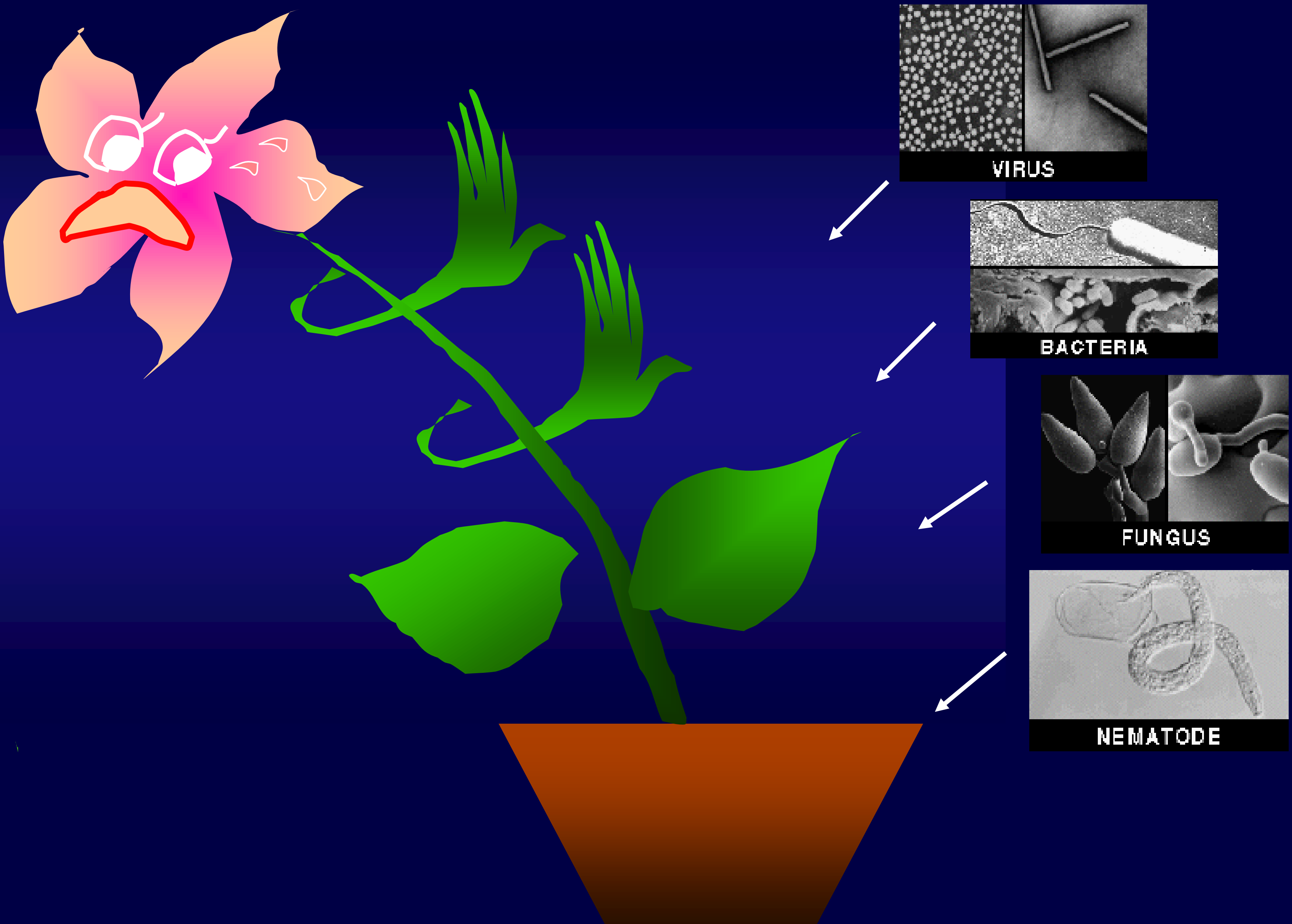
Apollo and Daphne

Plants Are Attacked By Diverse Pathogens



Multiple Layers of Plant Disease Resistance

Non-host resistance
PAMP-Triggered Immunity
Effector-Triggered Immunity

Non-host resistance

Non-host resistance is the most common form of plant disease resistance------but largely unexplored.

- **Can not get into the non-host plants**

The microorganism lacks essential genes that are required for it to penetrate the non-host.

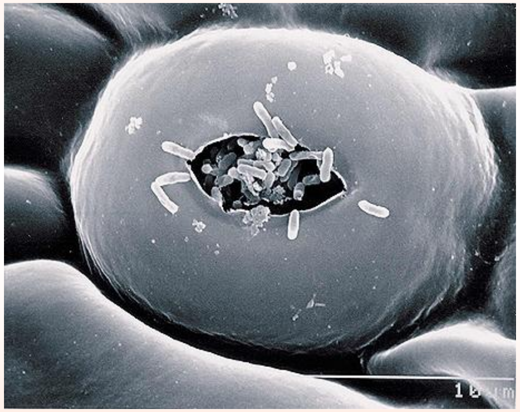
- **Can penetrate the non-host plants, but can not establish a feeding relationship to ensure reproduction**

The microorganism lacks an ability to induce accessibility and/or suppress host defense responses.

Plant-microbial interaction

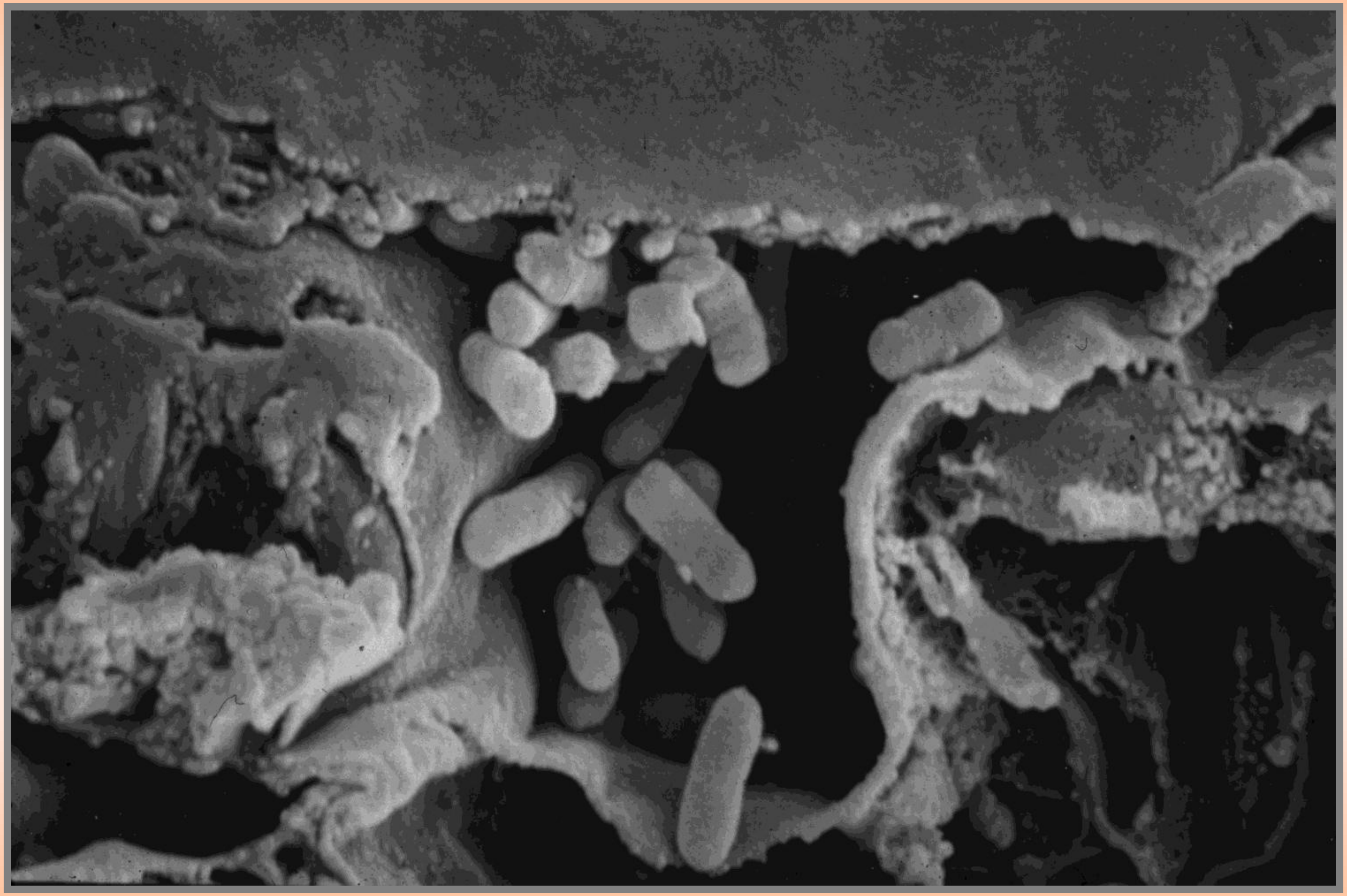
Plants have natural external barriers: cuticle and epidermal cell walls.
Microbes have their ways to overcome these barriers:

•Virus, bacteria and some fungi:
Depend on natural openings (stomata)
or wounds for invasion.



•Most fungi, oomycetes, nematodes and insects:
Secrete hydrolytic enzymes and/or penetrate mechanically for invasion.

Plant-bacterial pathogen interaction

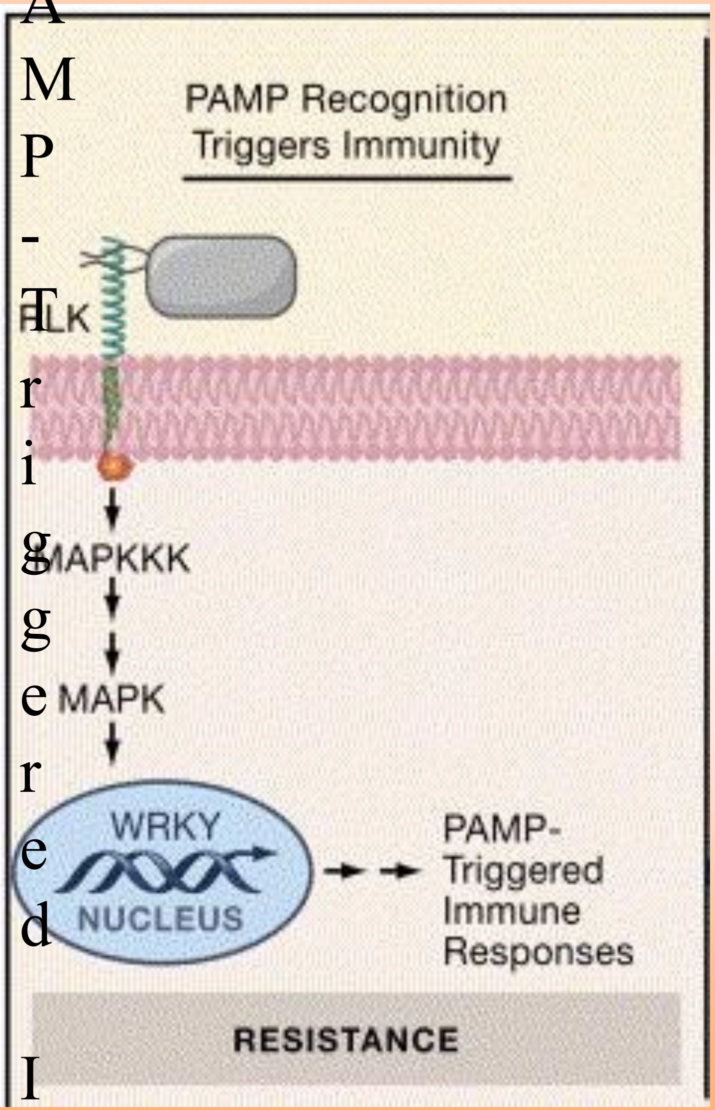


Arabidopsis thaliana* - *Pseudomonas syringae

Accumulated in the apoplast

The Evolution Of Bacterial Resistance In Plants

PAMP-Triggered I



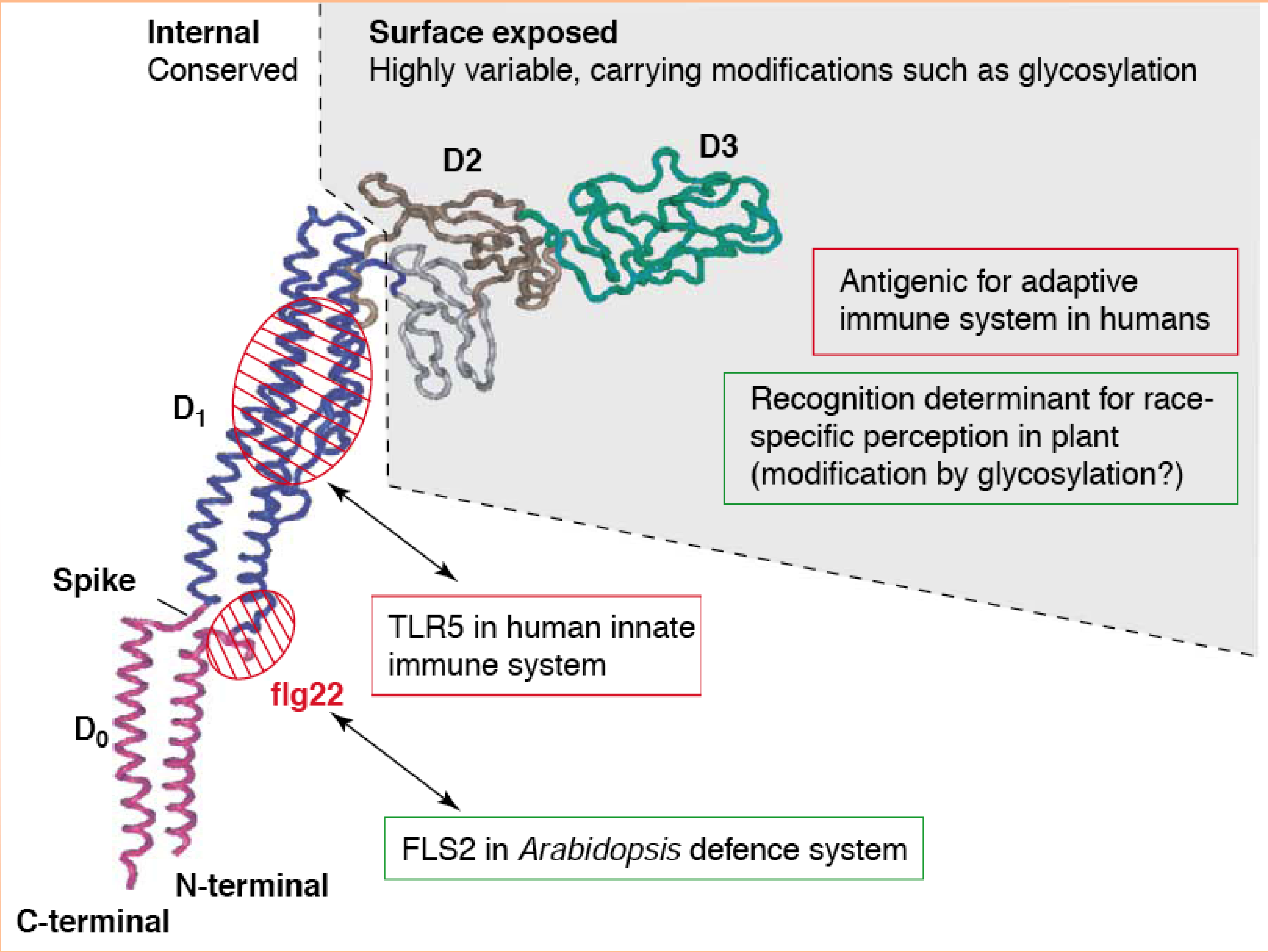
Chisholm et al., 2006

Pathogen-associated molecular pattern----PAMPs

Bacteria: Flagellin and LPS (Gram-negative)
Peptidoglycans (Cell wall of Gram-positive)

Fungi and Oomycetes: Ergosterol
Fungal-specific glycosylated protein
Chitin and beta-glucan (Cell wall components)

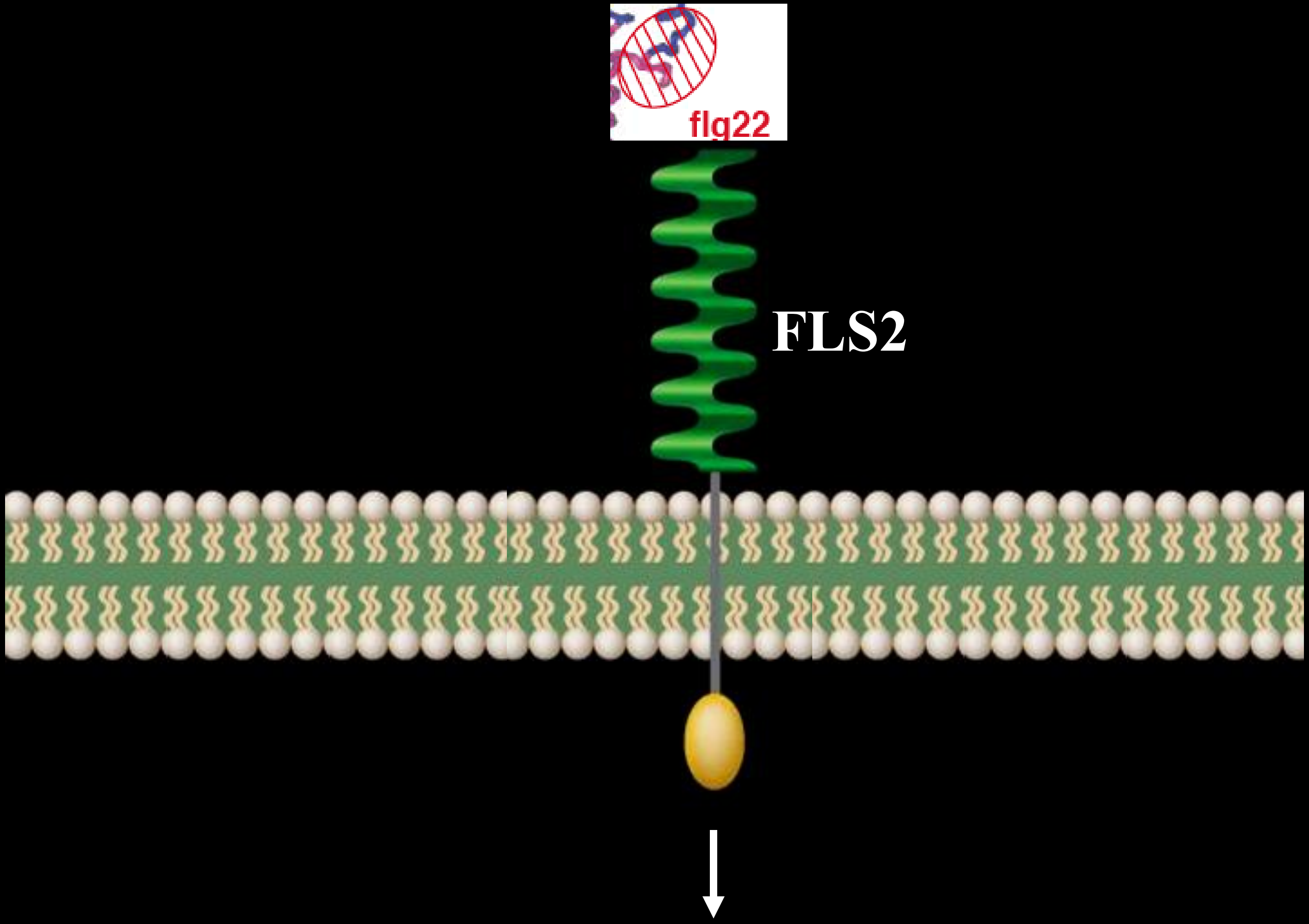
Structure of Flagellin monomer



(from Salmonella typhimurium)

