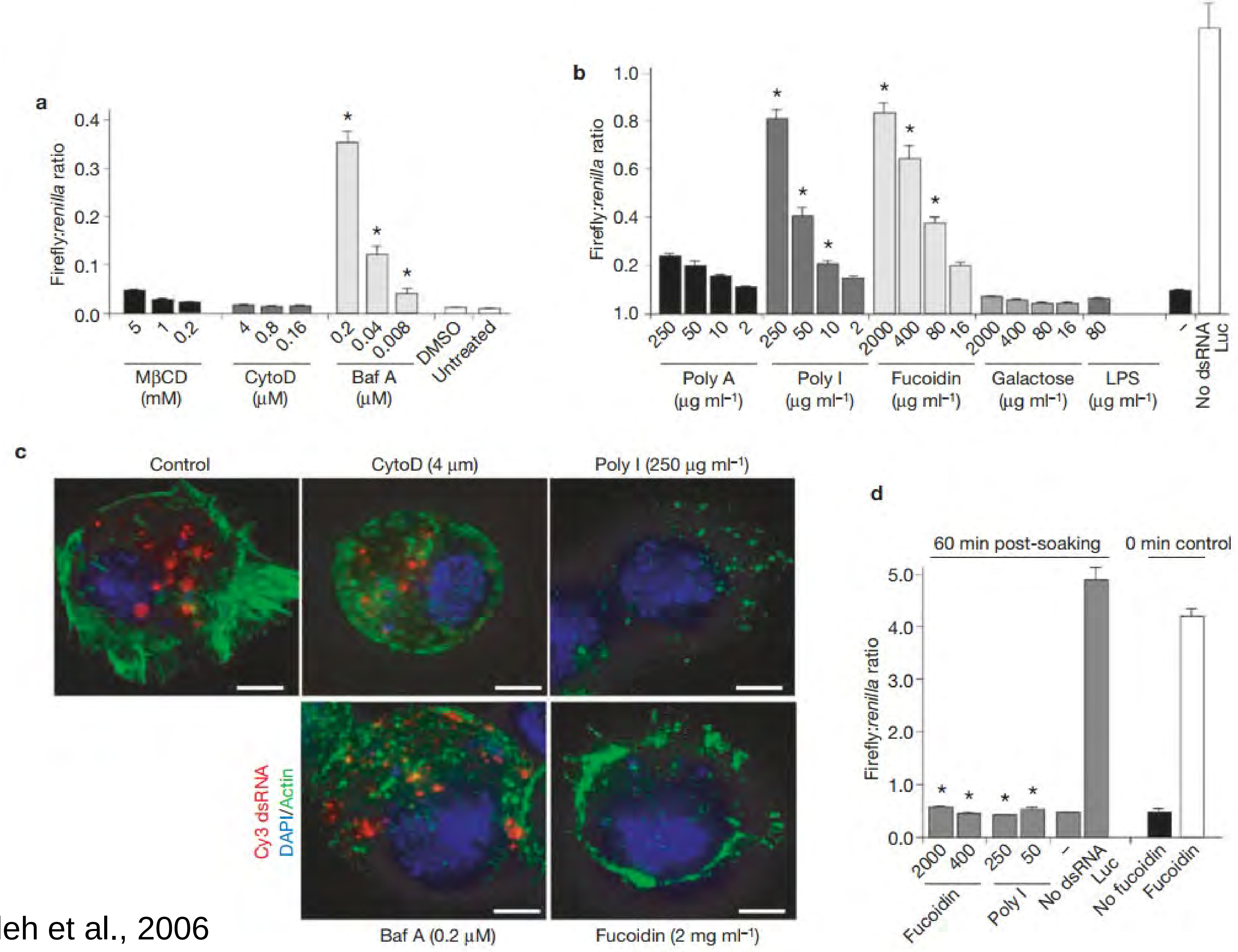


Da Xiao et al., 2015

Role of clathrin-dependent endocytosis genes in RNAi



Endocytic uptake of dsRNA into *Drosophila* S2 cells is mediated by scavenger receptors