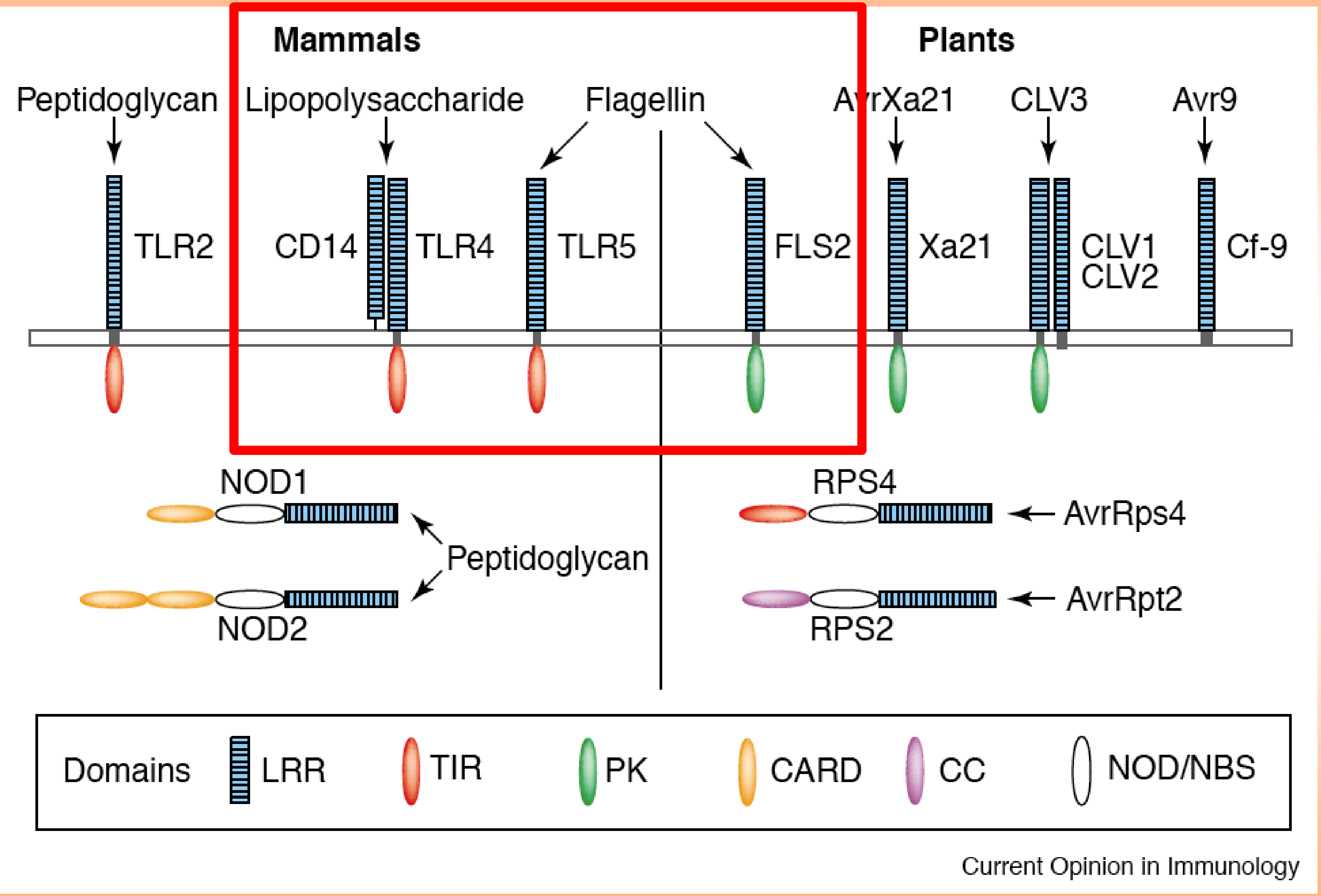
COPB 2005, 8:353-360

Plant Innate Immunity



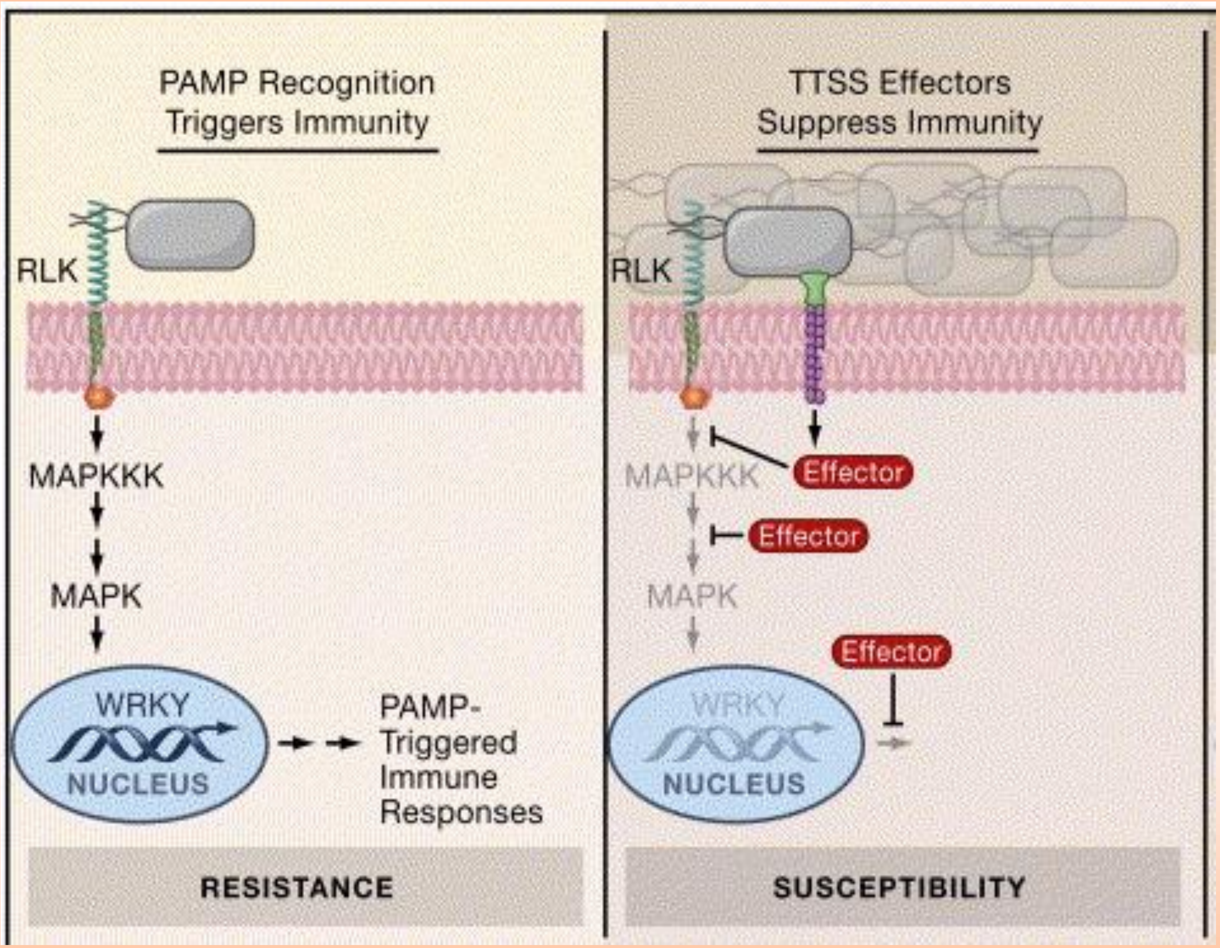
MAPK signaling and resistance responses

Similarity between animal and plant innate immunity and disease resistance



Jones et al., 2004

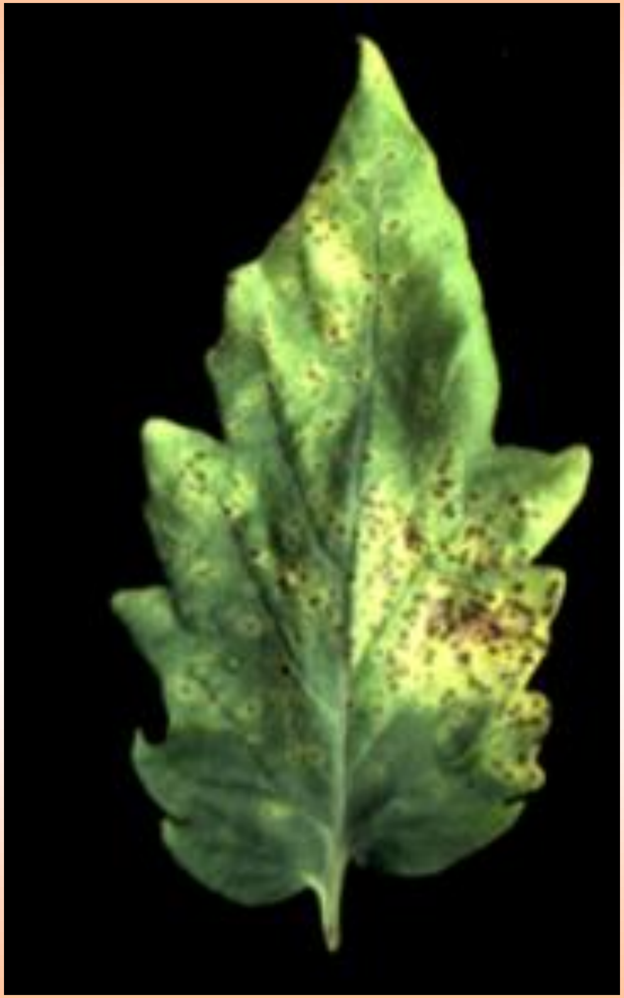
The Evolution Of Bacterial Resistance In Plants



Chisholm et al., 2006

Cloned Effectors from Bacteria

Effector	Organism
AvrRpt2	*Pseudomonas syringae*
AvrB	*Pseudomonas syringae*
AvrRpm1	*Pseudomonas syringae*
HopPtoD2	*Pseudomonas syringae*
AvrPphB	*Pseudomonas syringae*
AvrPtoB	*Pseudomonas syringae*
XopD	*Xanthomonas campestris*
AvrXv4	*Xanthomonas campestris*
AvrBsT	*Xanthomonas campestris*
Avr2	*Cladosporium fulvum*
Avr4	*Cladosporium fulvum*
Avr-Pita	*Magnaporthe grisea*
Pep-13	*Phytophthora sojae*
EPI10	*Phytophthora infestans*
EPI1	*Phytophthora infestans*

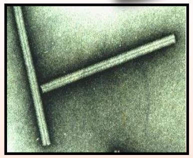


Chisholm et al., 2006. Cell 124,803-14

Tobacco Responses to TMV

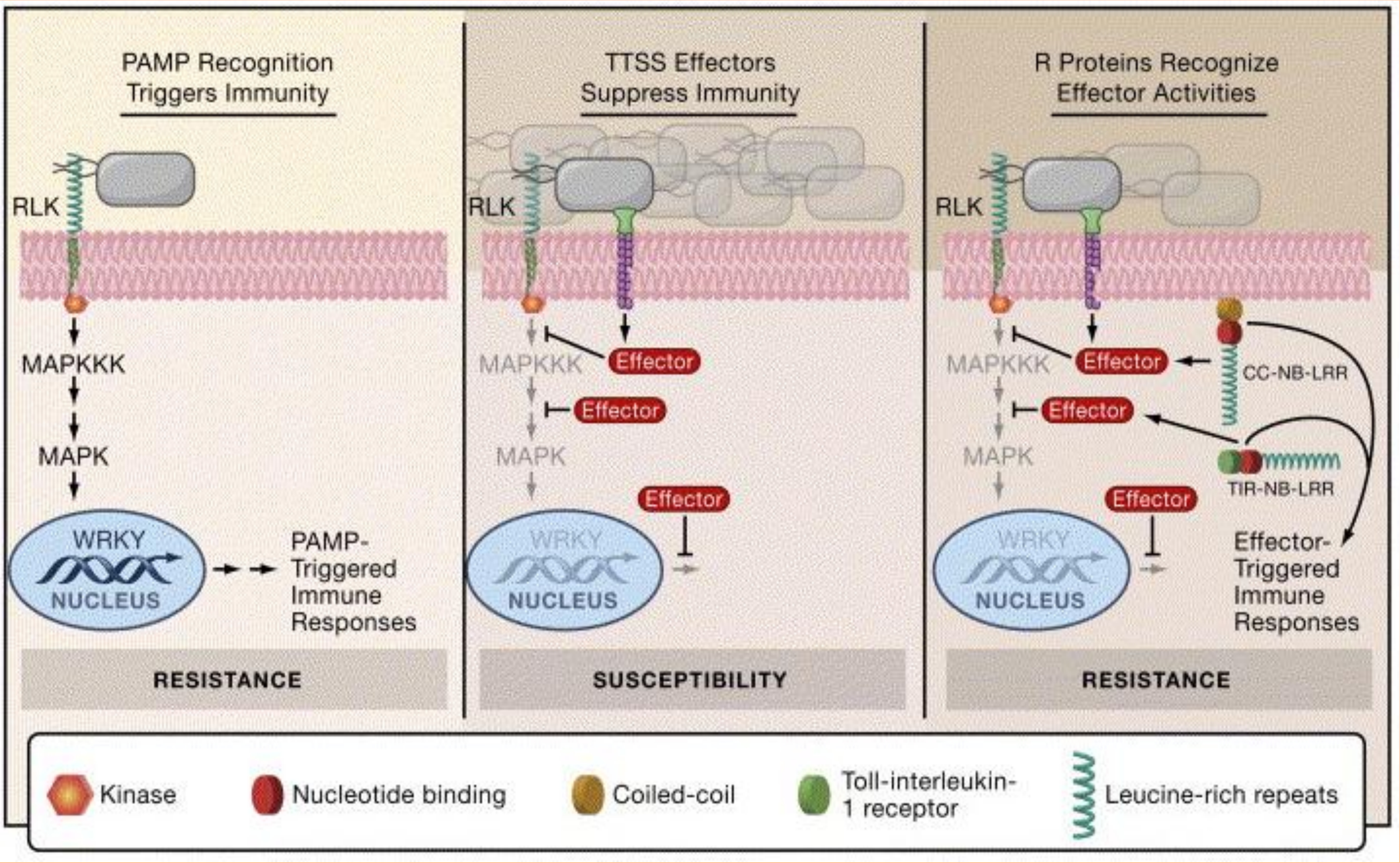
Susceptible (*nn*)

TMV



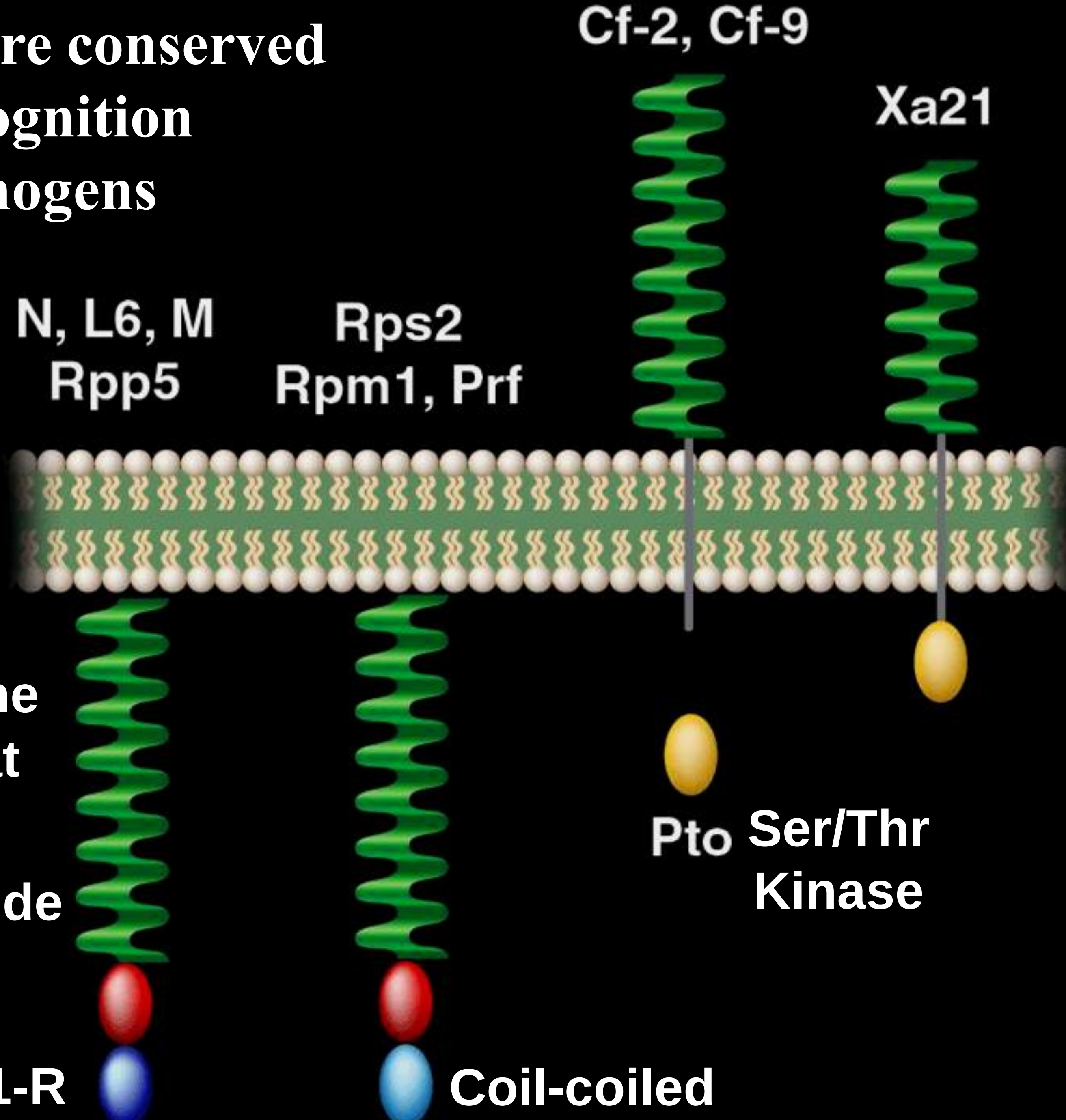
Mosaic disease

The Evolution Of Bacterial Resistance In Plants



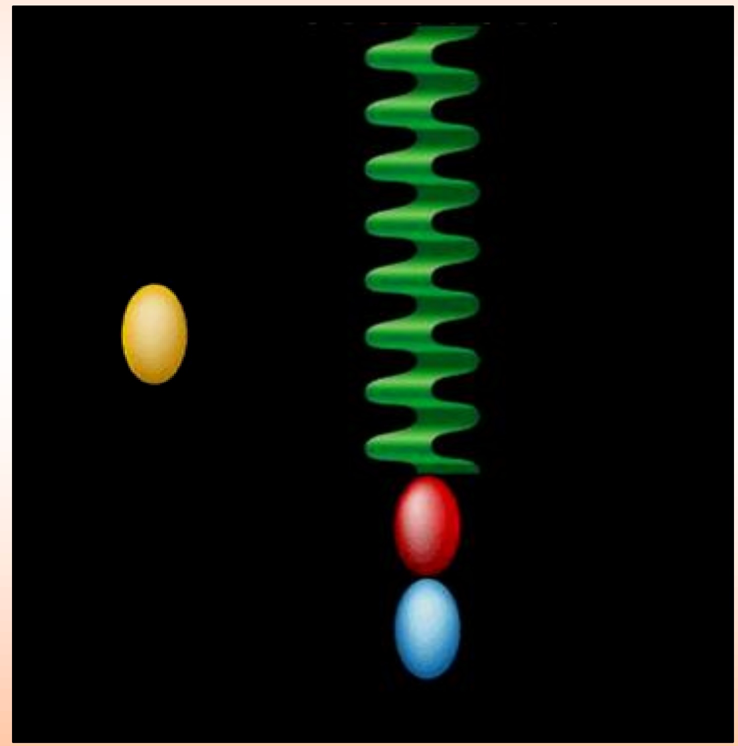
Chisholm et al., 2006

R proteins are conserved
Specific recognition
Diverse pathogens



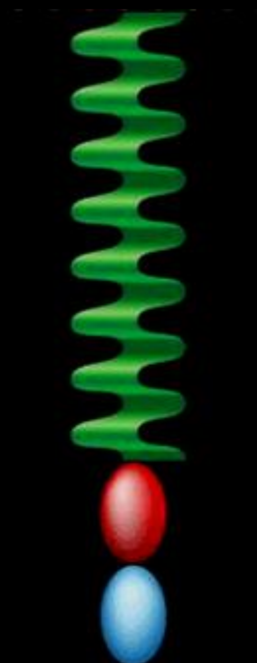
Pto and Prf genes encode resistance to bacterial speck

Pto Prf



Tobacco Responses To TMV

TMV



Susceptible (nn)

Mosaic disease

Gene-for-gene resistance (Flor, 1971)

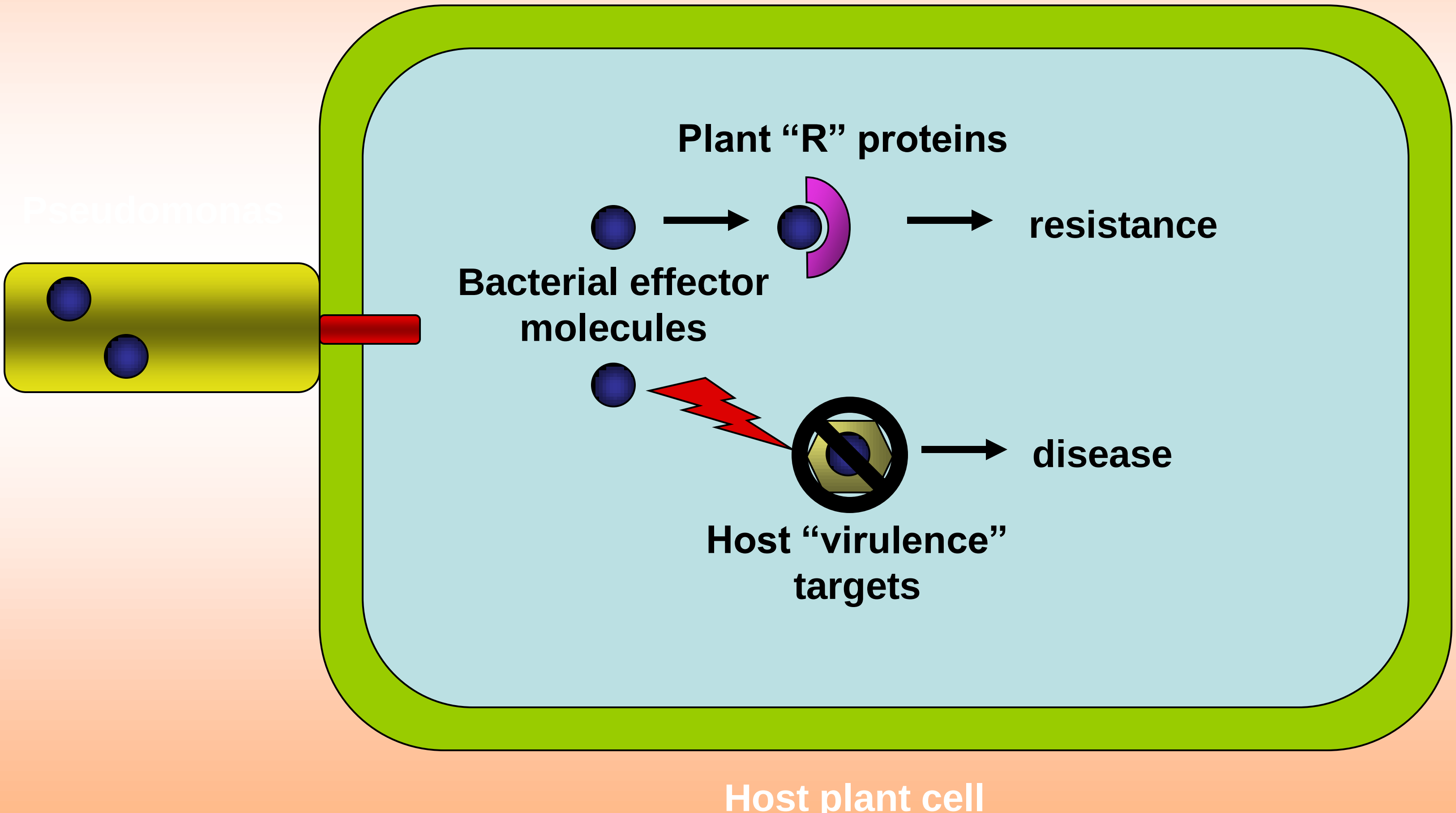
	Pathogen -Avr	Pathogen +Avr
Plants -R	Disease	Disease
Plants +R	Disease	Resistance

Dual Role for Microbial Pathogen-Derived Effector Proteins

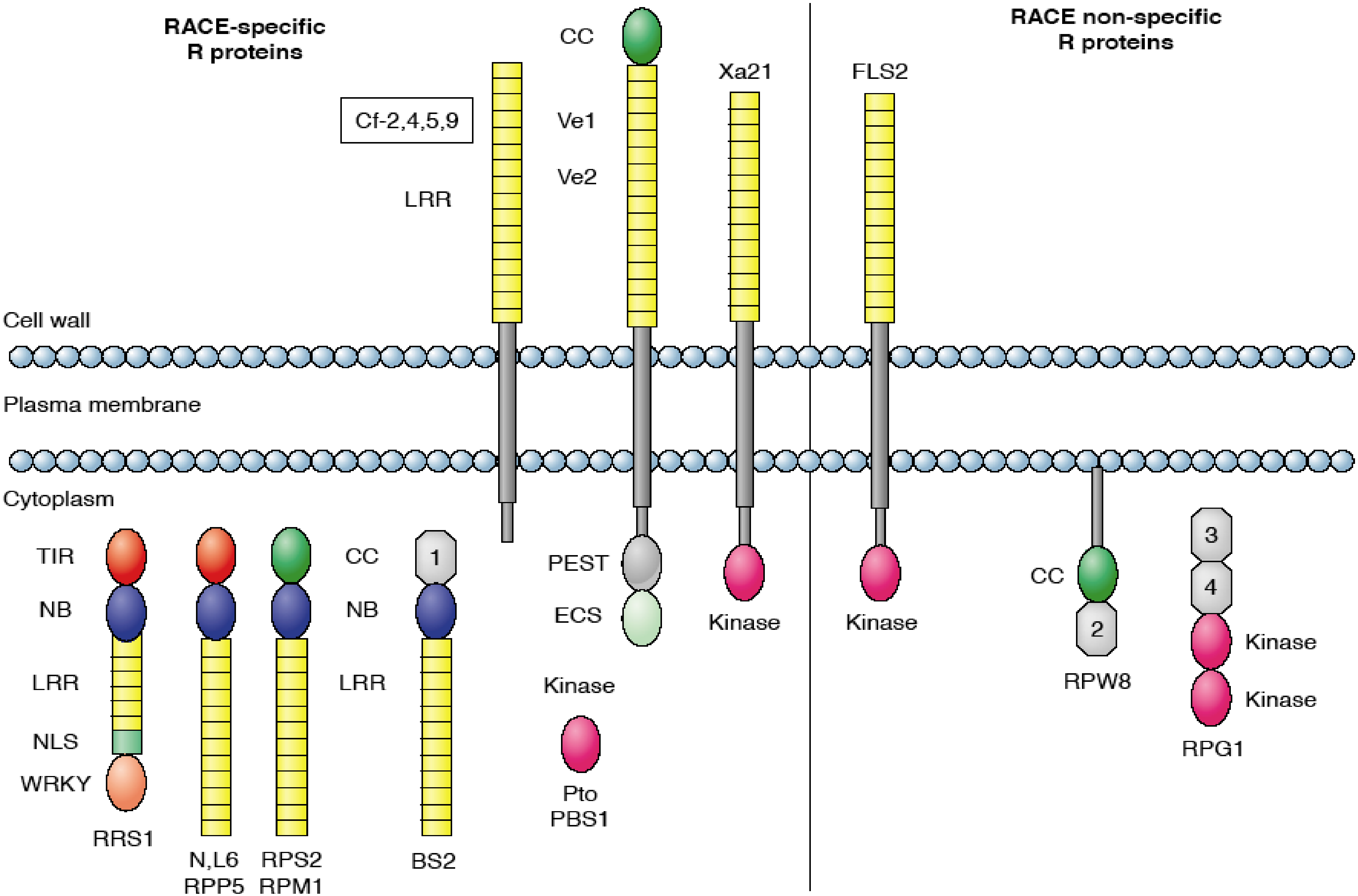
•Promote virulence and cause disease on the host species that lack a corresponding resistance genes

•Function as avirulence determinants by eliciting defense reactions in host expressing the appropriate resistance genes