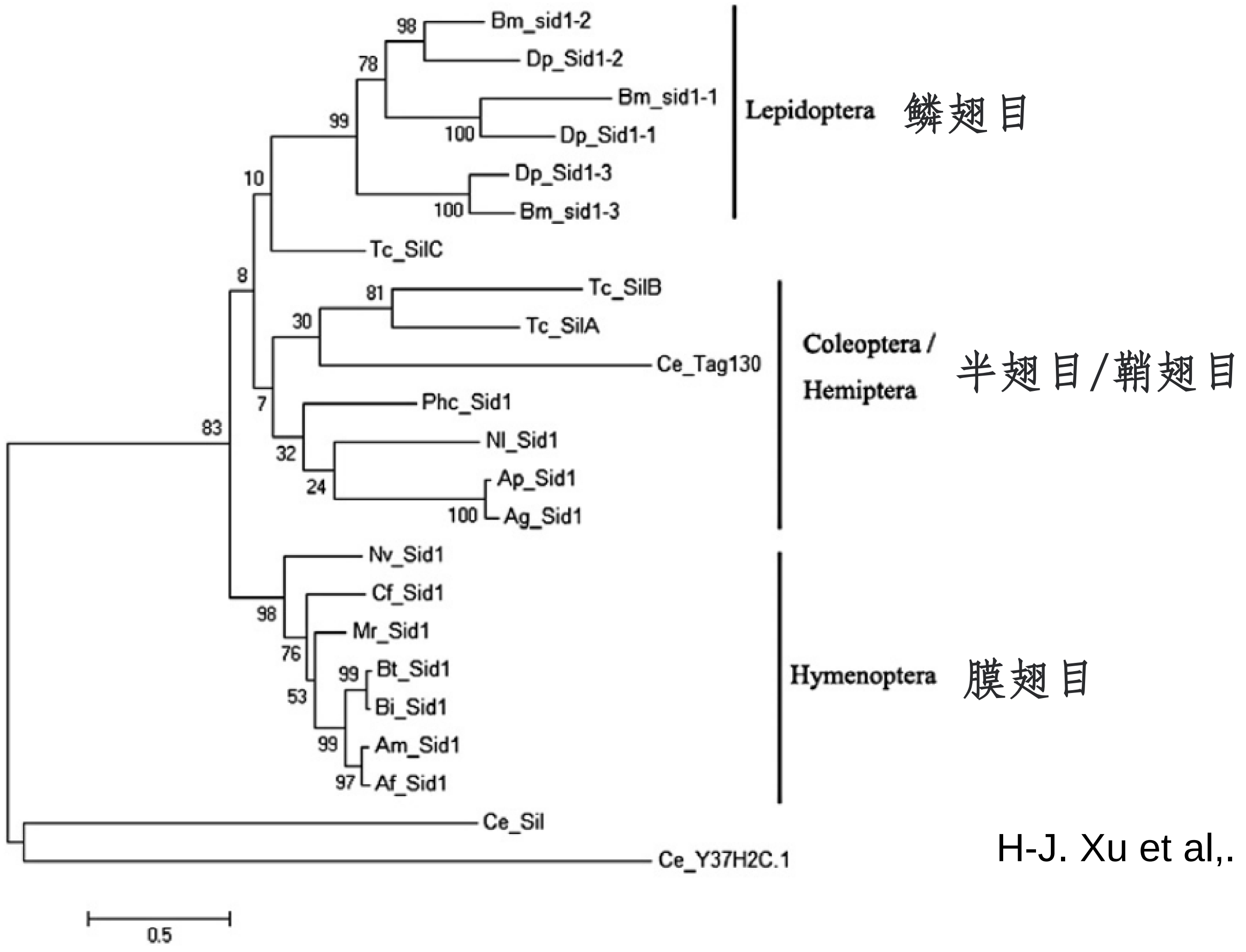


Maria-Carla Saleh et al., 2006

Part 2 Systemic RNAi

The mechanism(s) that mediate systemic RNAi in insects are largely unknown

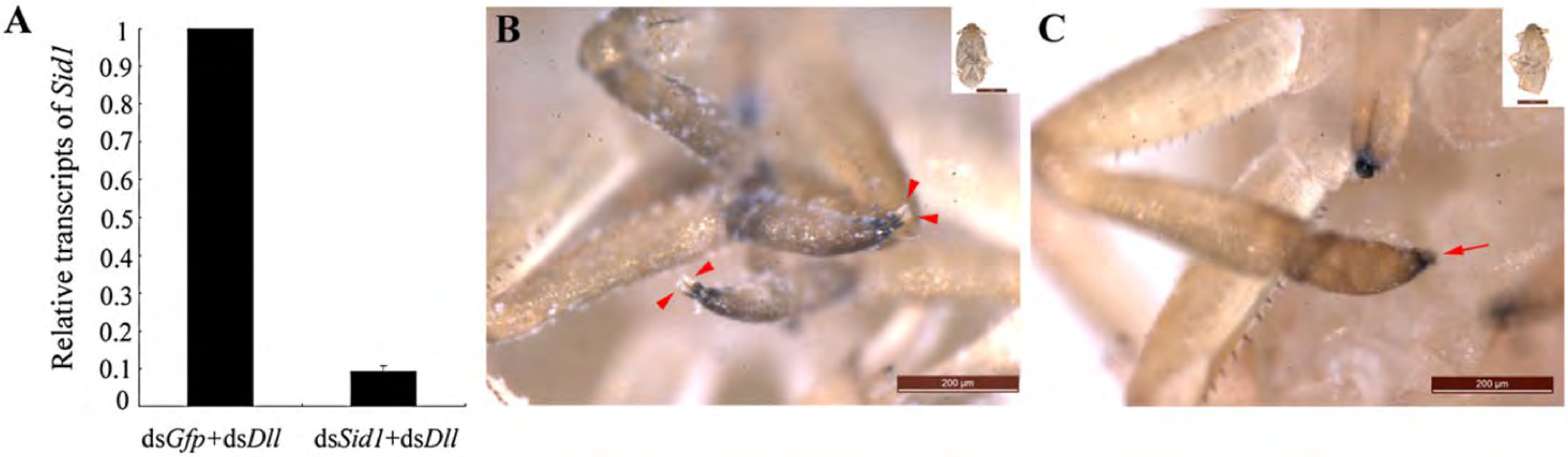
Phylogenetic analysis of Sid-1 proteins



H-J. Xu et al,. 2013

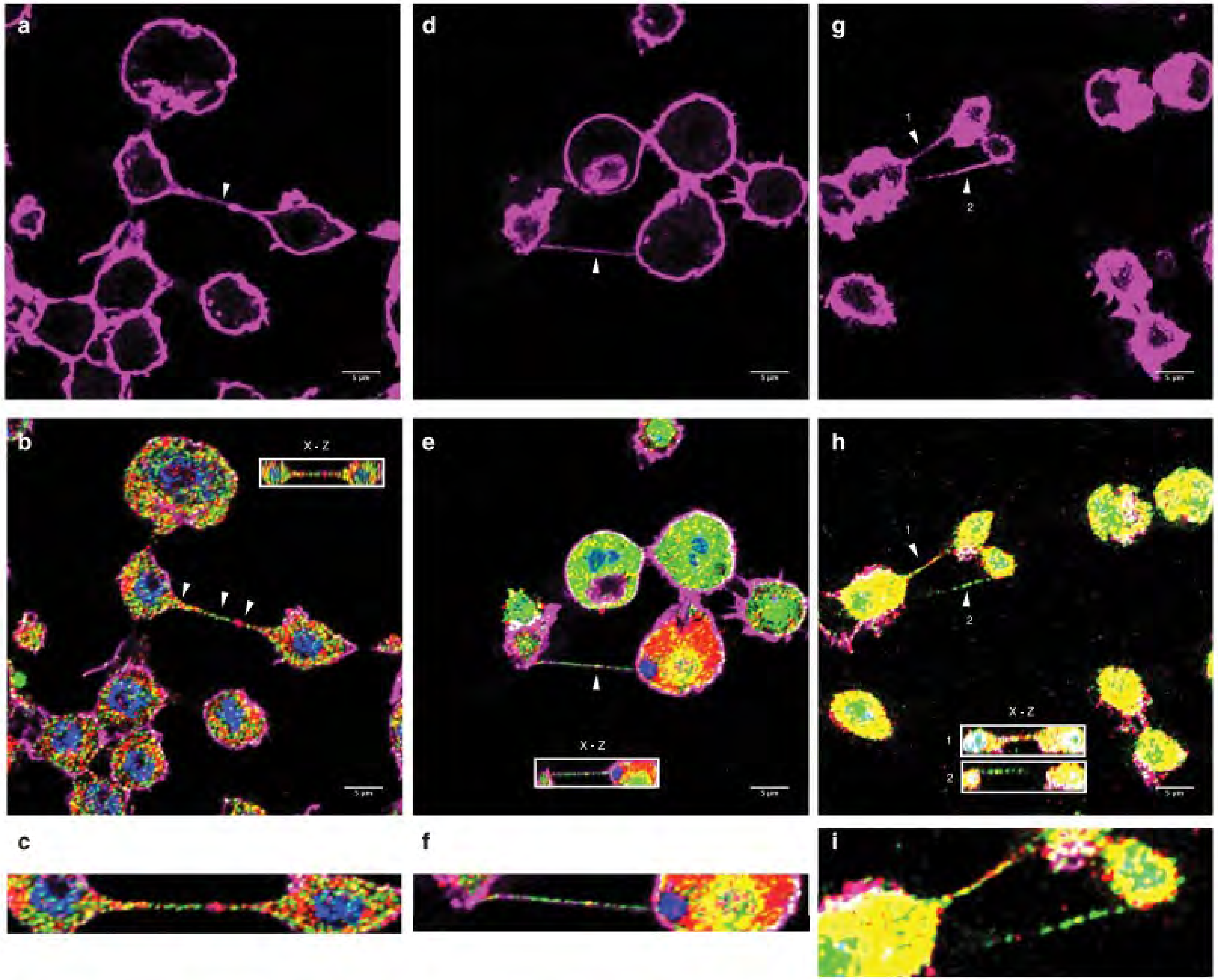
Sid-1 genes seem to be present in most insect species, but so far no *Sid-2* genes have been found in insect species whose genomes have been sequenced