Effector targets and Guard Hypothesis

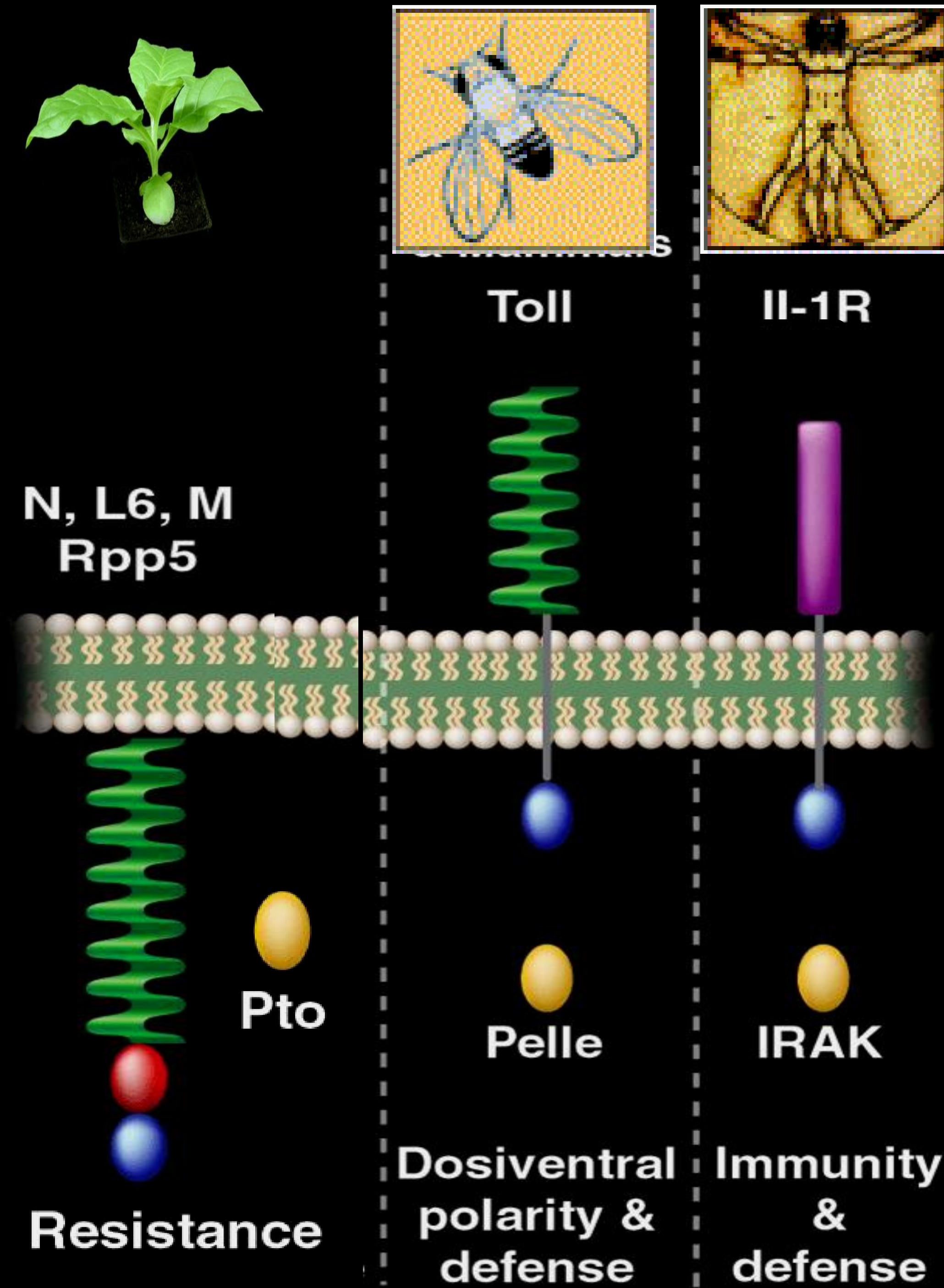


Resistance Gene Classes

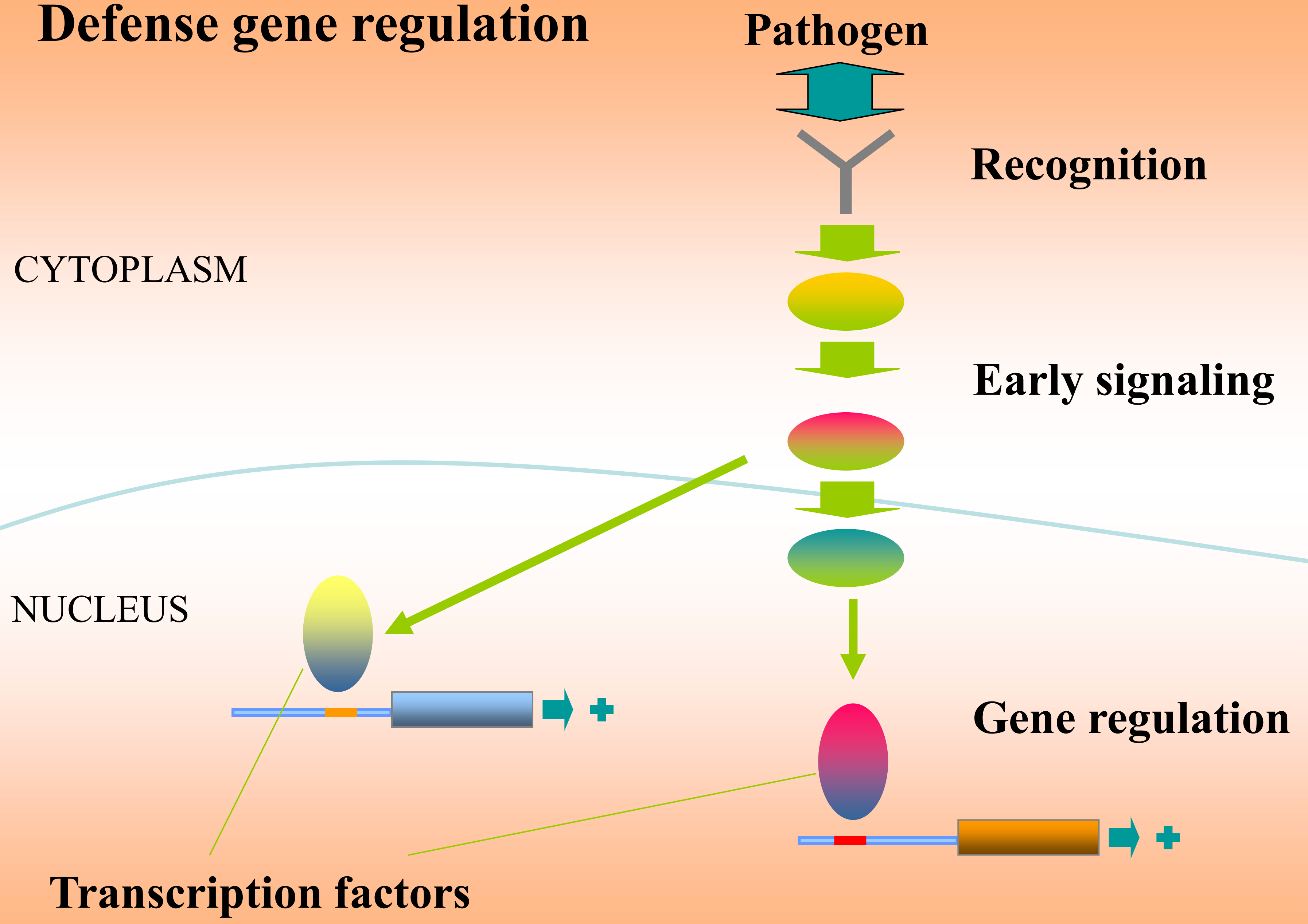


Hammond-Kosack and Parker, COBiotech, 2003, 14:177

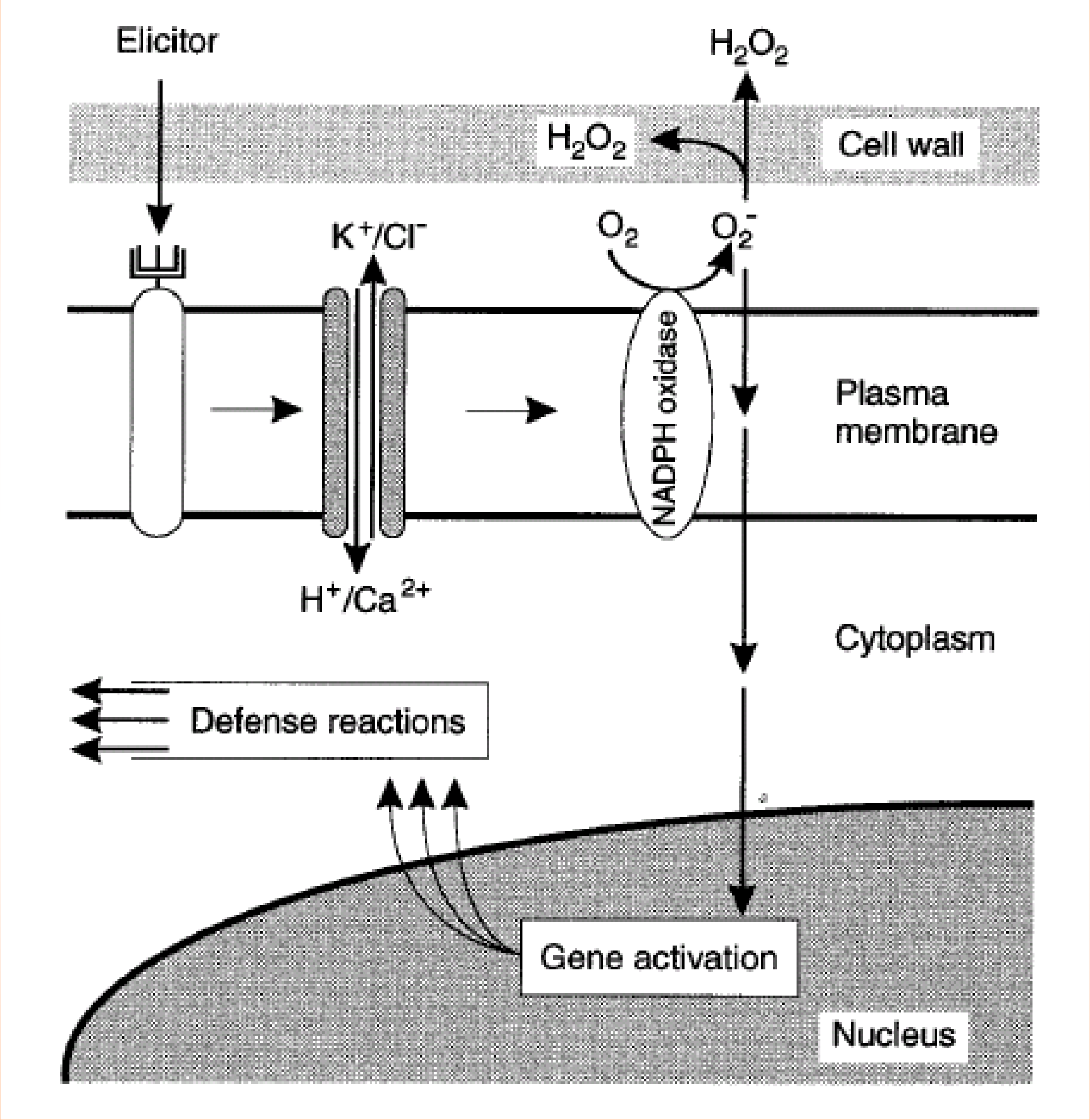
Receptor and receptor-like molecules of innate immunity in plants and animals are structurally similar



Defense gene regulation

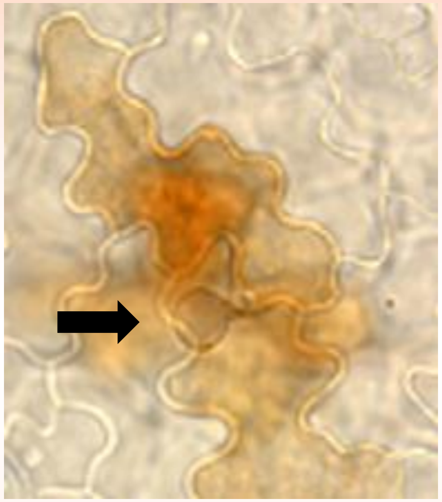


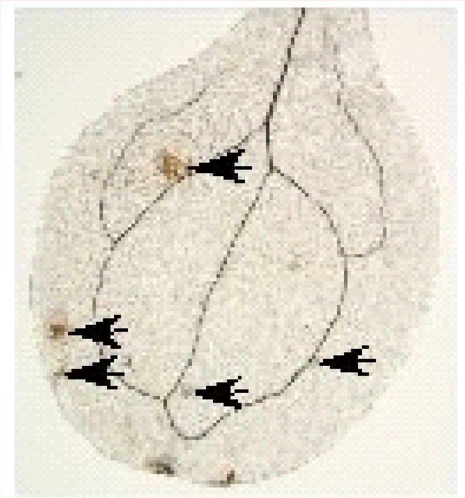
Ion fluxes



Jabs et al. (1997) Proc. Natl. Acad. Sci. USA, 94: 4800-4805

The oxidative burst

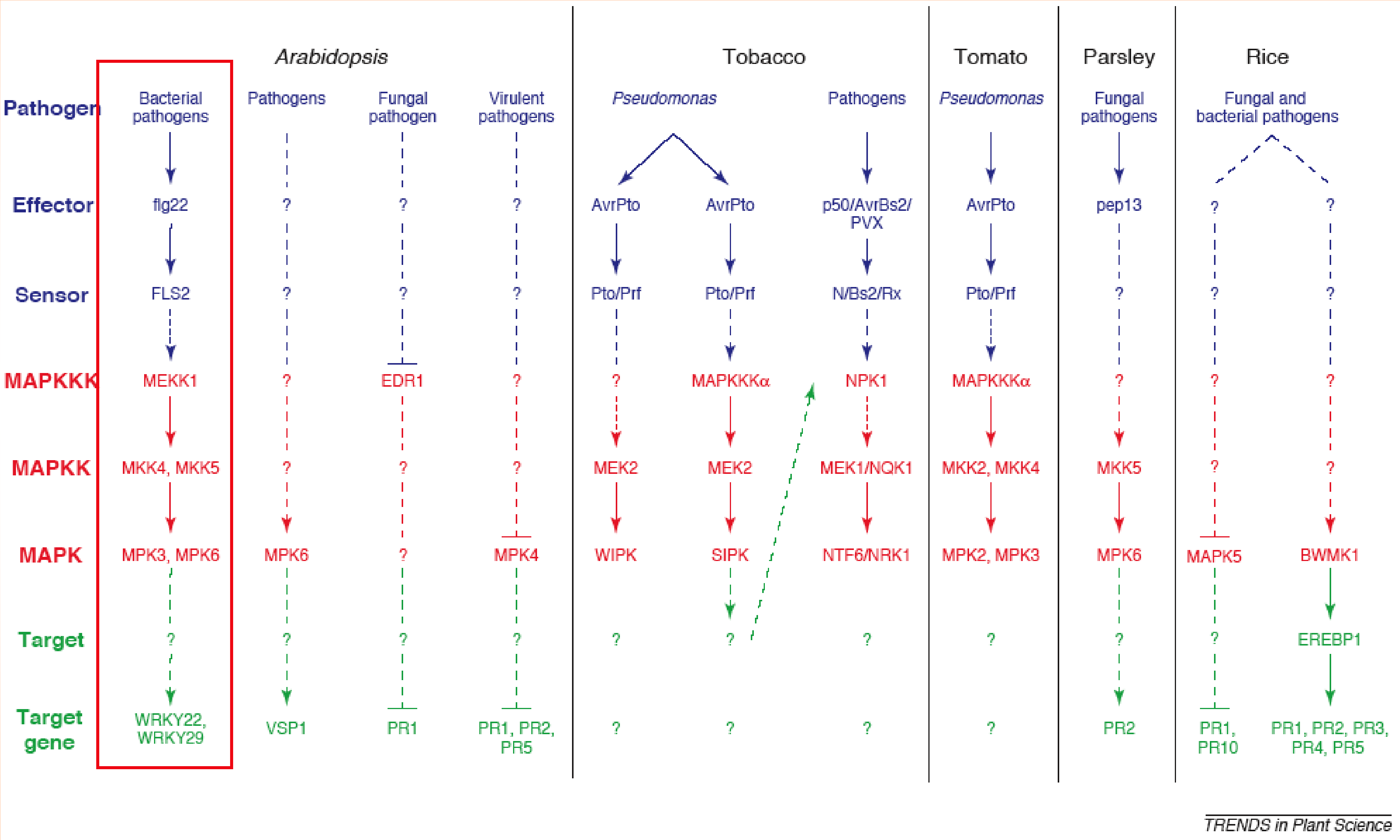




w.t.

Arabidopsis seedling stained with 2,4-Di-aminobenzidine (DAB) 24 hpi with *Peronospora parasitica* Hiks1; arrow points to germinated spore; Development of HR cell death begins ~ 24 hpi.

Plant MAPK Signaling



Pedley and Martin, Oct. 2005

Salicylic Acid

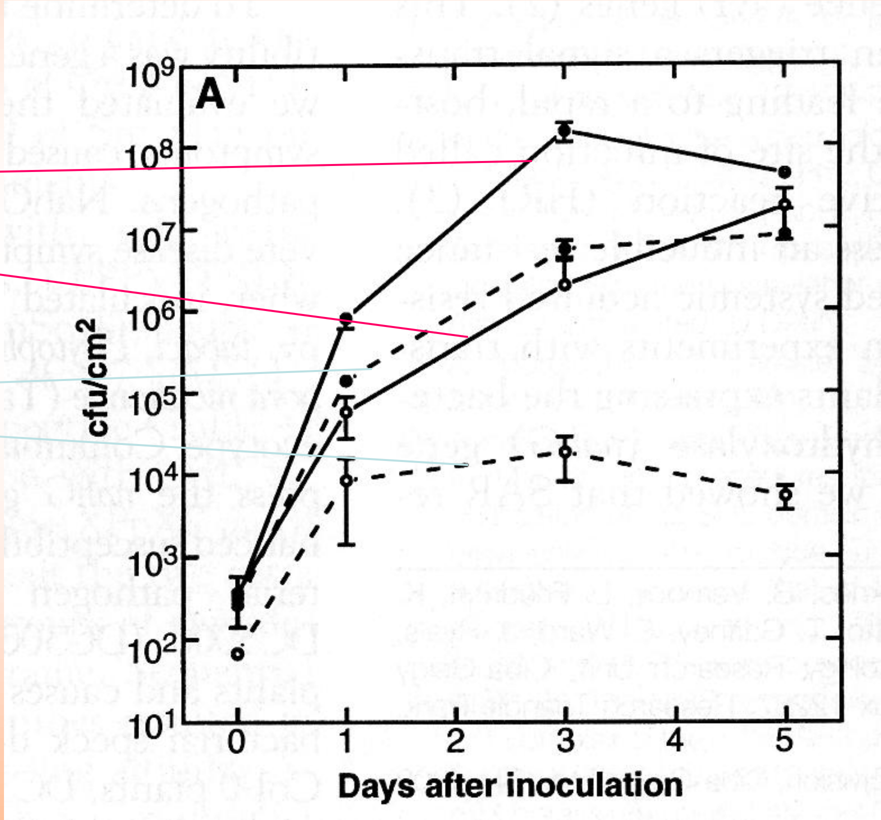
SA is required for basal defense and gene for gene resistance

virulent *P. syringae* / nahG

virulent *P. syringae* / Col-0

avirulent *P. syringae* (avrRpt2) / nahG

avirulent *P. syringae* (avrRpt2) / Col-0



From Delaney et al., 1994, Science, 266: 1247-1250

Gene Regulation and Plant Immunity

Plants response to pathogen attacks

Recognition

R & Avr

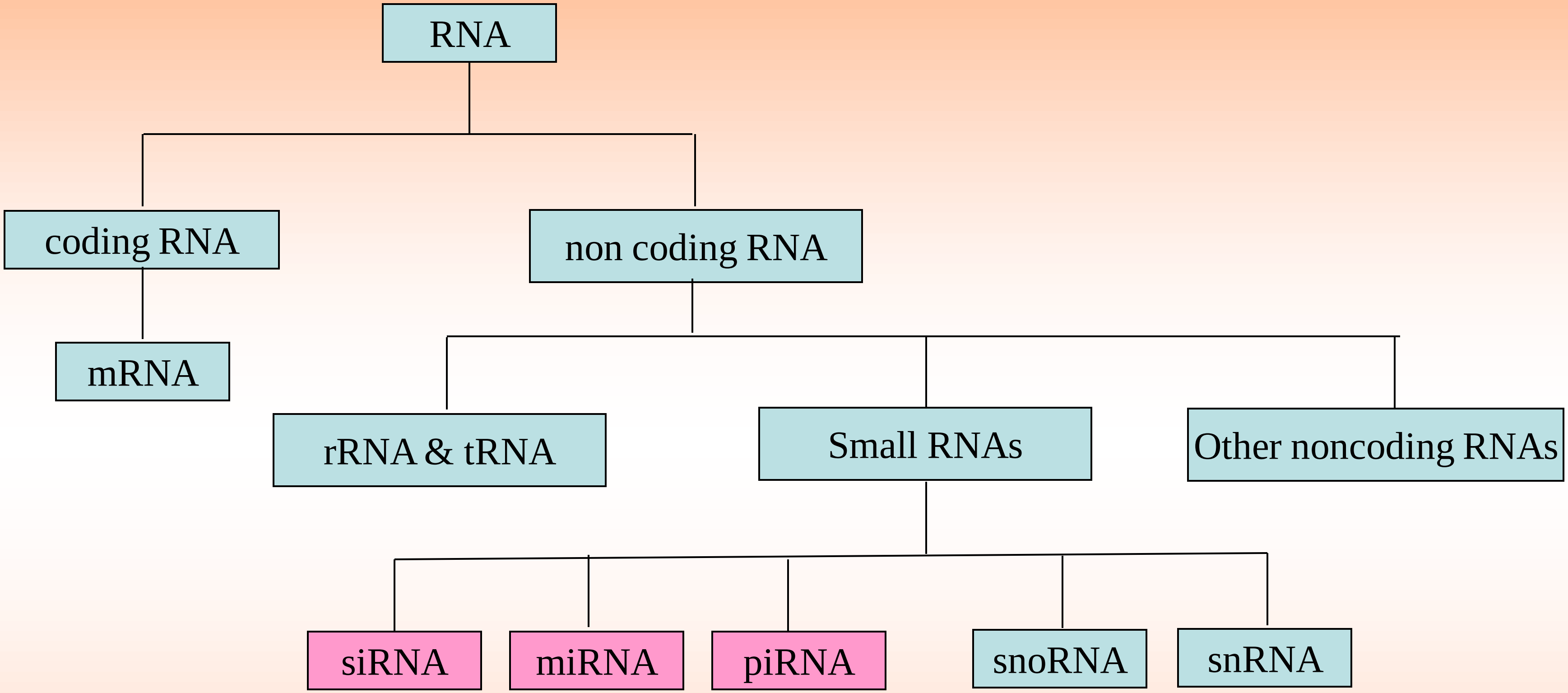
RLP & PAMPs

siRNA → **Gene Expression Reprogramming** ← **miRNA**

Race-specific Disease resistance

Basal defense

The RNA family



small non-coding RNAs
-snoRNAs (small nucleolar RNAs): constituents of spliceosome
-snRNAs (small nuclear RNAs): rRNA processing
-siRNAs, miRNAs and piRNAs: gene regulation

Overview

- **Introduction of endogenous small RNAs**

- **Small RNA pathways and pathway components**

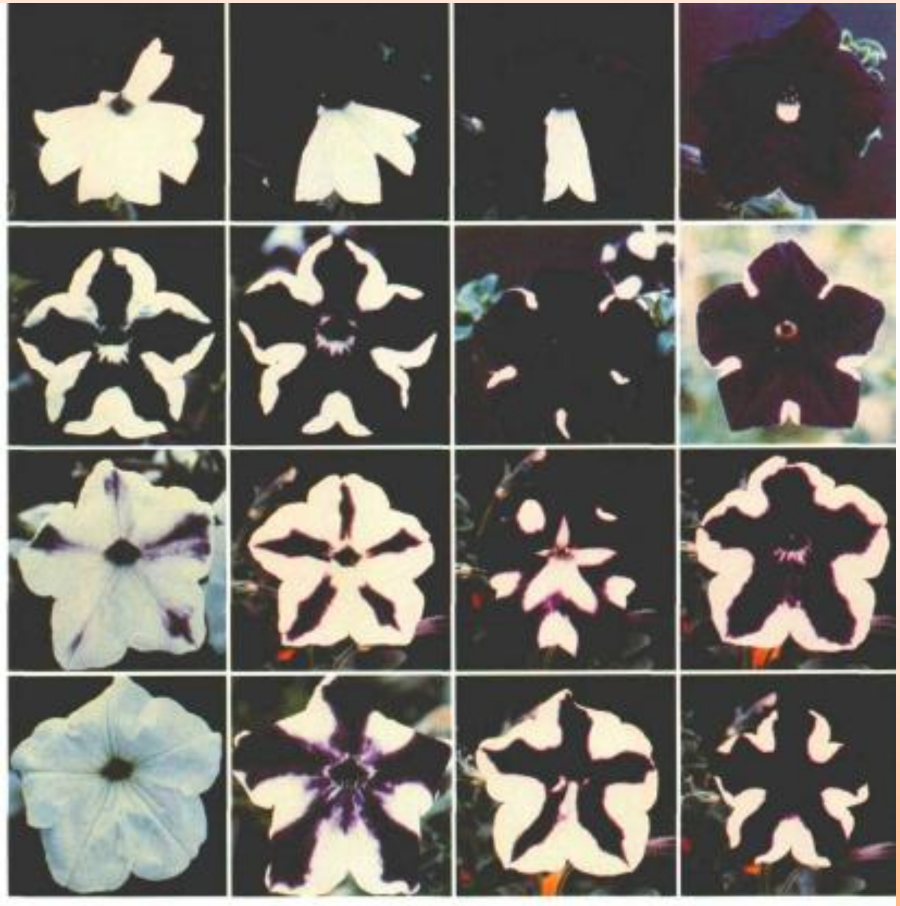
- **siRNA classes (Biogenesis and function)**
    - Endogenous siRNAs
        - Heterochromatin-associated siRNAs (hc-siRNAs)
        - Transacting siRNAs (ta-siRNAs)
        - Natural antisense transcripts associated siRNAs (nat-siRNAs)
    - Exogenous siRNAs
        - Viral and transgene derived-siRNAs

- **piRNAs**

- **Small RNA detection and cloning**

Silencing Discovery

1990-Co-suppression

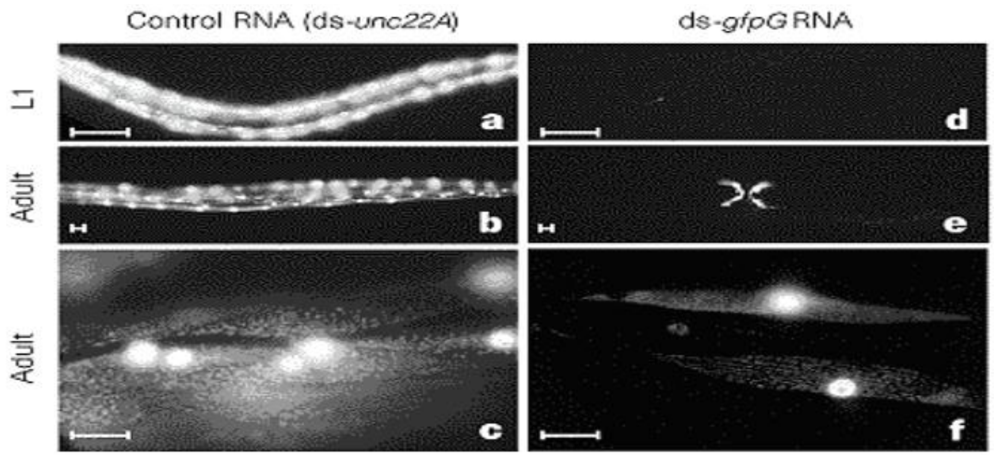


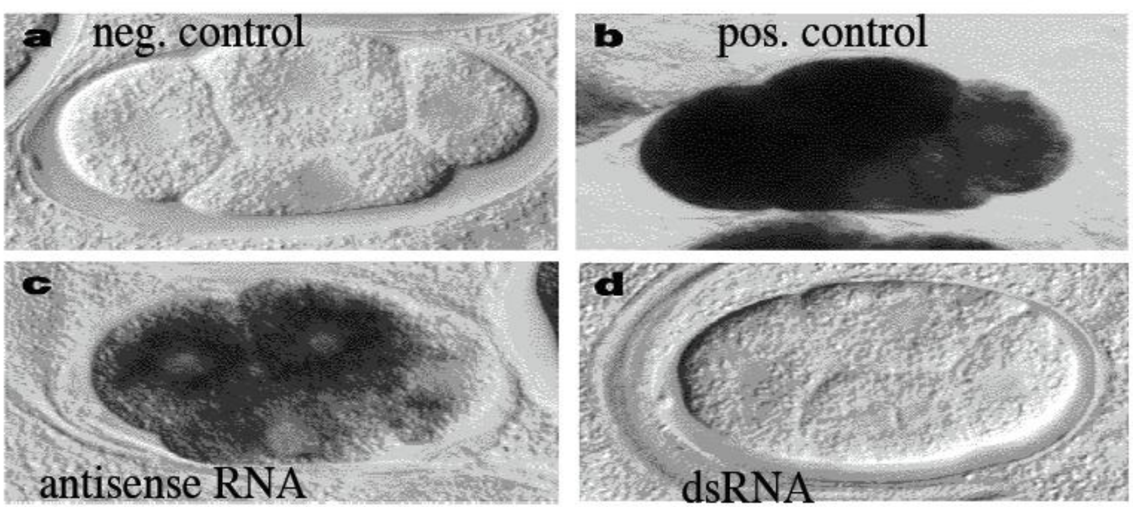
<u>Overexpression of chalcone synthase and dihydroflavonol reductase</u>

Napoli et al (1990) Plant cell 2, 279-289
Van der Krol et al., (1990) Plant cell 2, 291-299

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‡





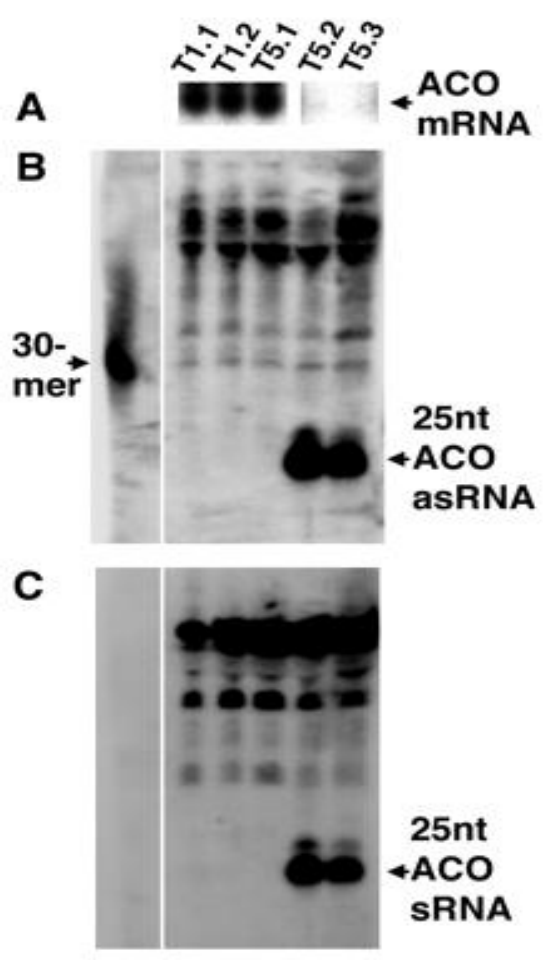
Fire et al., Nature 1998

Nobel Winning Discovery-----Double-Stranded RNA Is The Trigger For Gene Silencing

Co-suppression in plants	1990
Quelling in Neurospora Crassa	1992
RNAi in C. elegans	1995, 1998

Andy Fire and Craig Mello (1998, Nature 391:806-811)

Small RNA Discovery



- Small RNAs are associated with co-suppression
- Complementary to both the sense and antisense strands
- Derived from dsRNA
- The initiators of RNAi

Hamilton & Baulcombe, Science 286, 950 (1999).

Small RNAs That Induce Silencing

noncoding and small in size

siRNA	miRNA	piRNA
•Derived from long dsRNAs	•Derived from ssRNA hairpin structure	•Derived mainly from transposons and repeats
•Enormous and not conserved	•Limited in number and usually conserved	•Enormous and often not conserved
•Direct transcriptional and post-transcriptional gene silencing	•Direct post-transcriptional gene silencing	•Direct post-transcriptional gene silencing
•Dicer-dependent	•Dicer-dependent	•Dicer-independent
•Present in various tissues	•Present in various tissues	•Only present in germline cells

Types of RNA-silencing mechanisms

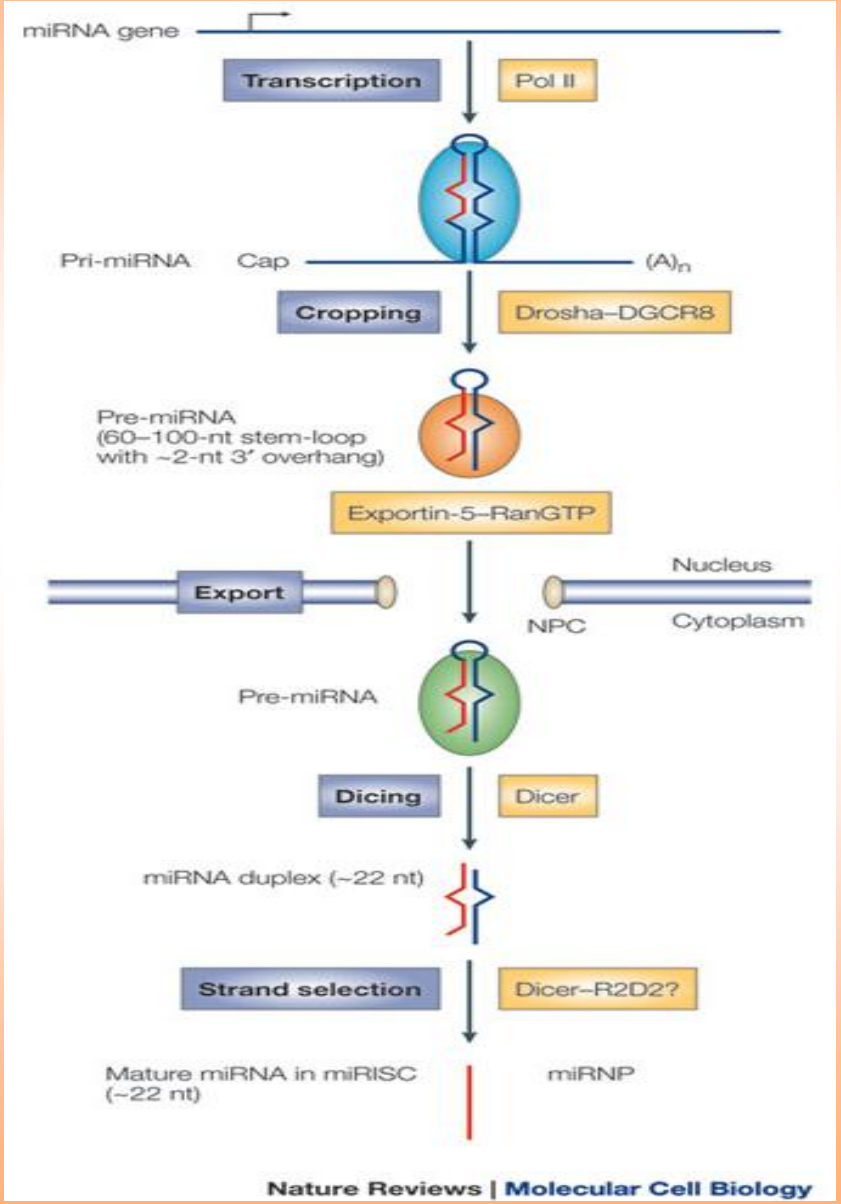
1) miRNA pathway — ***trans*** **targets**

2) siRNA pathways
- **PTGS**
  cis **and** ***trans*** **targets**
- **TGS**

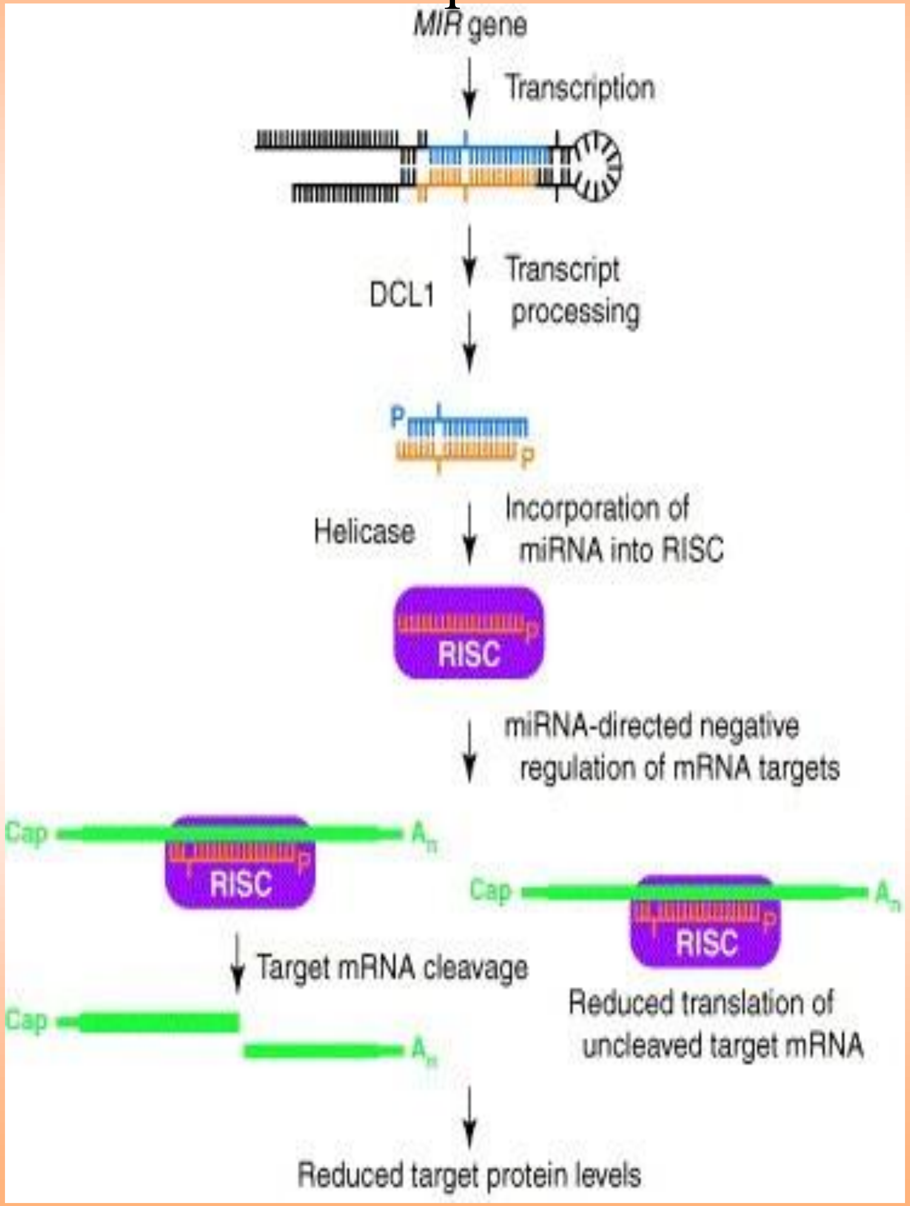
3) piRNA pathway — ***cis*** **and** ***trans*** **targets**

miRNA pathways

In animals



In plants



siRNA classes

Endogenous small RNAs

Chromatin-associated siRNAs
Transacting-siRNAs
Natural antisense transcripts-derived siRNAs

Exogenous small RNAs

Viral and transgene-derived siRNAs

Functions of siRNAs

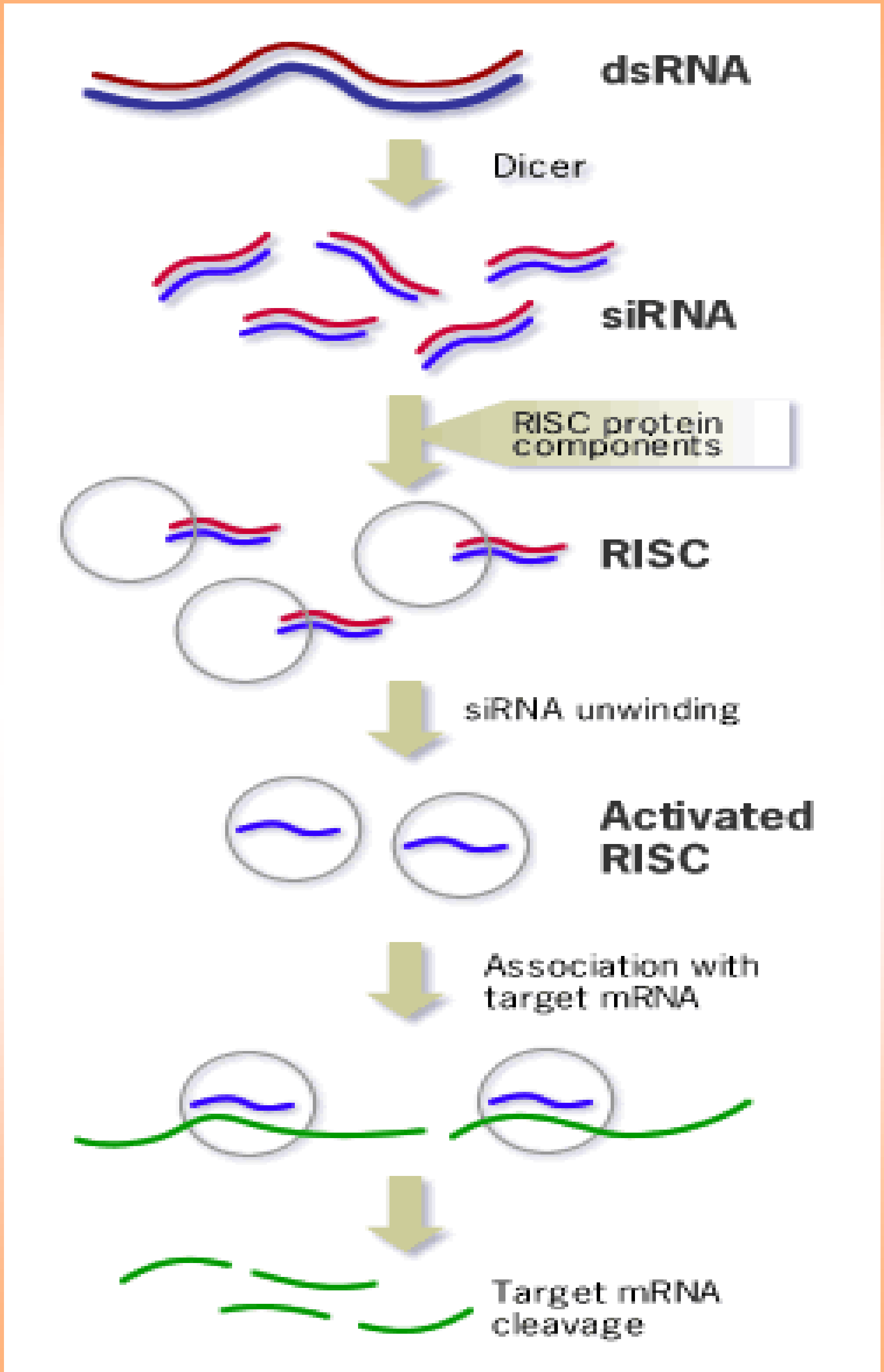
1) **Defense---Antiviral and antibacterial defenses**
   abiotic stress responses

2) **Genome stability---Silencing transposons,**
   retroelements and repeats

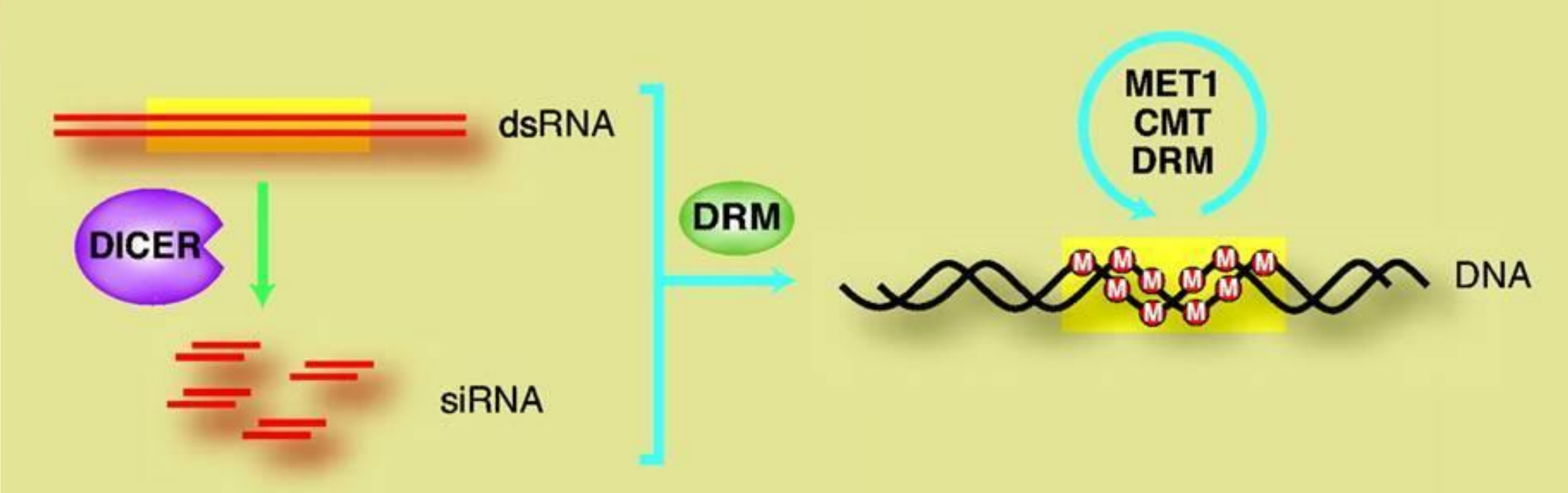
3) **Development and cellular processes---Gene regulation**

4) **DNA elimination**
   (27–30-nt small RNAs in *Tetrahymena*)

siRNA pathway- PTGS



siRNA pathway -TGS



-RNA-directed DNA methylation (RdDM) is triggered by dsRNA.