Knockdown of *sid-1* abolished systemic RNAi in BPH



H-J. Xu et al,. 2013

The RNAi machinery localizes along nanotube-like structures



Rab7 / Ago2 / DAPI / Phalloidin

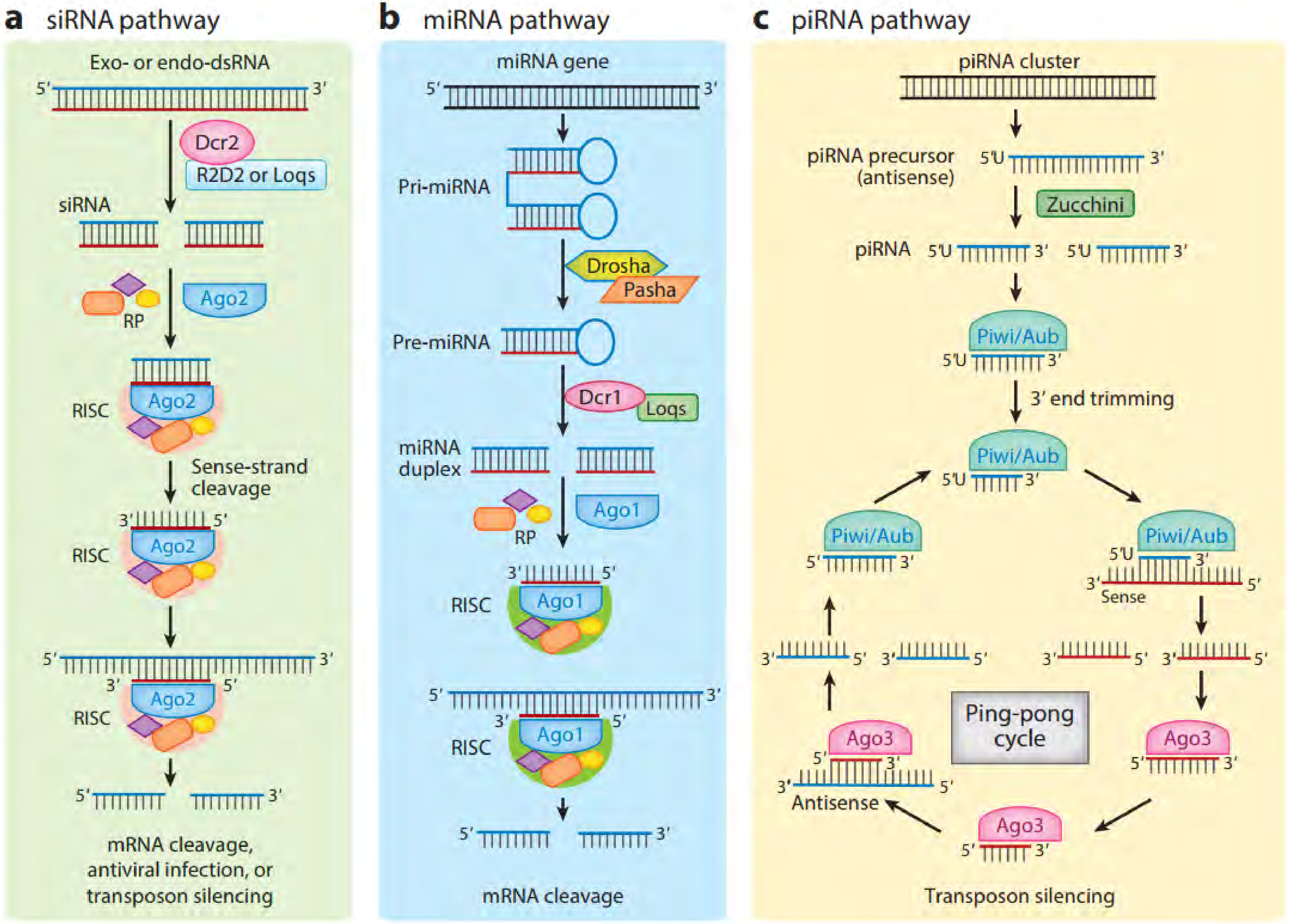
dsRNA / Ago2 / DAPI / Phalloidin

CG4572 / Ago2 / DAPI / Phalloidin

Margot Karlikow et al,. 2016

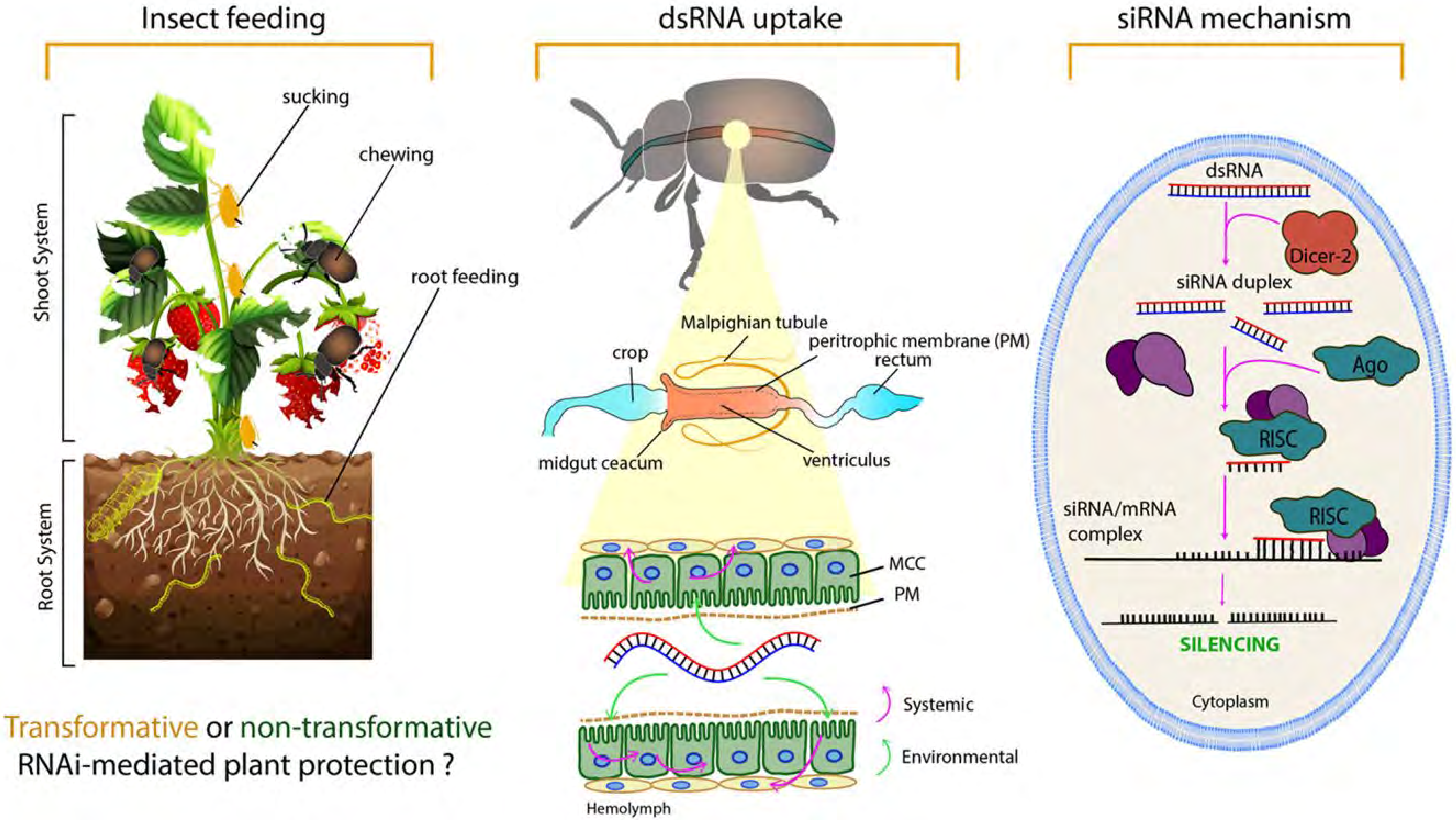
Cell autonomous RNAi

Mechanisms of different RNA interference (RNAi) pathways identified in insects



Kun Yan Zhu and Subba Reddy Palli,. 2023

Conclusions



Transformative or non-transformative RNAi-mediated plant protection ?

Mallikarjuna R. Joga et al,. 2016

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[2] J R Kennerdell 1, R W Carthew. Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. Cell. 1998 Dec 23;95(7):1017-26. doi: 10.1016/s0092-8674(00)81725-0.

[3] Honggang Tian et al. Developmental Control of a Lepidopteran Pest Spodoptera exigua by Ingestion of Bacteria Expressing dsRNA of a Non-Midgut Gene. Plos one. . 2009 Jul 13;4(7):e6225. doi: 10.1371/journal.pone.0006225.

[4] James A Baum et al. Control of coleopteran insect pests through RNA interference. Nature Biotechnol. 2007 Nov;25(11):1322-6.doi:10.1038/nbt1359. Epub 2007 Nov 4.

[5] Renata Bolognesi et al. Characterizing the Mechanism of Action of Double Stranded RNA Activity against Western Corn Rootworm(Diabrotica virgifera virgifera LeConte). Transgenic Res. 2013 Dec;22(6):1207-22.doi: 10.1007/s11248-013-9716-5. Epub 2013 Jun 8.

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[7] Jiang Zhang et al. Pest control. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science. 2015 Feb 27;347(6225):991-4.doi: 10.1126/science.1261680.

[8] K. Cappelle et al. The involvement of clathrin-mediated endocytosis and two SID transmembrane proteins in double-stranded RNA uptake in the Colorado potato beetle midgut. Insect Mol Bio. 2016 Jun;25(3):315-23.doi: 10.1111/imb.12222. Epub 2016 Mar 9.

[9] Maria-Carla Saleh et al. The endocytic pathway mediates cell entry of dsRNA to induce RNAi silencing. Nat Cell Bio. 2006 Aug;8(8):793-802. doi: 10.1038/ncb1439.

[10] Margot Karlikow et al. Drosophila cells use nanotube-like structures to transfer dsRNA and RNAi machinery between cells. Sci Rep. 2016 Jun 3:6:27085.doi: 10.1038/srep27085.

[11] Kun Yan Zhu and Subba Reddy Palli. Mechanisms, Applications, and Challenges of Insect RNA Interference. Annu Rev Entomol. 2020 Jan 7:65:293-311.doi: 10.1146/annurev-ento-011019-025224. Epub 2019 Oct 14.

[12] Mallikarjuna R. Joga et al. RNAi Efficiency, Systemic Properties, and Novel Delivery Methods for Pest Insect Control: What We Know So Far. Front Physiol. 2016 Nov 17:7:553.doi: 10.3389/fphys.2016.00553. eCollection 2016.

Thanks for your listening!

Challenges of oral RNAi in Agricultural Pest Control

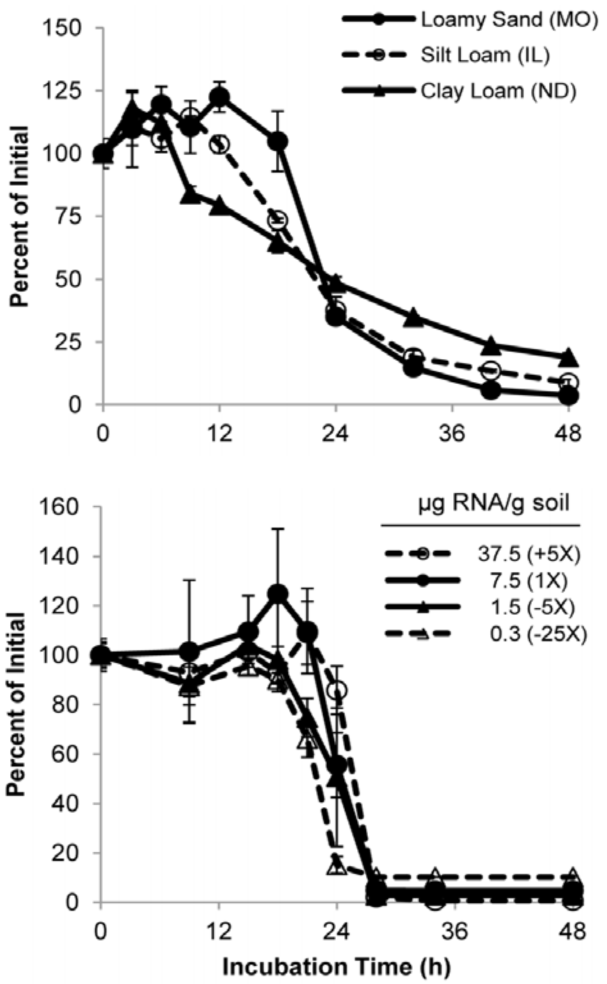
纪小小

Challenges

- Stability of dsRNA
  - In the environment
  - In the gut of insects
  - In insect hemolymph
- Oral RNAi efficiency
  - Different species
  - Length of dsRNA molecule
  - Nontarget effects of dsRNA
  - Resistance development
- The cost of oral RNAi

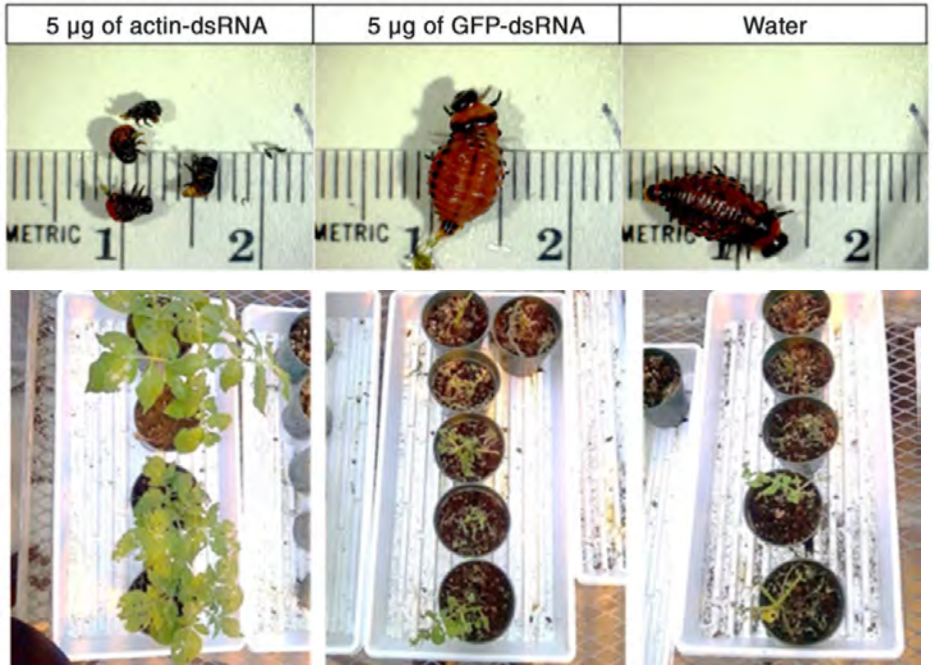
Stability of dsRNA in the environment

Put DvSnf7 dsRNA into soil



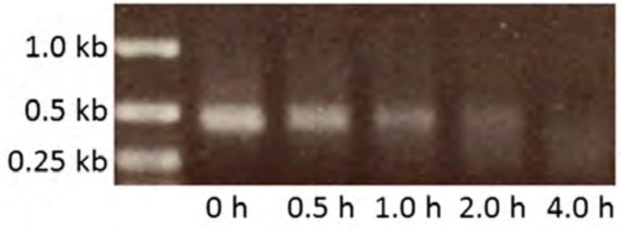
(Dubelman et al., *PLoS One*, 2014)