-dsRNAs are cleaved by enzymes of the Dicer family to generate small, 21-26 nt siRNAs.

-siRNAs are involved in RdDM, mediated by three classes of methyltransferases:
DRMs, CMT and MET1.

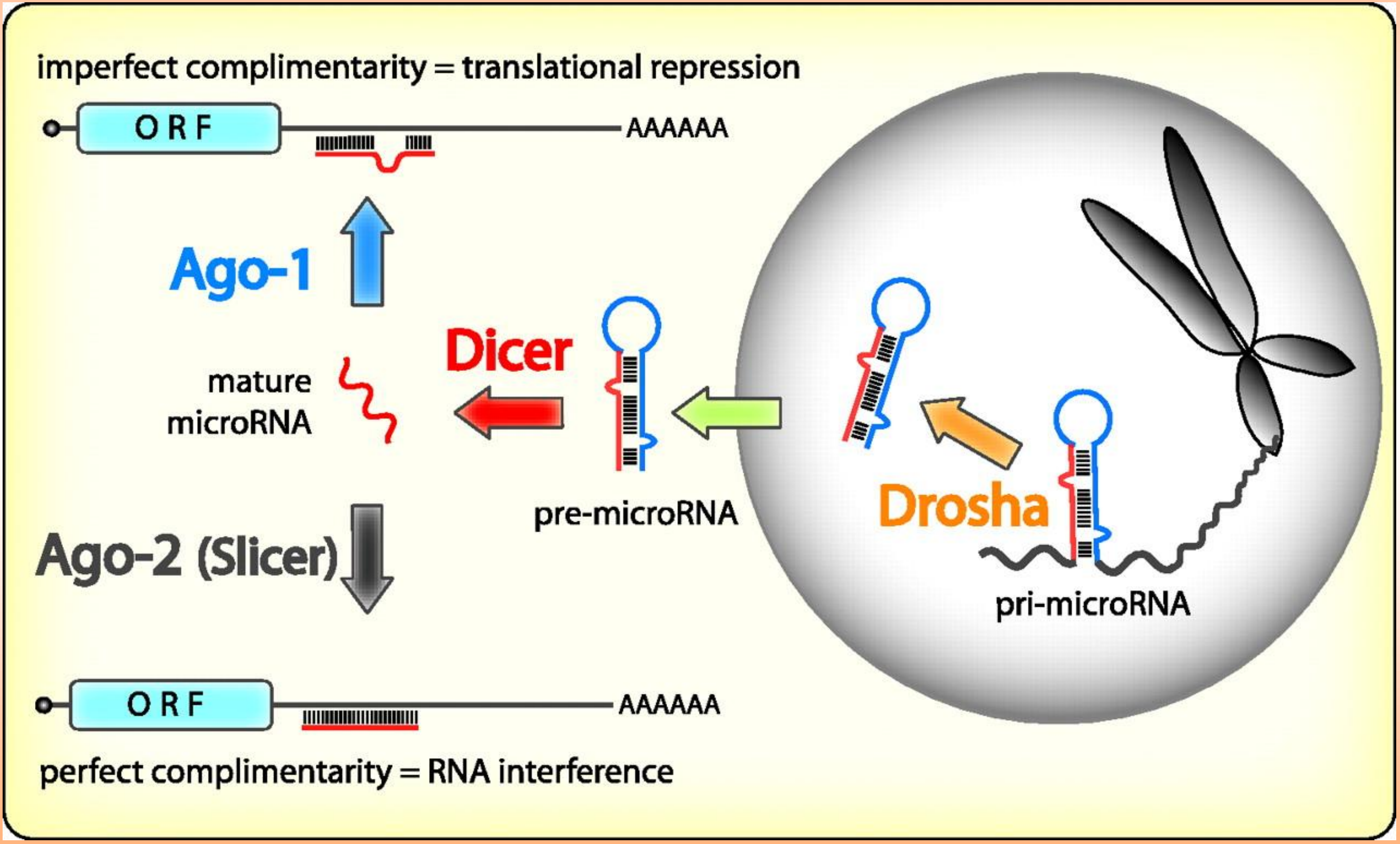
Key Components for Small RNA Silencing Pathways

Dicers

Argonautes

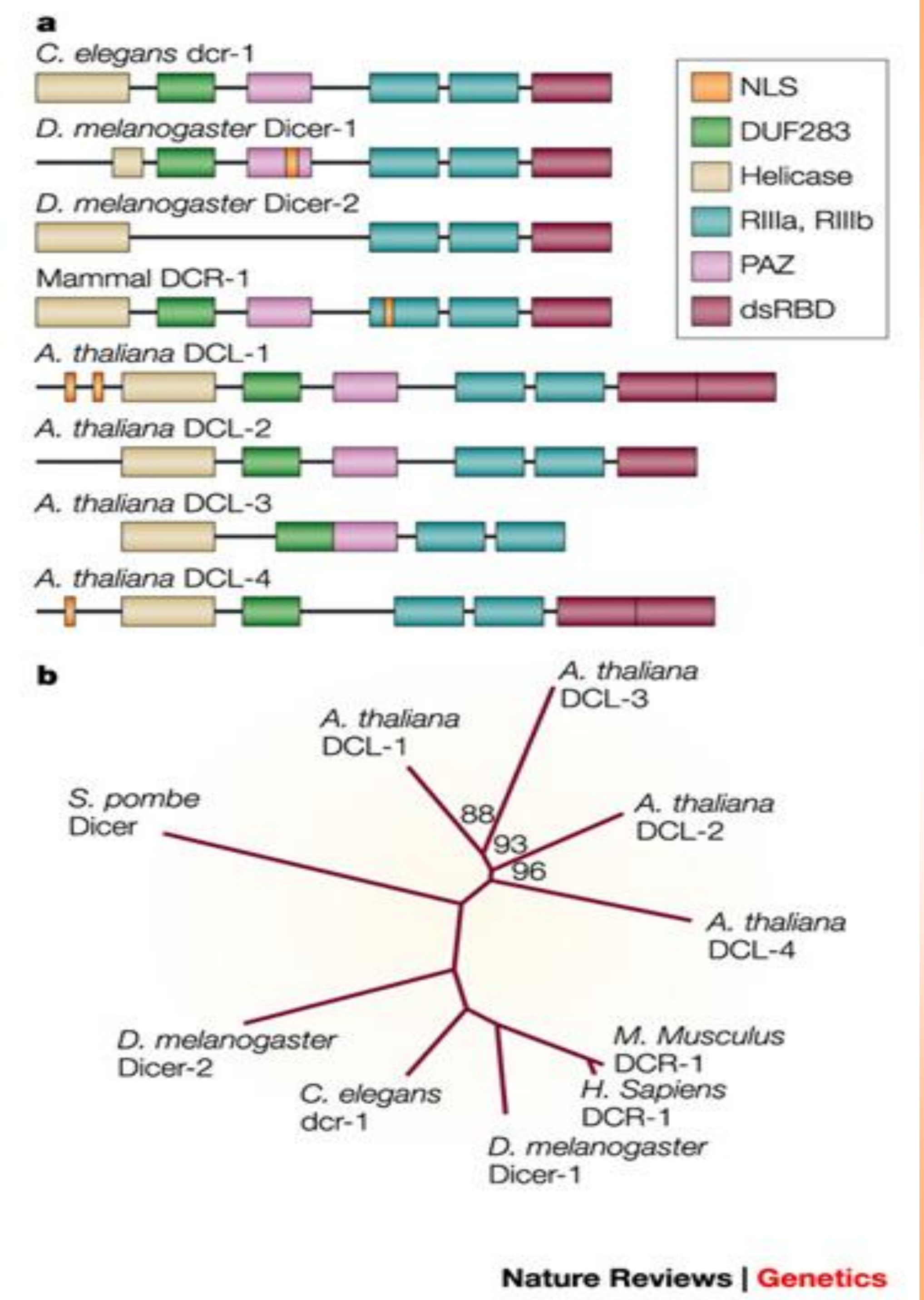
RNA Dependent RNA Polymerases

Dicers & Argonautes

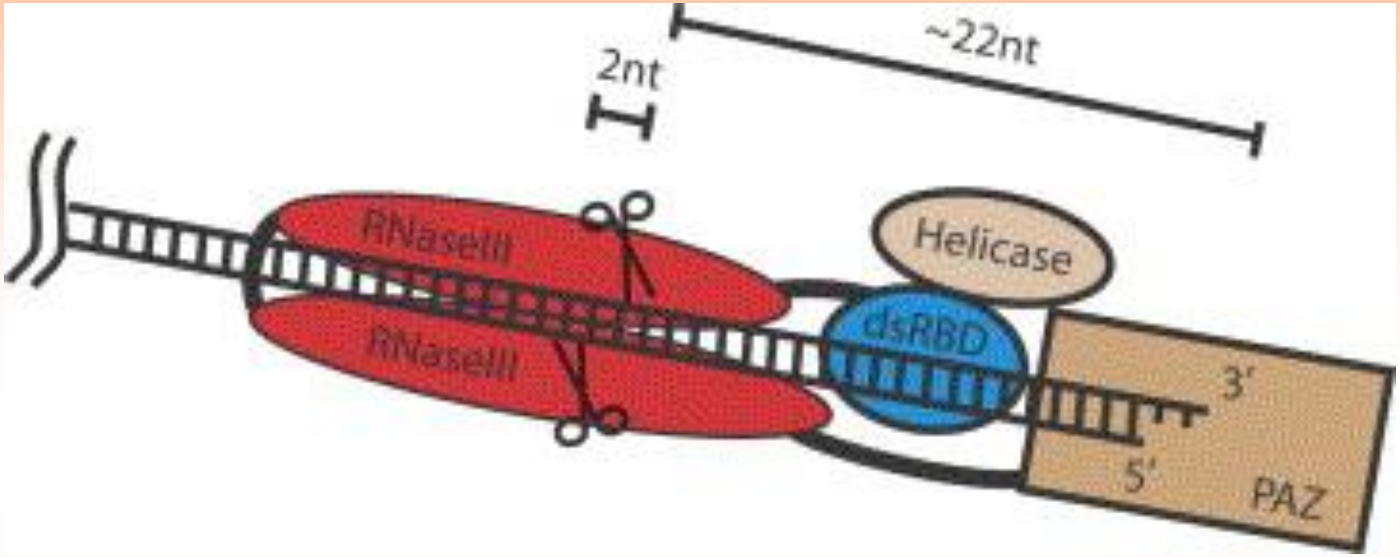


a. The domain structure of Dicer homologues in worms, flies, mammals and plants.

b. The phylogenetic tree of the Dicer protein family.



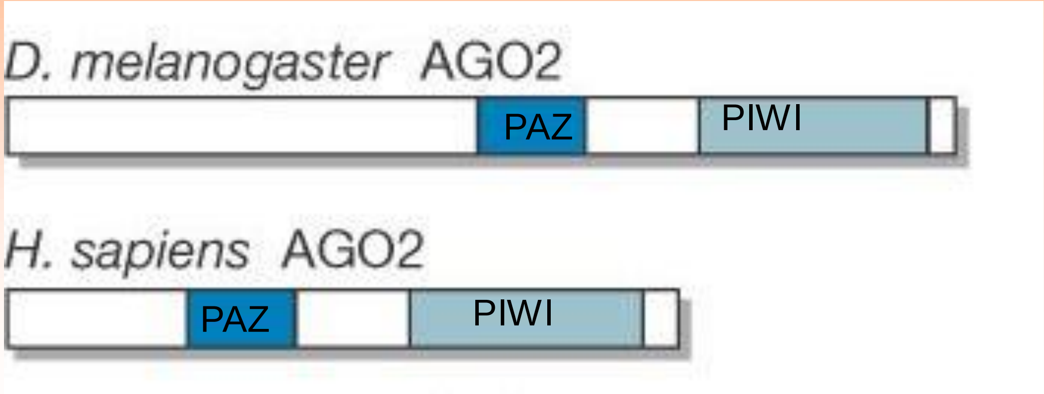
Model for Dicer catalysis



The PAZ domain binds the 2 nt 3′ overhang of a dsRNA terminus.
The RNaseIII domains form a pseudo-dimer. Each domain hydrolyzes one strand of the substrate. The binding site of the dsRBD is not defined.

Hammond (2005) FEBS Lett.,579-5822-5829

Structure of Argonautes



-All RISCs contain a member of the Argonaute (Ago) family of proteins, as defined by the presence of PAZ and PIWI domains.

-The PIWI domain has homology to ribonuclease-H enzymes, which implicates it as the endonuclease that cleaves the target mRNA.

Nature Reviews Molecular Cell Biology **6**, 127-138 (2005) E Sontheimer

In *Arabidopsis*

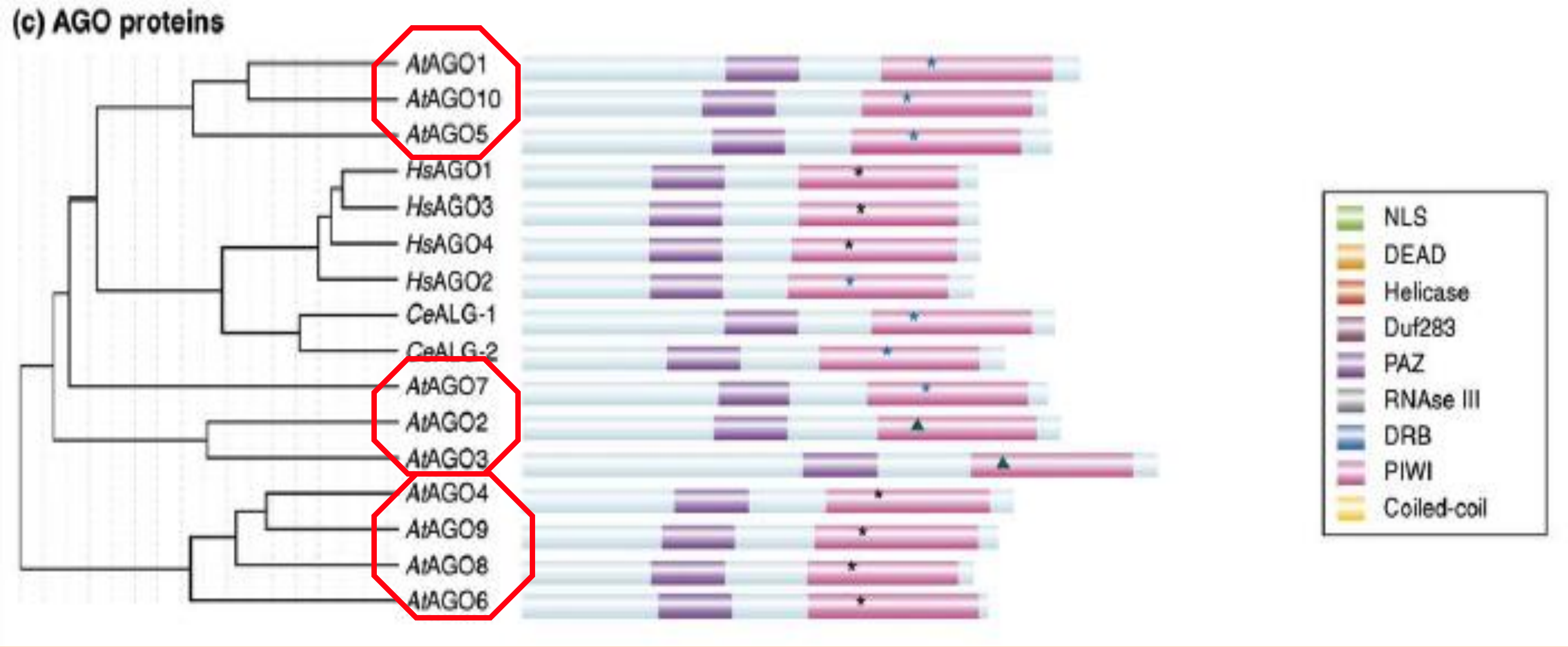
-10 members

-*AGO1* mutants are defective in miRNA-directed silencing.

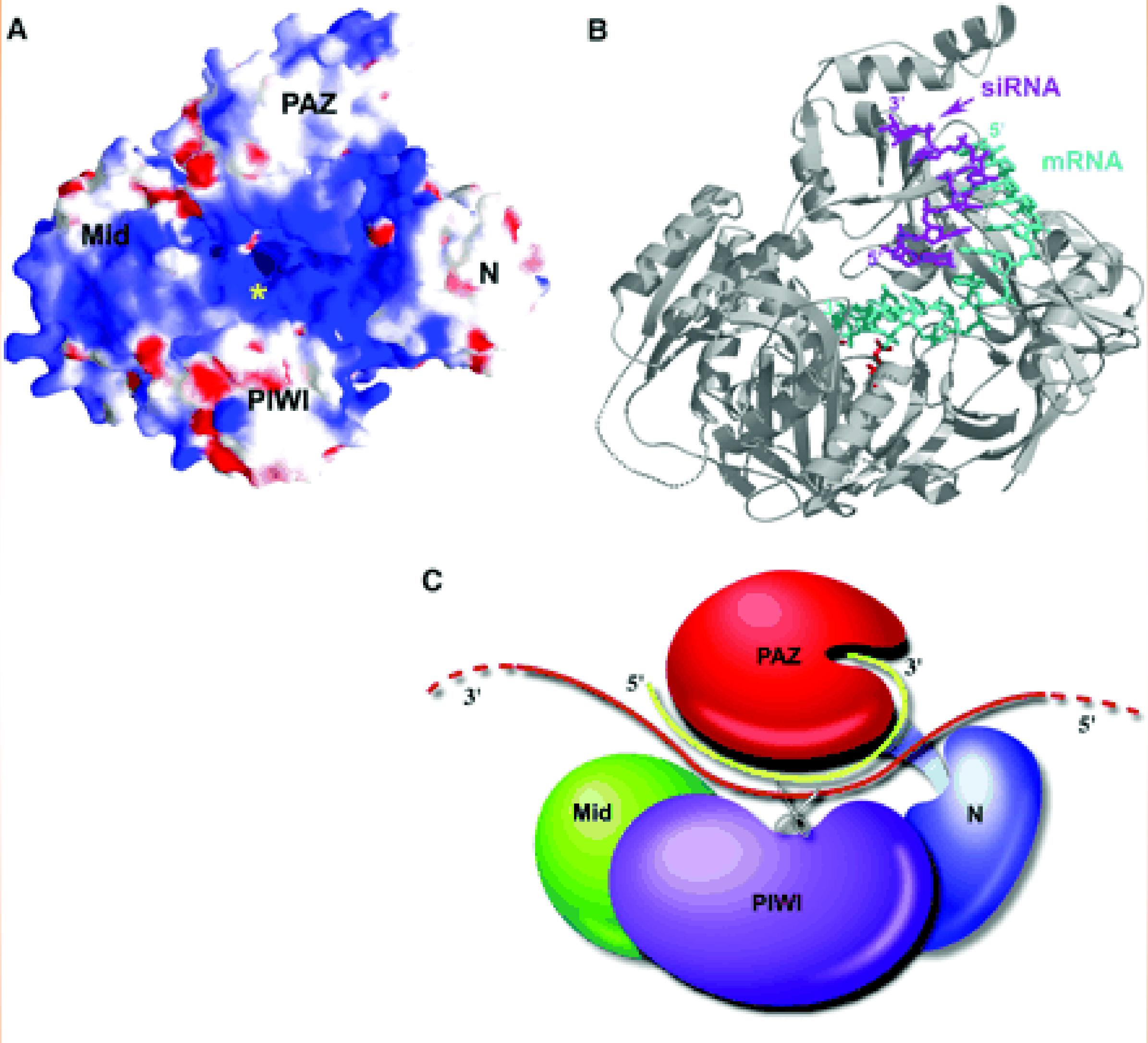
-*AGO4 and AGO6 are* implicated in chromatin silencing.

***AGO1, AGO4* proteins shown to be a Slicer.**

(Baumberger, and Baulcombe, D. 2005 Proc. Natl. Acad. Sci. USA; Qi et al., 2006)



Phylogenetic tree of AGOs from human, C. elegans and *Arabidopsis*



The phosphate between nucleotides 10 and 11 from the 5' end of the small RNA falls near the active site residues.

model for siRNA-guided mRNA cleavage

RITS and RISC

RITS: RNA-induced transcriptional silencing complex

Nuclear localized

Functions in heterochromatic siRNA pathways

RISC: RNA-induced silencing complex

Cytoplasmic

Functions in miRNA, endogenous siRNA, tasiRNA, nat-siRNA pathways

Small RNA pathways

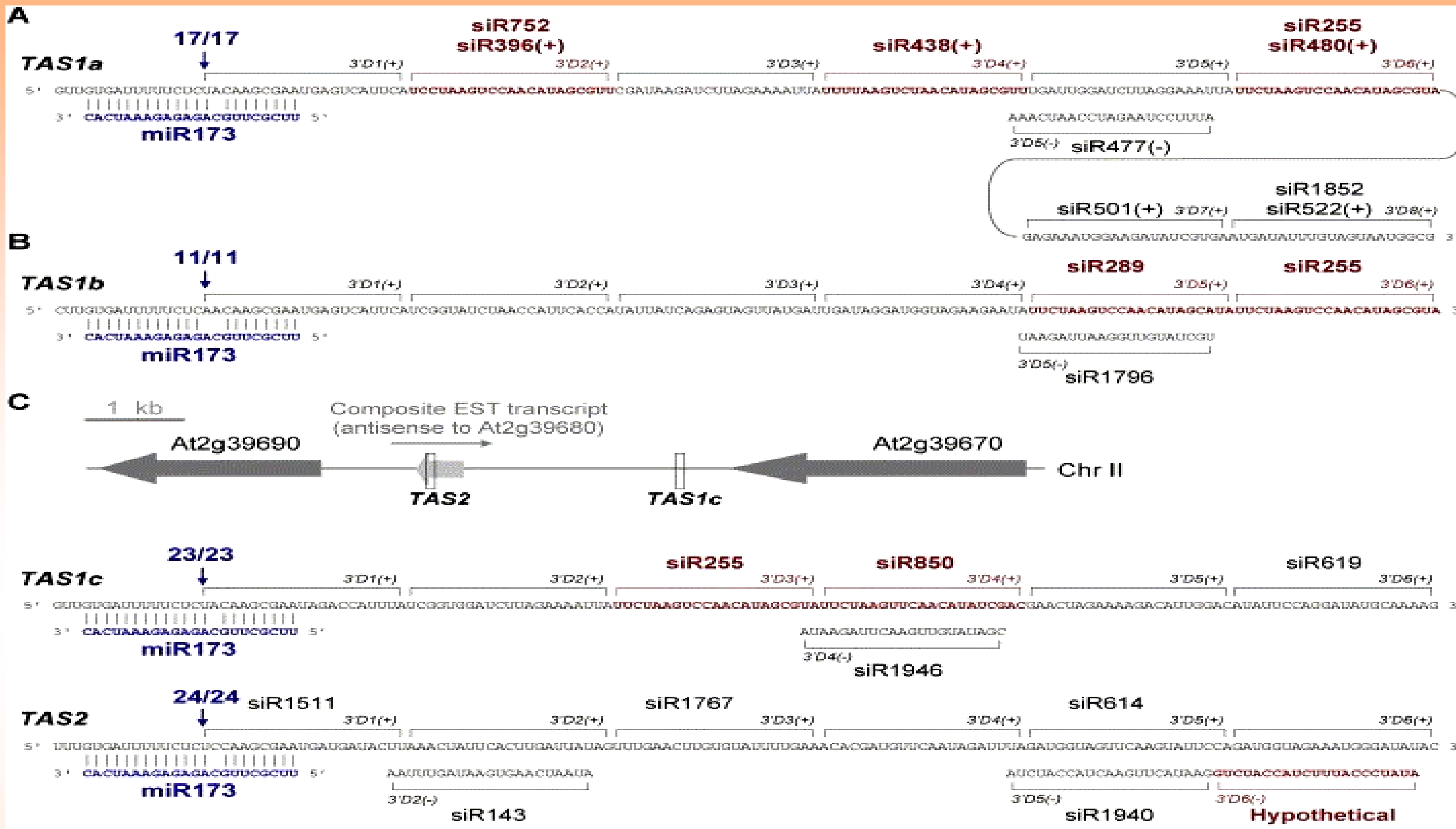
1) miRNA pathway

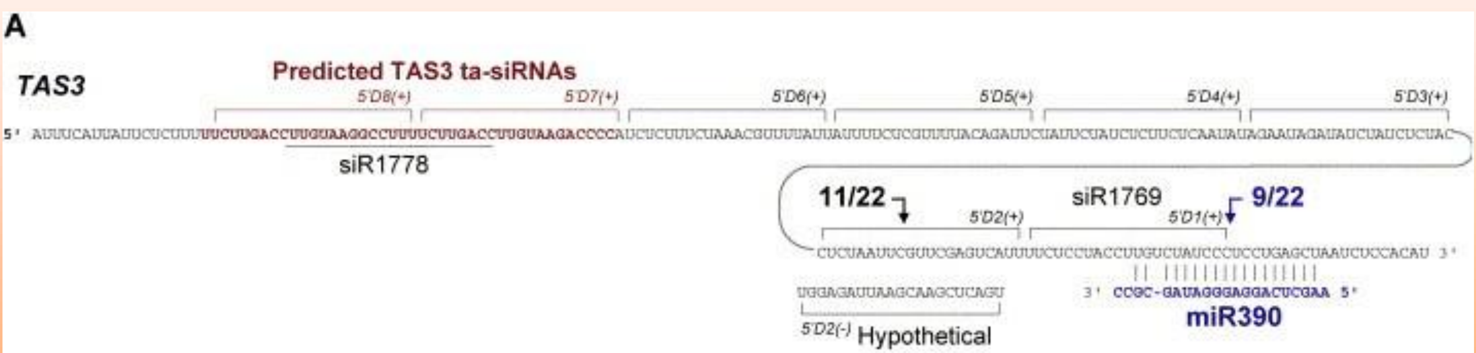
2) siRNA pathways

- 1) tasiRNAs
  2) nat-siRNAs
  3) transgene/viral siRNAs
  } PTGS

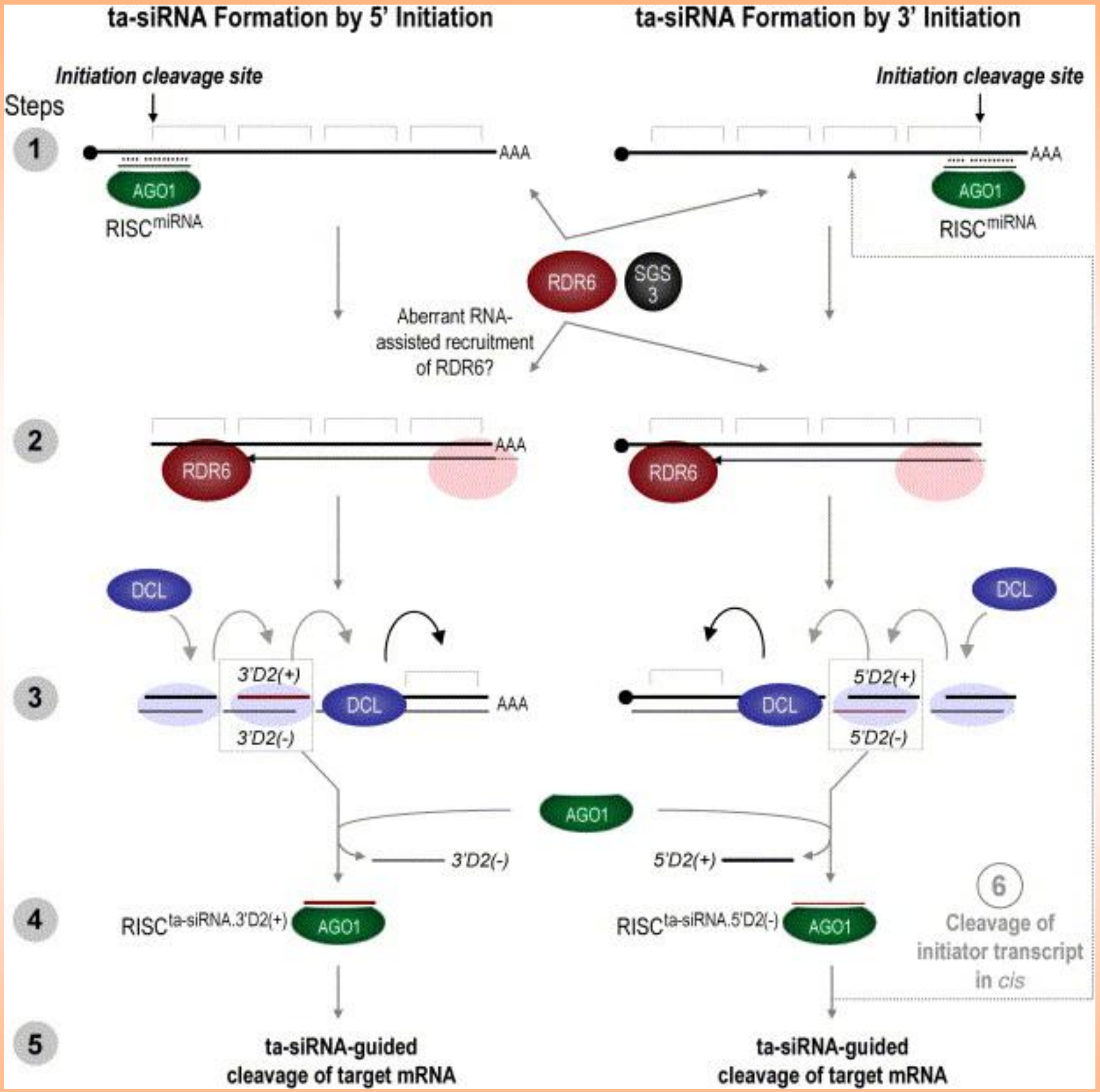
- heterochromatic siRNAs
  (rasiRNAs and others)
  } TGS

TAS1, TAS2 and TAS3 tasiRNAs





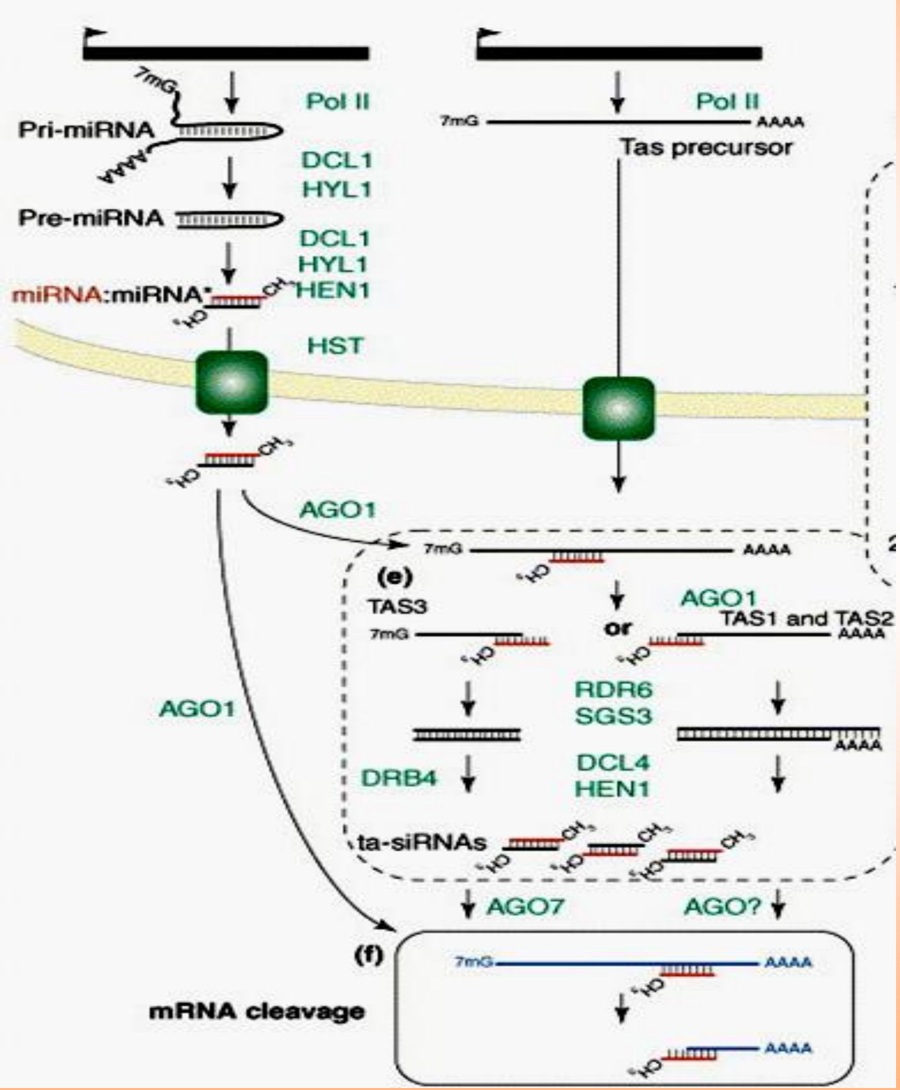
Model for miRNA-guided tasiRNA biogenesis



Allen et al. (2005) Cell 121: 207-221

The Biogenesis Of Small RNAs In Plants

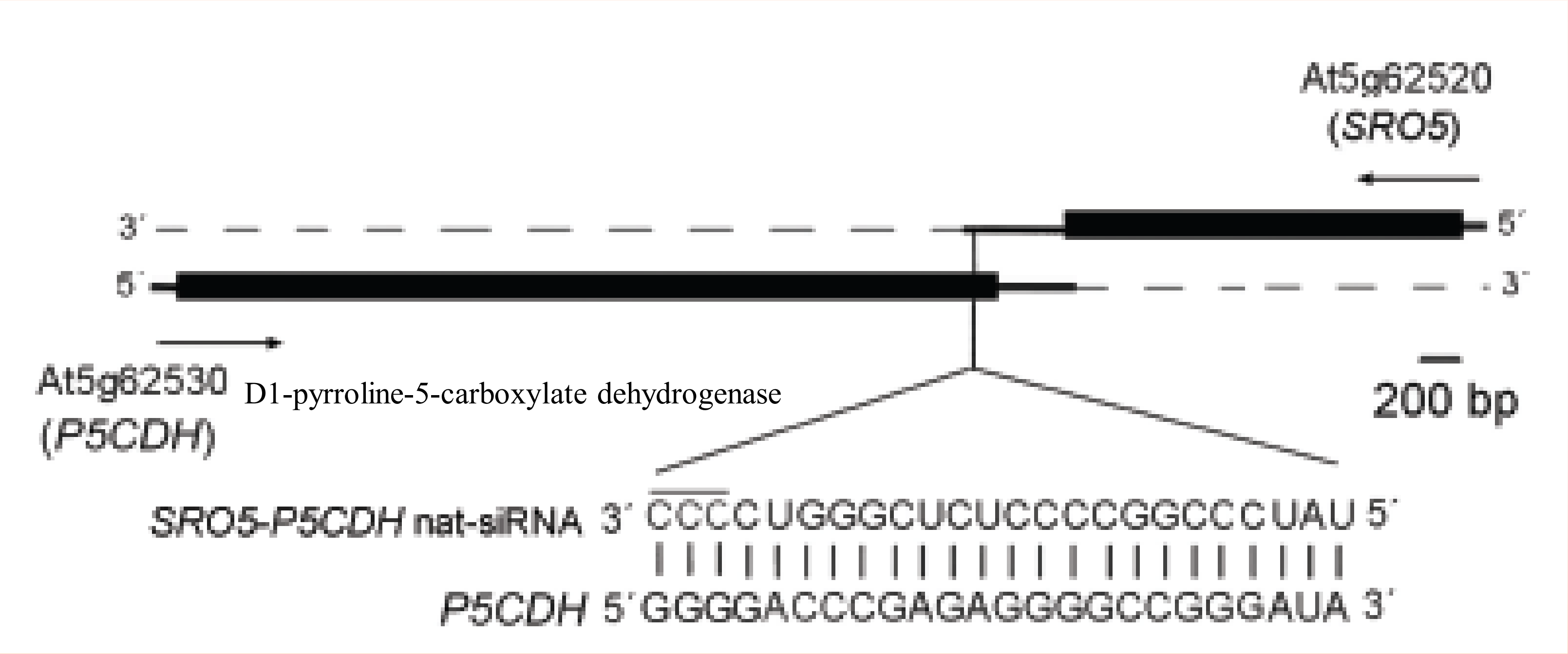
microRNA **ta-siRNAs**



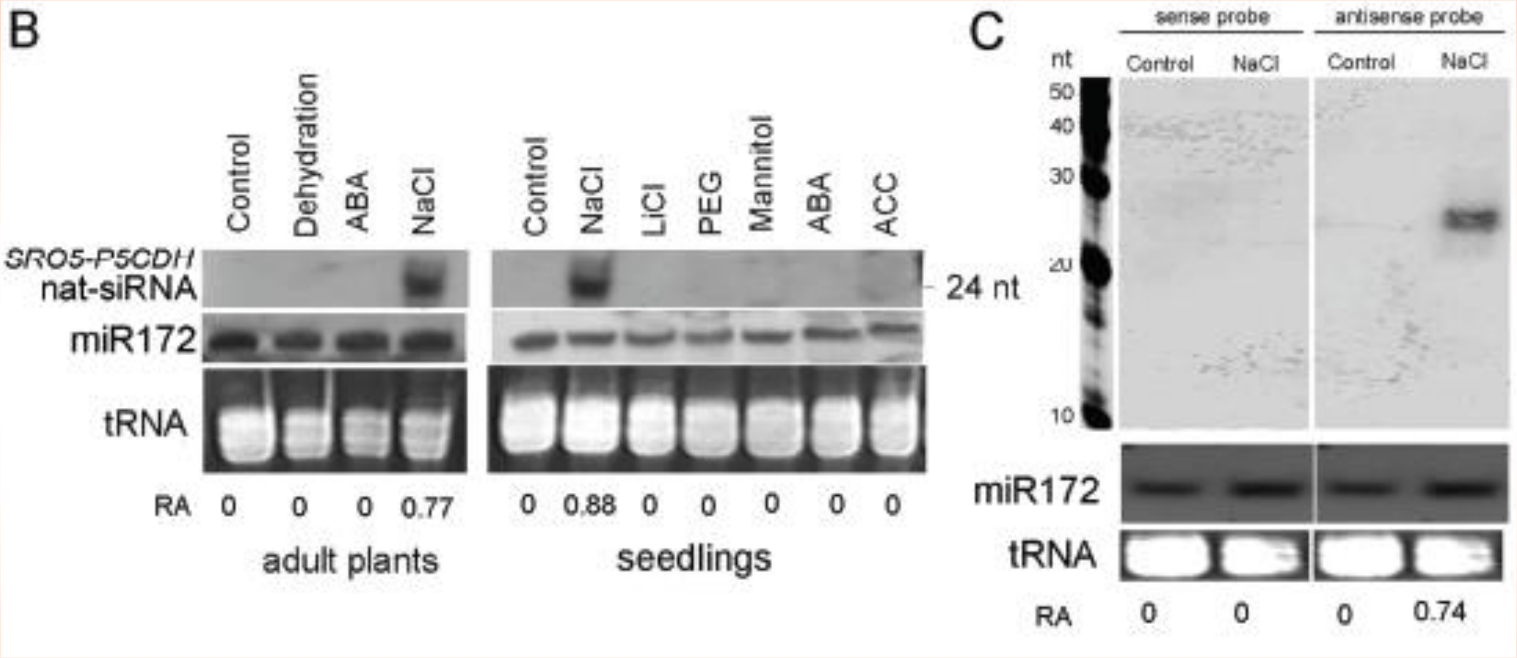
Vazquez 2006

nat-siRNAs

nat-siRNAs: **siRNAs derived from natural *cis*-antisense transcripts produced by convergent overlapping genes**