Colorado potato beetle larvae fed leaves treated with CPB actin-dsRNA



7 days

24 days

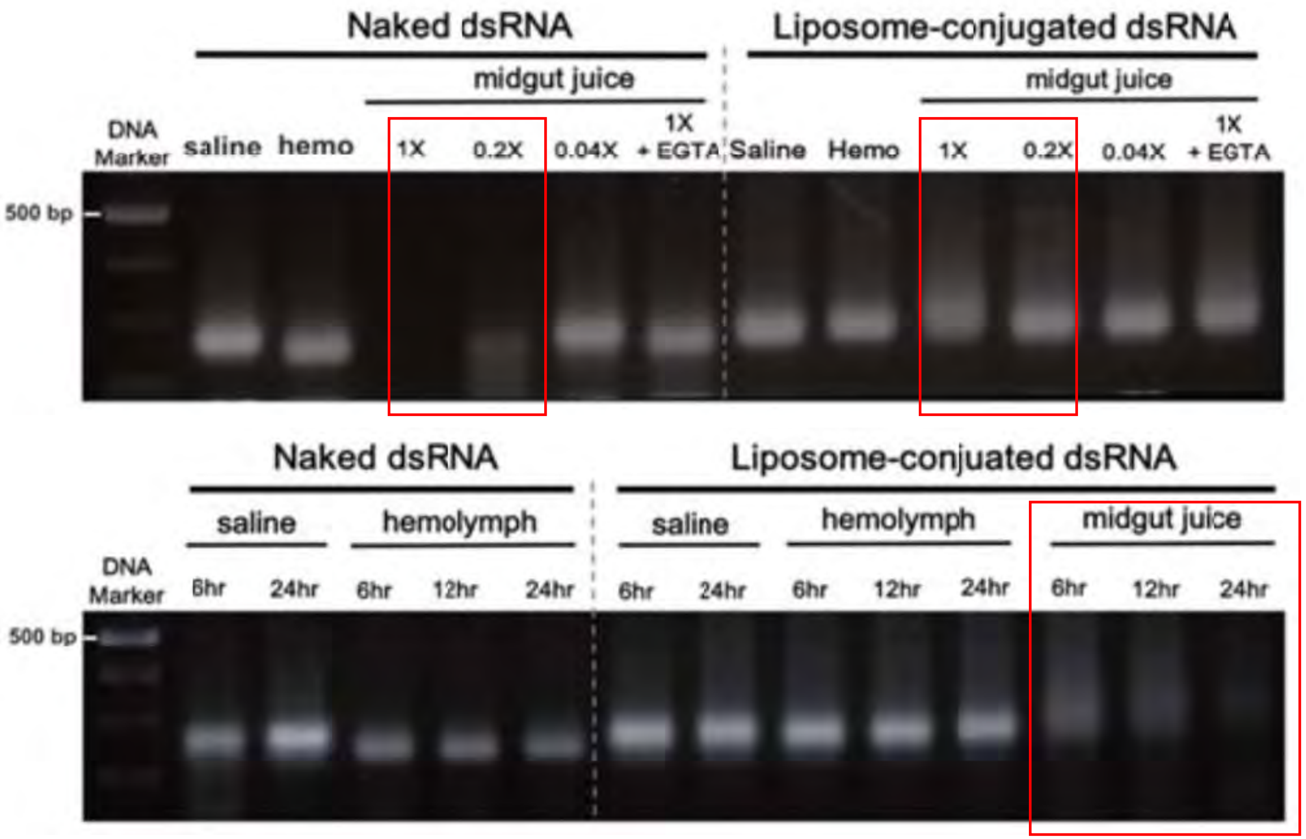


UV treatment (254 nm, 1500 µW cm^{-2})

(San Miguel and Scott, *Pest management science*, 2016)

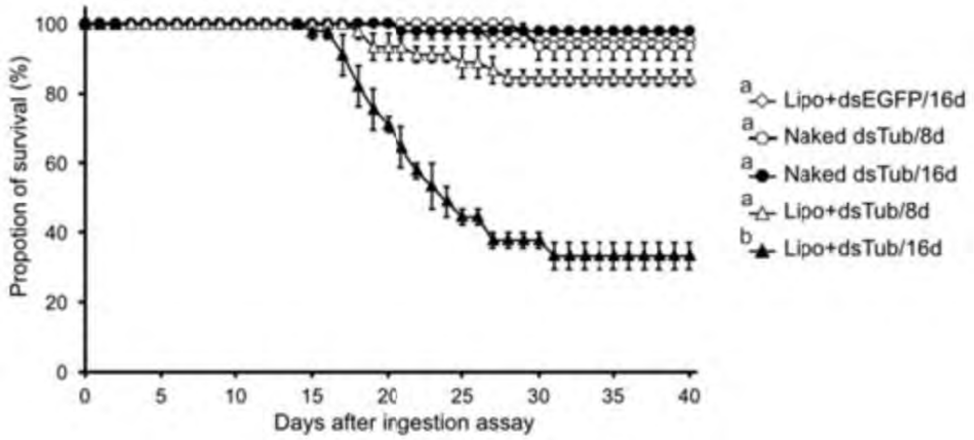
Stability of dsRNA in the gut of insects

- Digestion of dsRNA by insect gut nucleases
- Chemical hydrolysis of dsRNA in insect gut



1 h

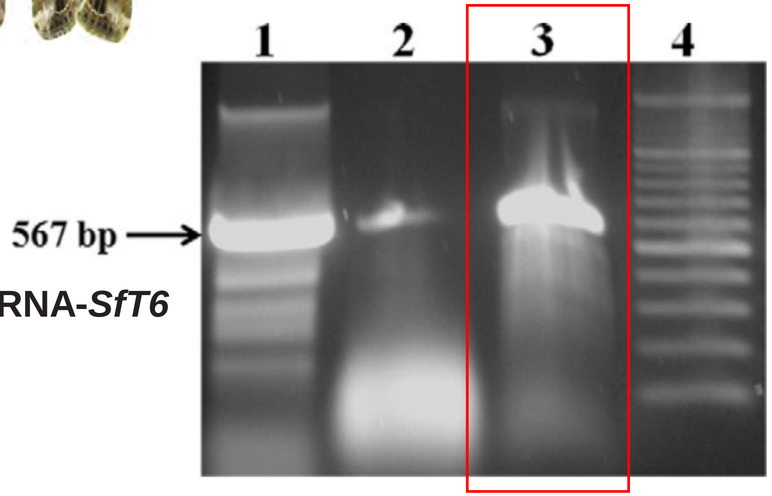
German cockroach
α-tubulin (*tub*) gene



(Lin et al., *Pest management science*, 2017)

Stability of dsRNA in the gut of insects

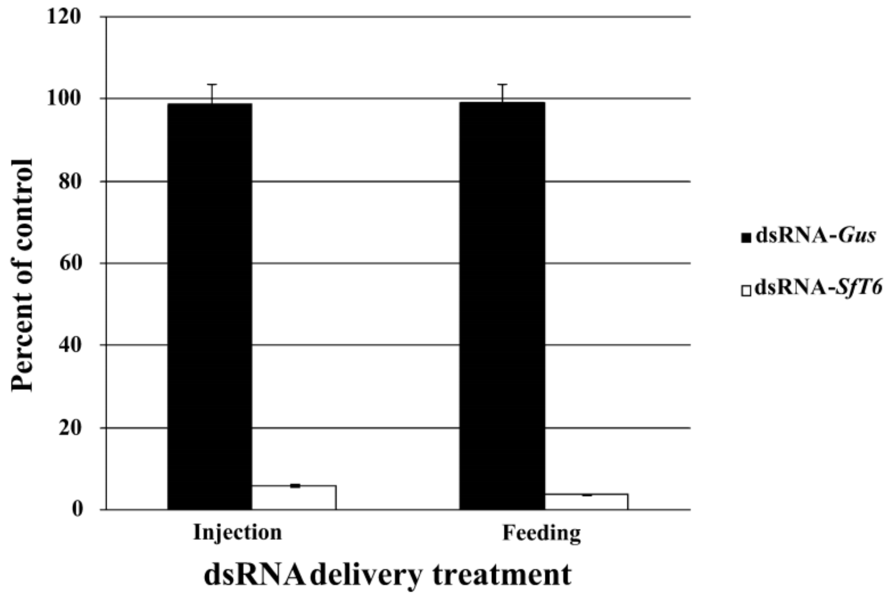
Spodoptera frugiperda
SfT6, a serine-protease gene



dsRNA-*SfT6* in:

1. control: delivery buffer
2. midgut juice non-starved
3. midgut juice 24h starved
4. DNA ladder

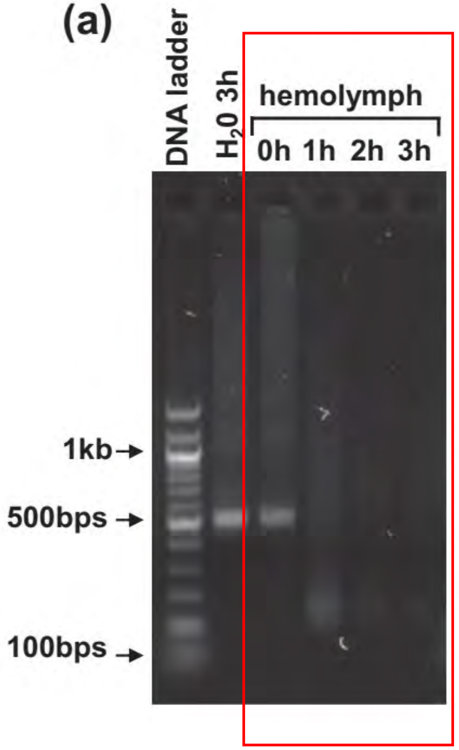
Newly molted 4th-instar *S. frugiperda* larvae were subjected to the **24 h starvation** treatment and **dsRNA-SfT6 droplet–feeding**.

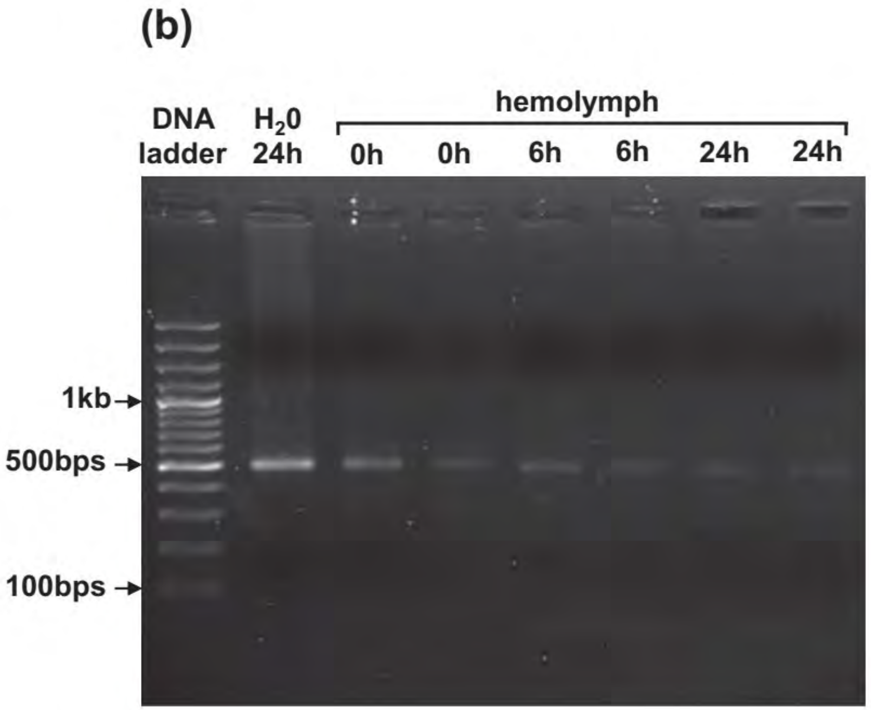


Stress conditions such as starvation may facilitate oral RNAi.

(Rodríguez-Cabrera et al., *Environmental microbiology*, 2010)

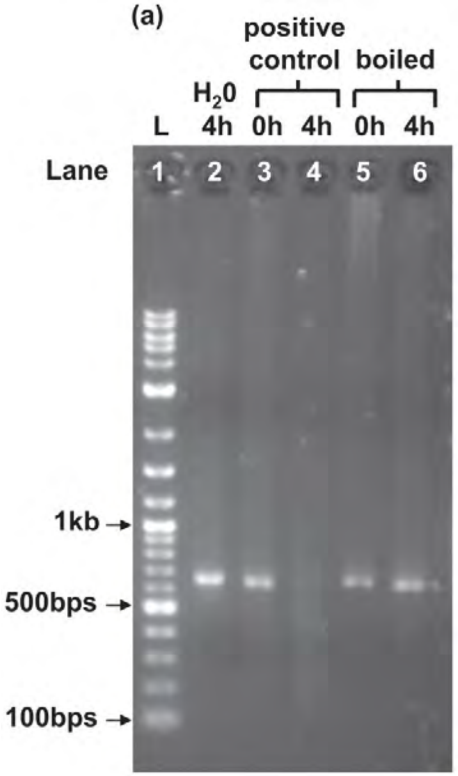
Stability of dsRNA in insect hemolymph



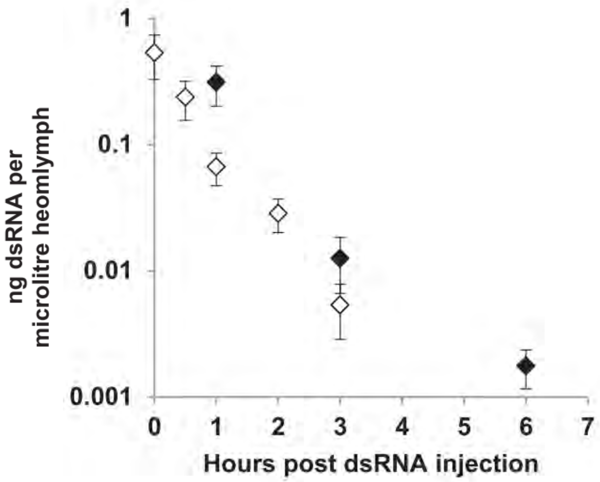


Manduca sexta

Blattella germanica
German cockroach

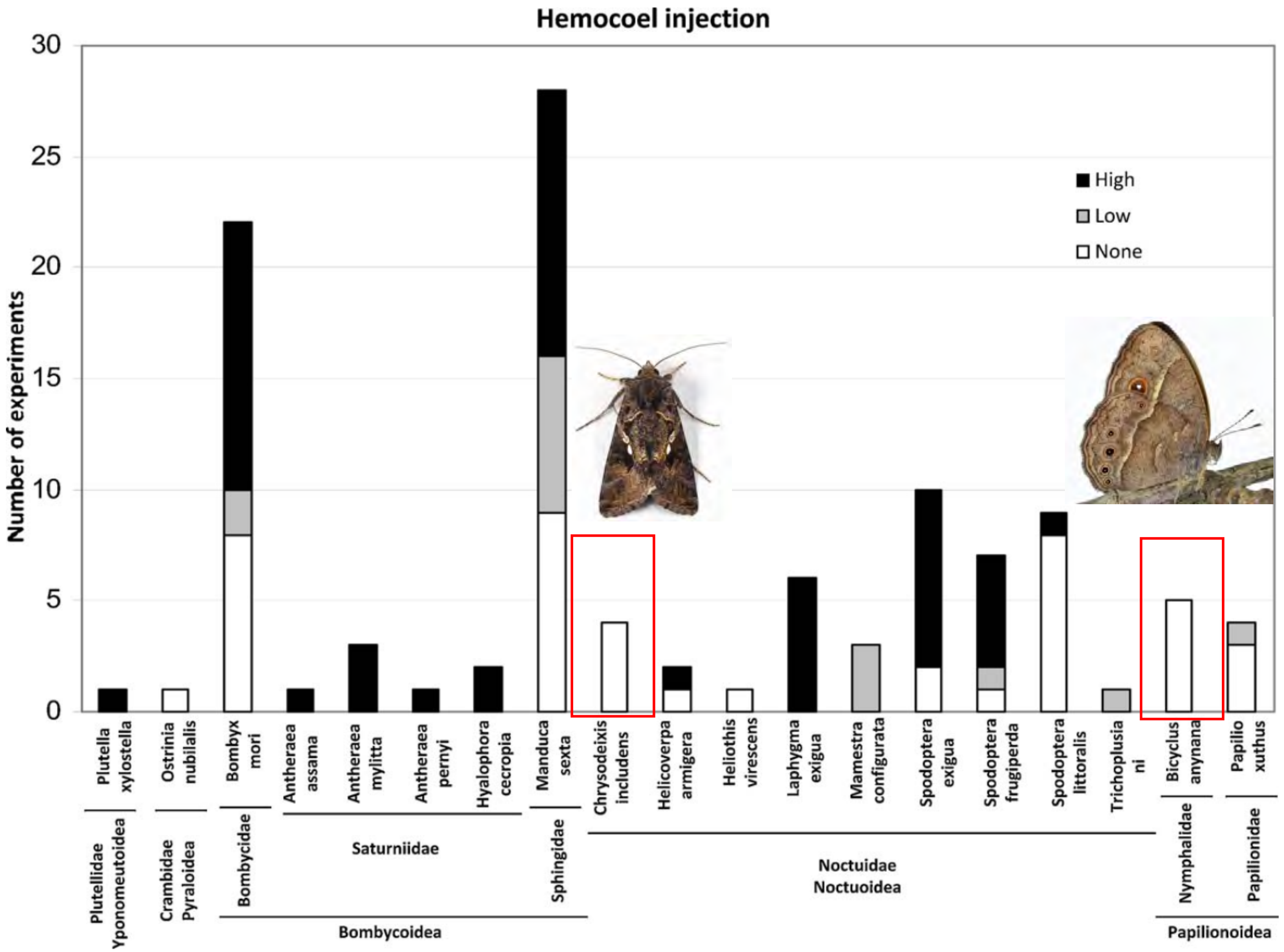


Persistence of dsRNA in *Manduca sexta* hemolymph

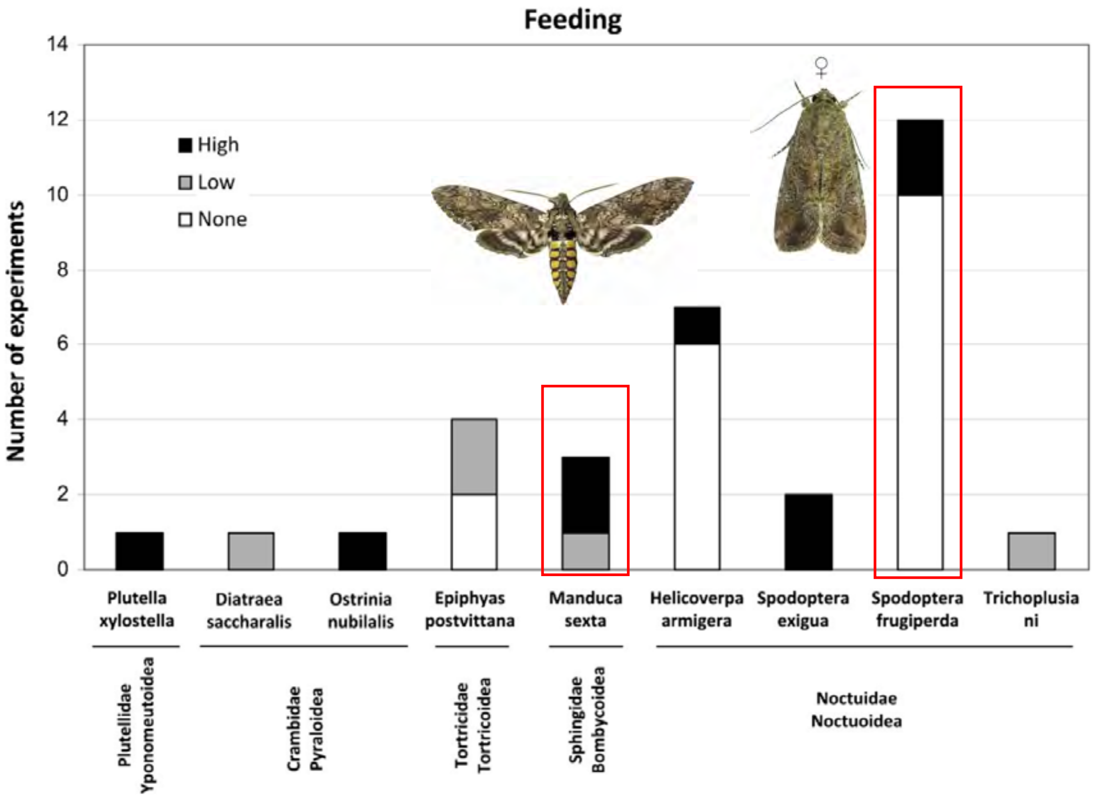


(Garbutt et al., *Journal of insect physiology*, 2013)

RNAi efficiency in different species



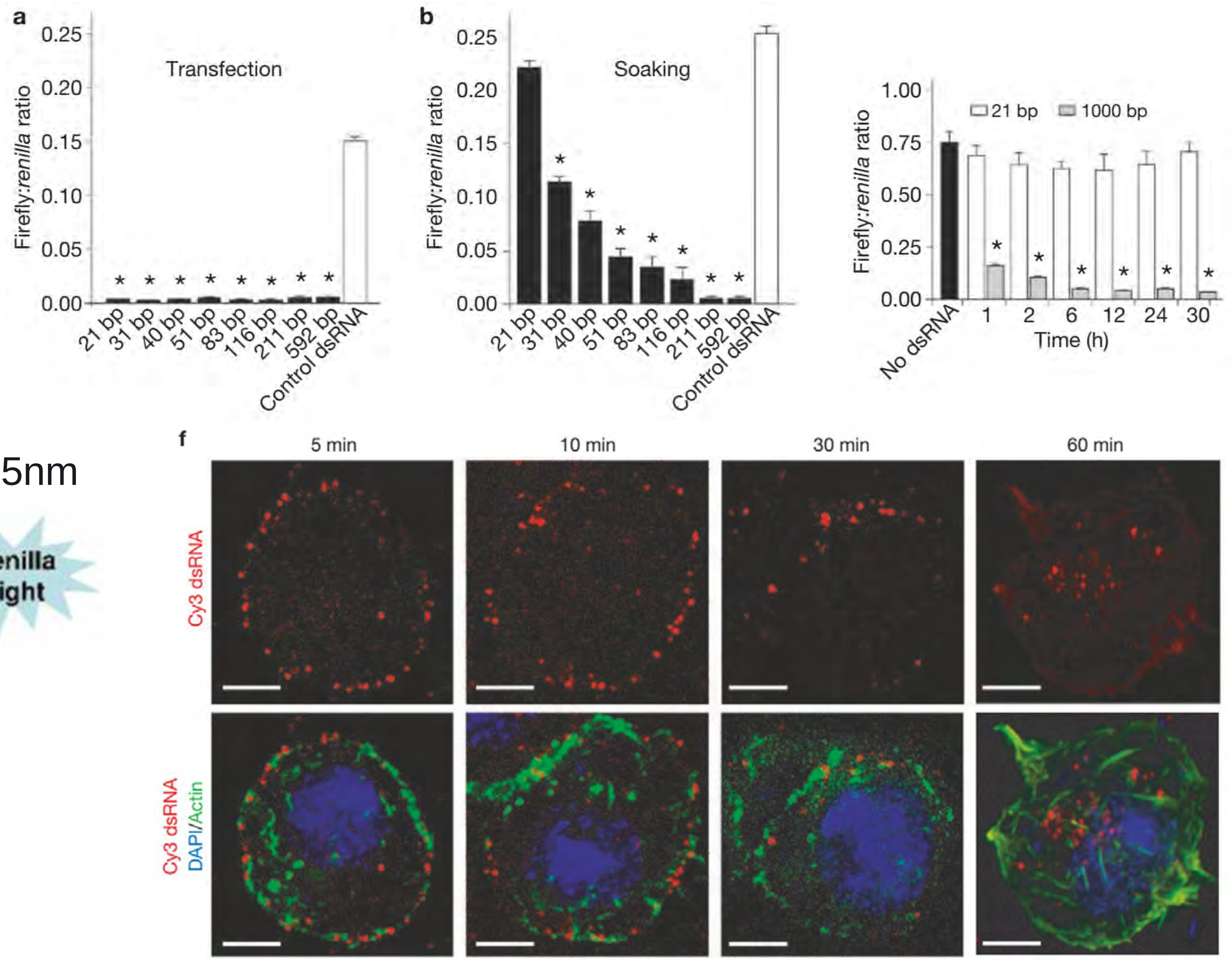
cellular uptake proteins;
core RNAi machinery enzymes;
dsRNA degrading enzymes…



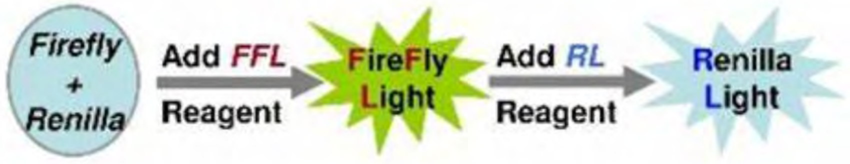
(Terenius et al., *Journal of insect physiology*, 2011)

Length of dsRNA molecule influences RNAi efficiency

S2 cells were cotransfected with expression plasmids encoding firefly and *Renilla luciferase*.



560nm 465nm



+ firefly *luciferase dsRNA*

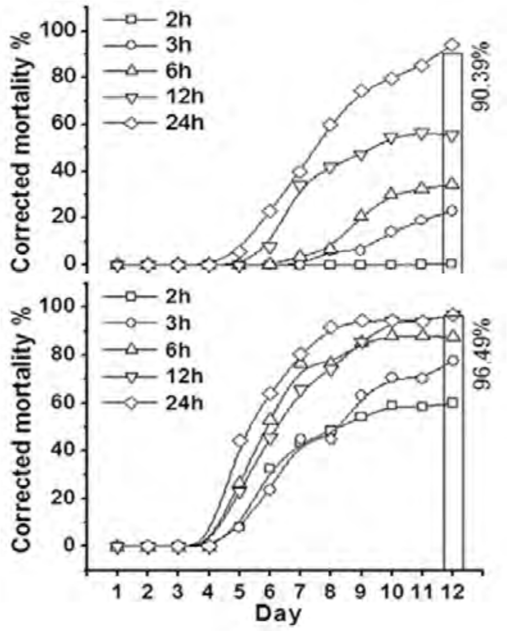
⬇

Firefly:*Renilla* Light ↓

(Saleh et al., *Nature cell biology*, 2006)

Length of dsRNA molecule influences RNAi efficiency

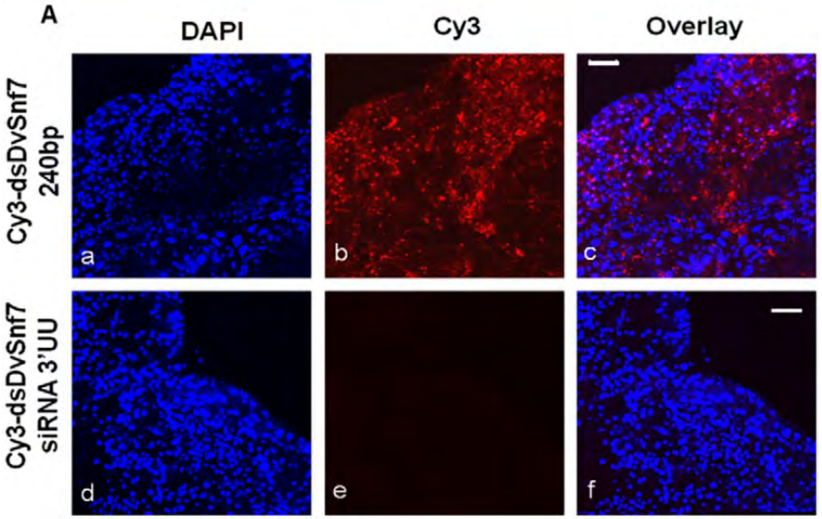
Western Corn Rootworm **second instar larvae** fed on diet overlaid with 1000 ng/mL DvSnf7 dsRNA for **5 days**.

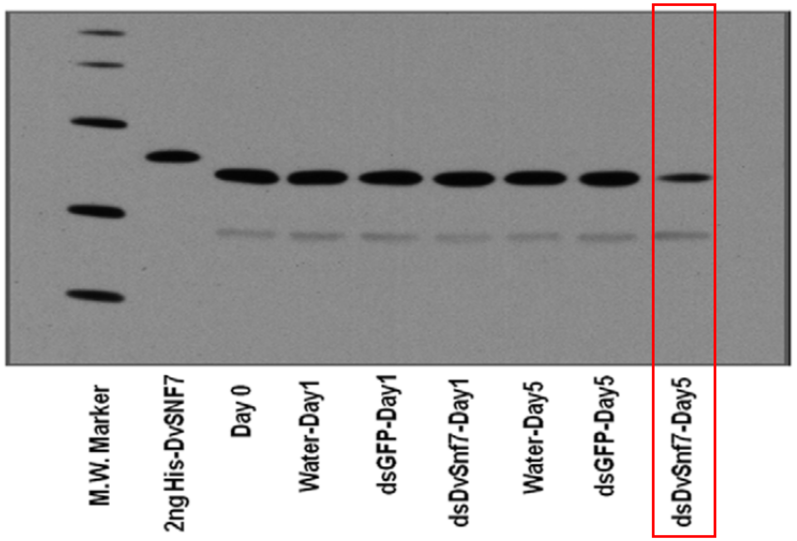


50 ng/mL

1000 ng/mL

dsRNA Tested	Description	Percent Mortality[1, 2]
27	27 bp without carrier	0
27_40	27 bp in neutral carrier to 40 bp	0
27_50	27 bp in neutral carrier to 50 bp	16
27_60	27 bp in neutral carrier to 60 bp	68*
27_70	27 bp in neutral carrier to 70 bp	95*
27_80	27 bp in neutral carrier to 80 bp	95*
27_90	27 bp in neutral carrier to 90 bp	95*
27_100	27 bp in neutral carrier to 100 bp	95*
27_150	27 bp in neutral carrier to 150 bp	96*
27_240	27 bp in neutral carrier to 240 bp	95*
240 bp	DvSnf7 full 240 bp, no carrier	95*
240 Filler	Neutral carrier sequence, 240 bp	0

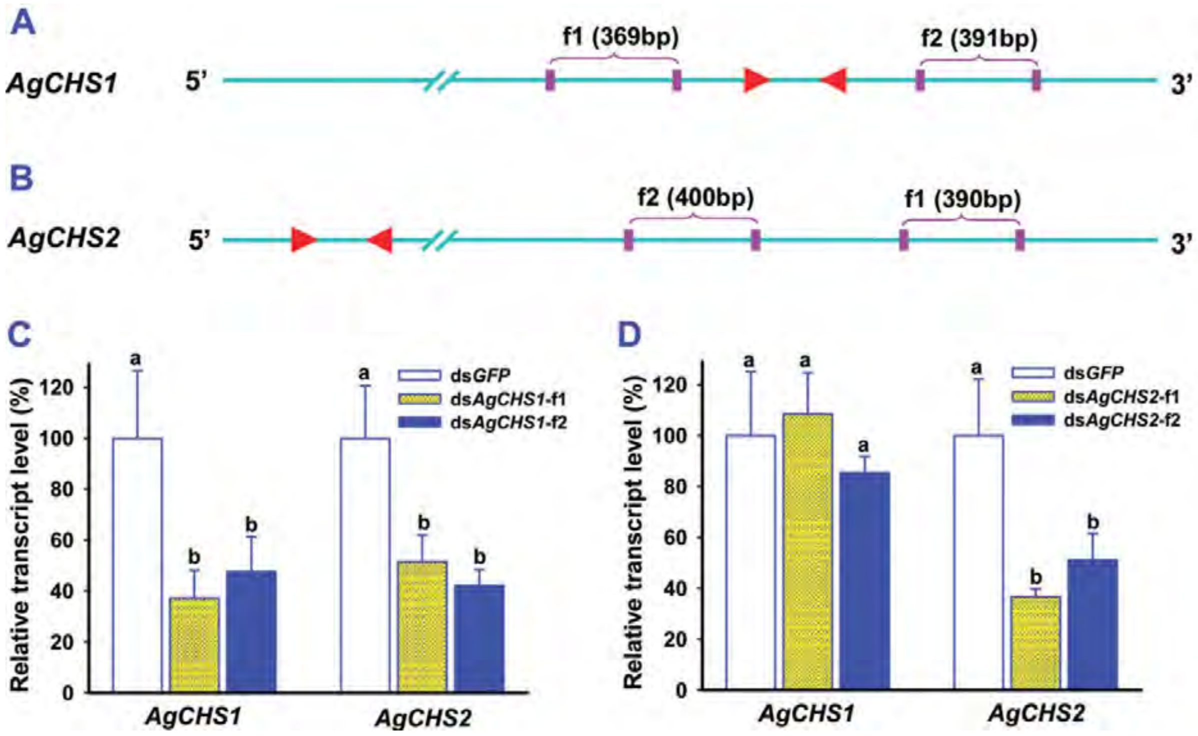




The majority of studies demonstrate success with dsRNA ranging from **140 to 520 nucleotides** in length.

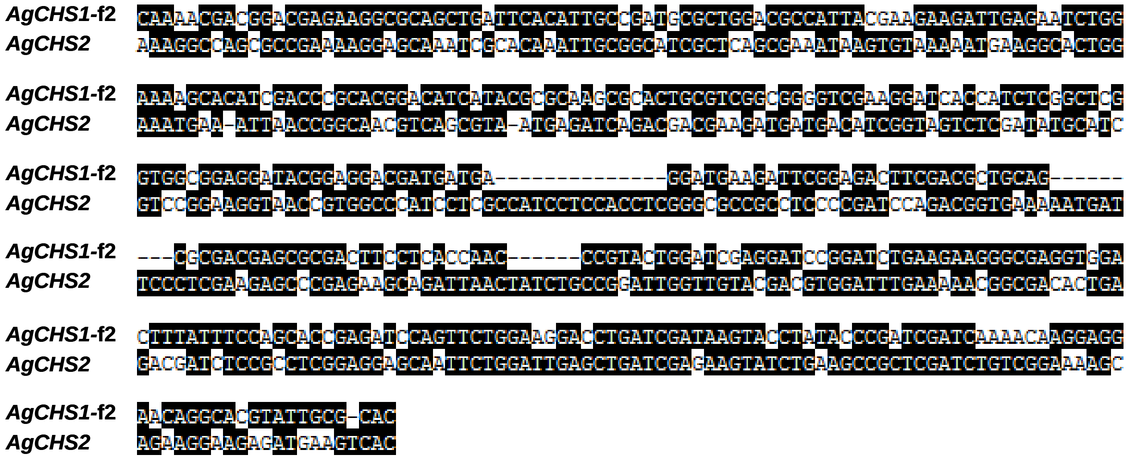
(Bolognesi et al., *PloS one*, 2012)

Nontarget effects of dsRNA



AgCHS1-f1 and *AgCHS2* (identity 70.4%)

AgCHS1-f2 and *AgCHS2* (identity 54.8%)



AgCHS2-f1 and *AgCHS1* (identity 63.7%)

AgCHS2-f2 and *AgCHS1* (identity 53.8%)

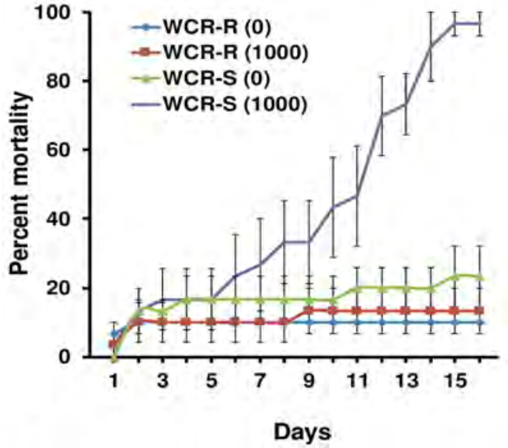
RNAi triggered by one or more short but less conserved sequences in their dsRNAs.

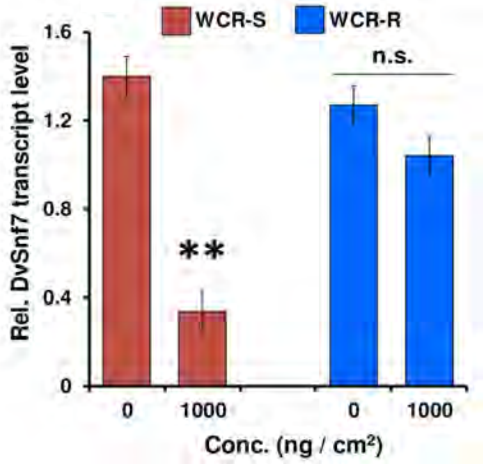
(Zhang et al., *Insect Molecular Biology*, 2010)

Resistance of RNAi

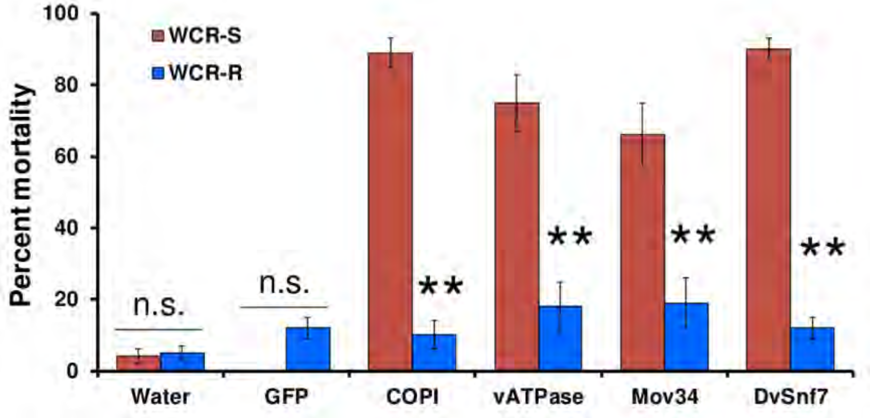
WCR exposed to diets overlaid with:

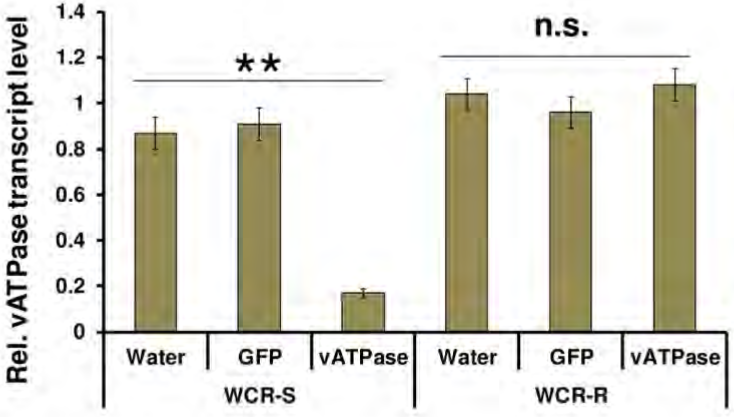
DvSnf7 dsRNA (1000 ng/cm^2)
or water (0 ng/cm^2)



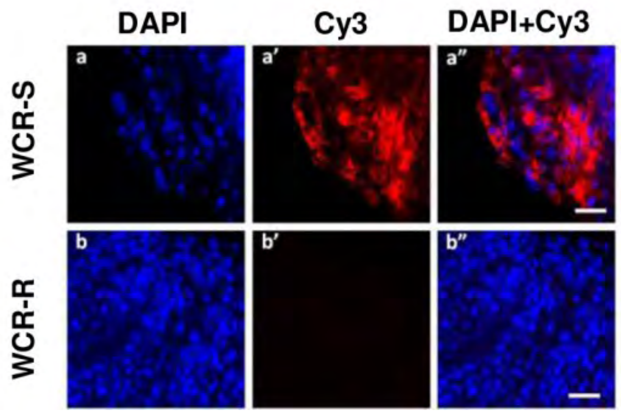


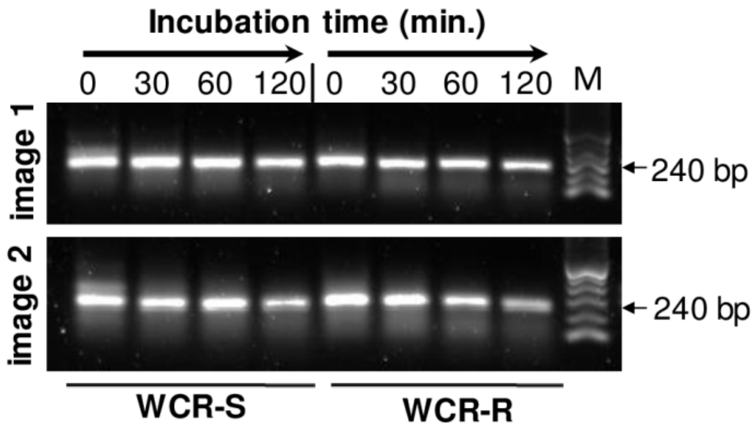
300 ng/cm^2 dsRNA
(GFP, COPIβ, vATPaseA, mov34 or DvSnf7)
or water (0 ng/cm^2)





Cy3-labeled 240bp DvSnf7 dsRNA

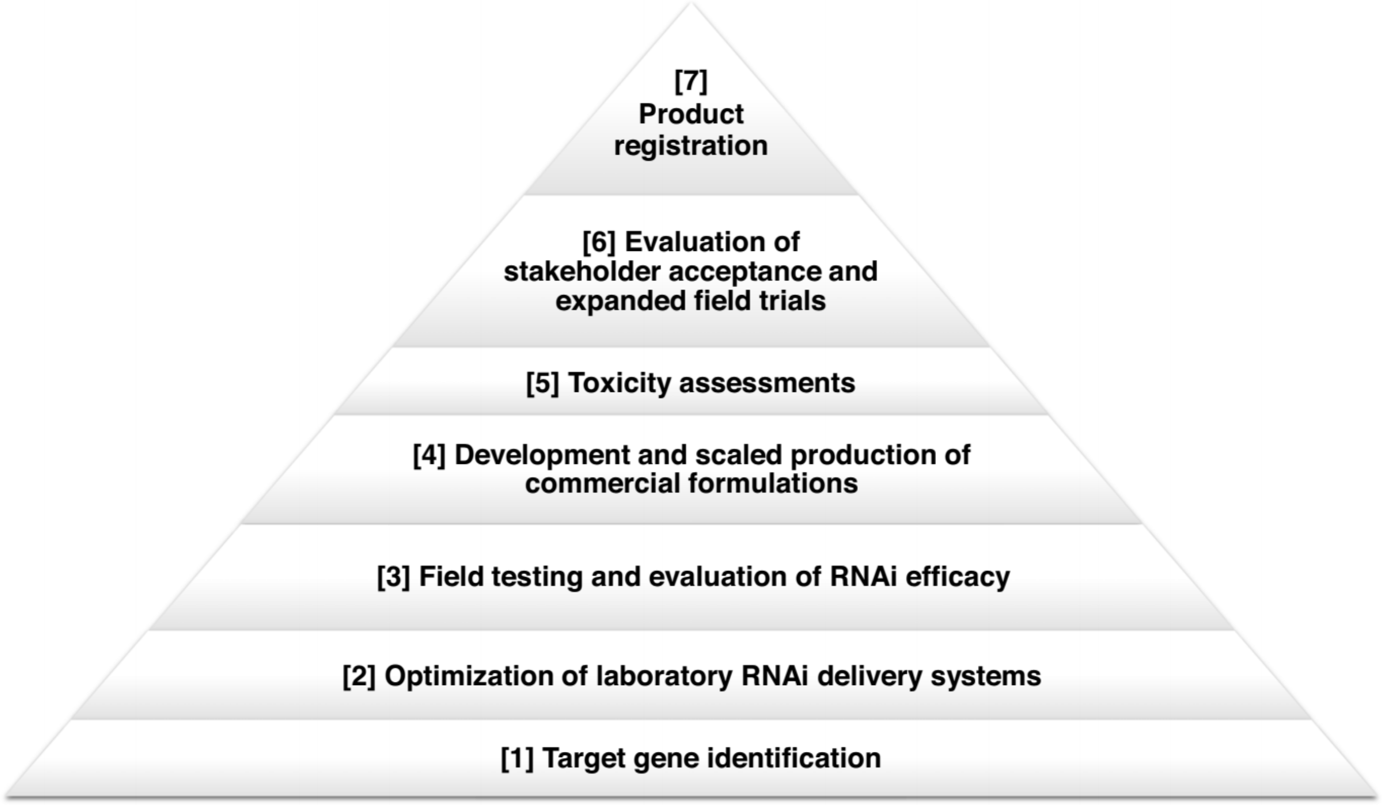




(Khajuria et al., *PloS one*, 2018)

The cost of dsRNA

- RNAi transgenic plants.
- T7 polymerase promoter mediated dsRNA production in vitro (in research).
- Microbially-expressed vector systems (such as bacteria and yeast).



(Wiltshire and Duman-Scheel, *Current opinion in insect science*, 2020)

Challenges

- Stability of dsRNA
  - In the environment (soil 24h, leaf 28d and UV 0.5h)
  - In the gut of insects
    - dsRNA in liposome but not naked dsRNA still exists after 1h midgut juice treatment.
    - Stress conditions such as starvation may facilitate oral RNAi.
  - In insect hemolymph (dsRNA in *Manduca sexta* hemolymph degrades fast)
- Oral RNAi efficiency
  - Different species
    - cellular uptake proteins; core RNAi machinery enzymes; dsRNA degrading enzymes
  - Length of dsRNA molecule
    - The majority of studies demonstrate success with dsRNA ranging from **140 to 520 nucleotides** in length
  - Nontarget effects of dsRNA (design specific dsRNA sequence of target gene)
  - Resistance development (mutations of target genes, mutations of RNAi core machinery genes, enhanced dsRNA degradation, **decreased dsRNA uptake** and transport…)
- The cost of dsRNA
  - RNAi transgenic plants
  - Microbially-expressed vector systems

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Thanks！