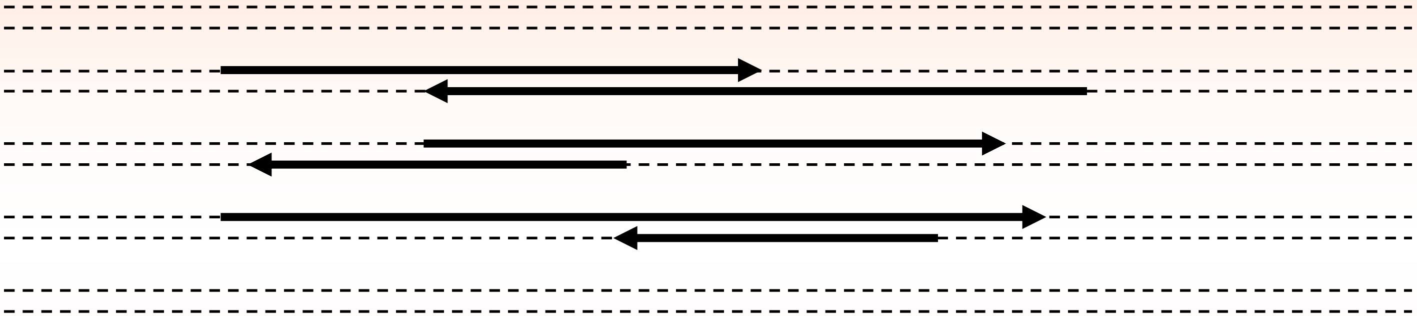


Salt Stress induces a 24-nt nat-siRNA from the *SRO5-P5CDH cis*-antisense transcripts



Requires
DCL2, DCL1, HYL1,
RDR6, SGS3, NRPD1a

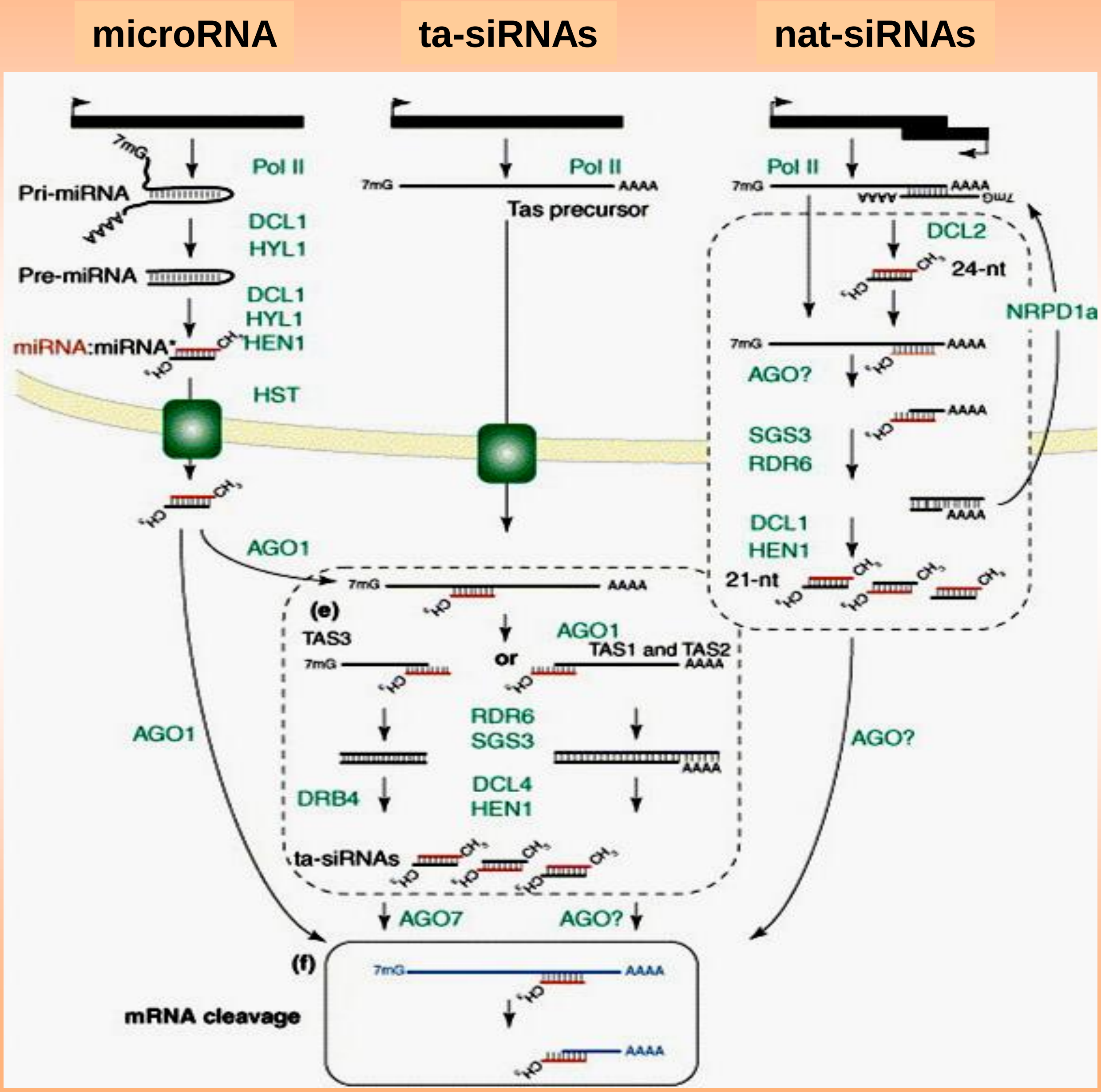
Natural Antisense Transcripts (NATs) May Serve As a Major Sources For Gene Targeting siRNAs



	NAT	Total	Percentage	Reference(s)
Human	2,940	26,741	22%	(16, 72)
Mouse	2,481	33,409	14.9%	(49)
Drosophila	1,027	13,379	15.4%	(44)
Rice	601	20,447	5.9%	(50)
Arabidopsis	1,340	29,993	8.9%	(68)

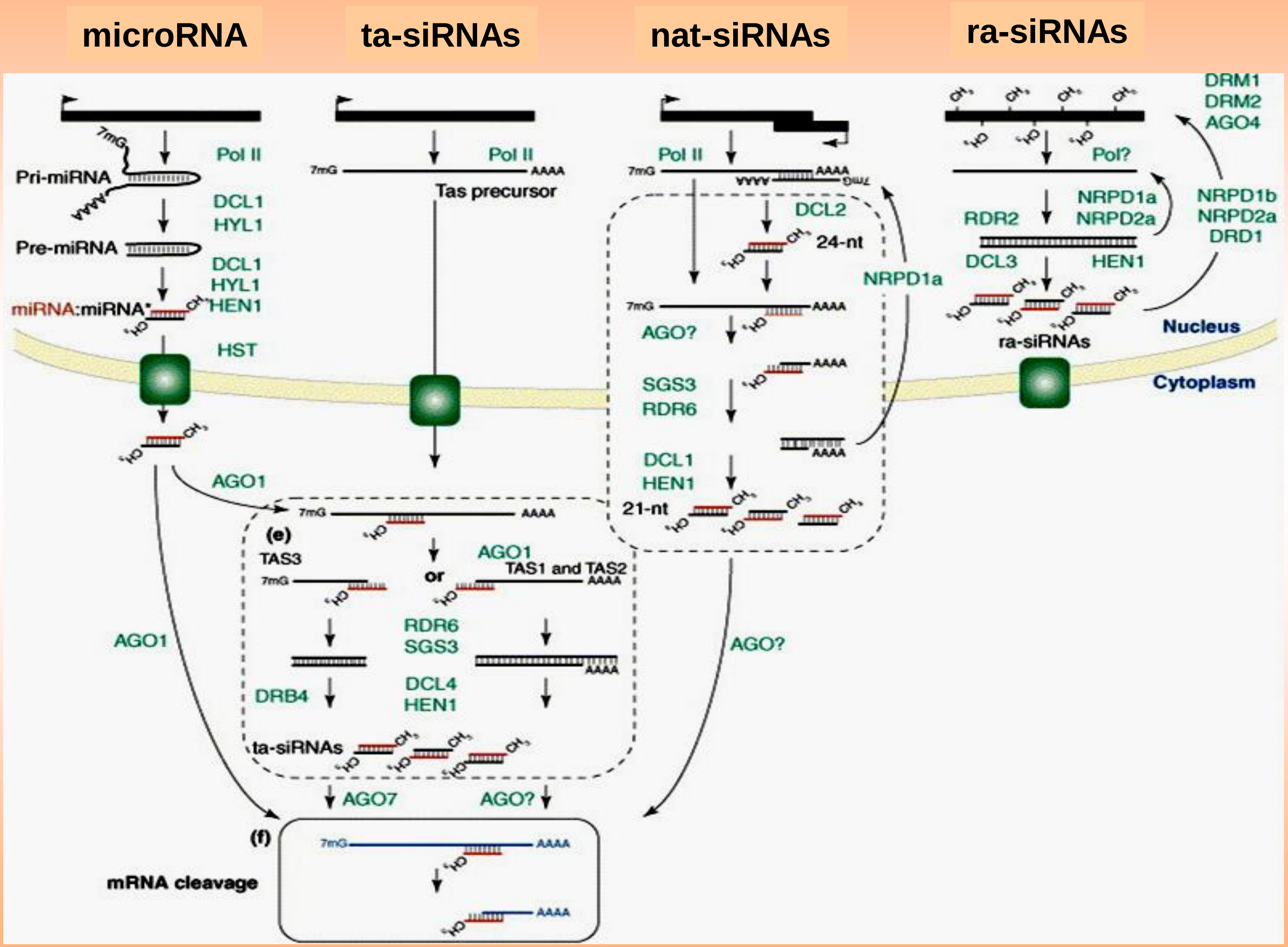
Werner et al., 2005

The Biogenesis Of Small RNAs In Plants



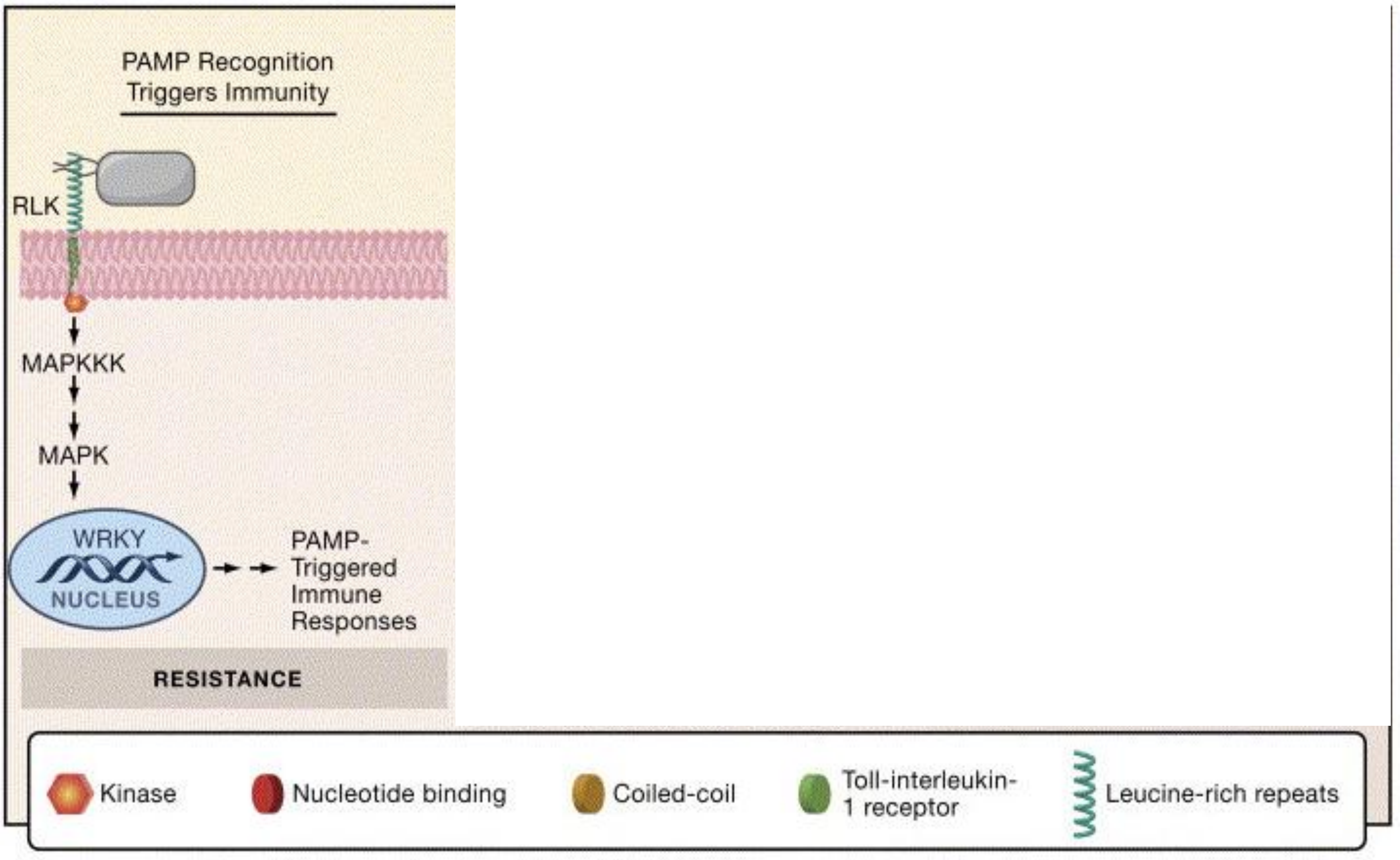
Vazquez 2006

The Biogenesis Of Small RNAs In Plants



Vazquez 2006

The Evolution Of Bacterial Resistance In Plants



Chisholm et al., 2006

Gene Regulation and Plant Immunity

Plants response to pathogen attacks

Recognition

R & Avr

RLP & PAMPs

siRNA

Gene Expression Reprogramming

miRNA

Race-specific Disease resistance

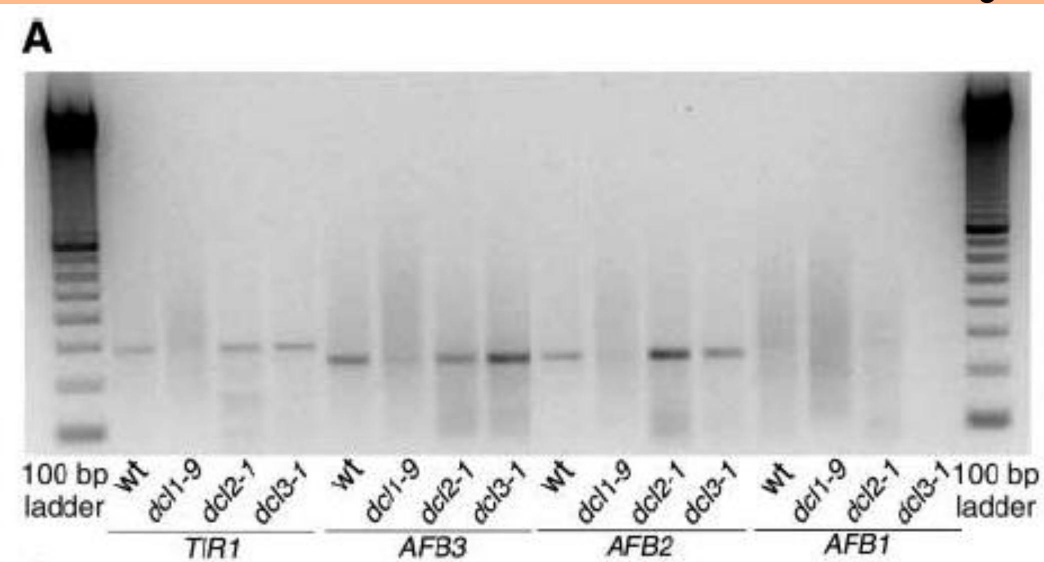
Basal defense

A Plant miRNA Contributes to Antibacterial Resistance by Repressing Auxin Signaling

Lionel Navarro,[1,2] Patrice Dunoyer,[2] Florence Jay,[2] Benedict Arnold,[3] Nihal Dharmasiri,[4] Mark Estelle,[4] Olivier Voinnet,[2]*† Jonathan D. G. Jones[1]*†

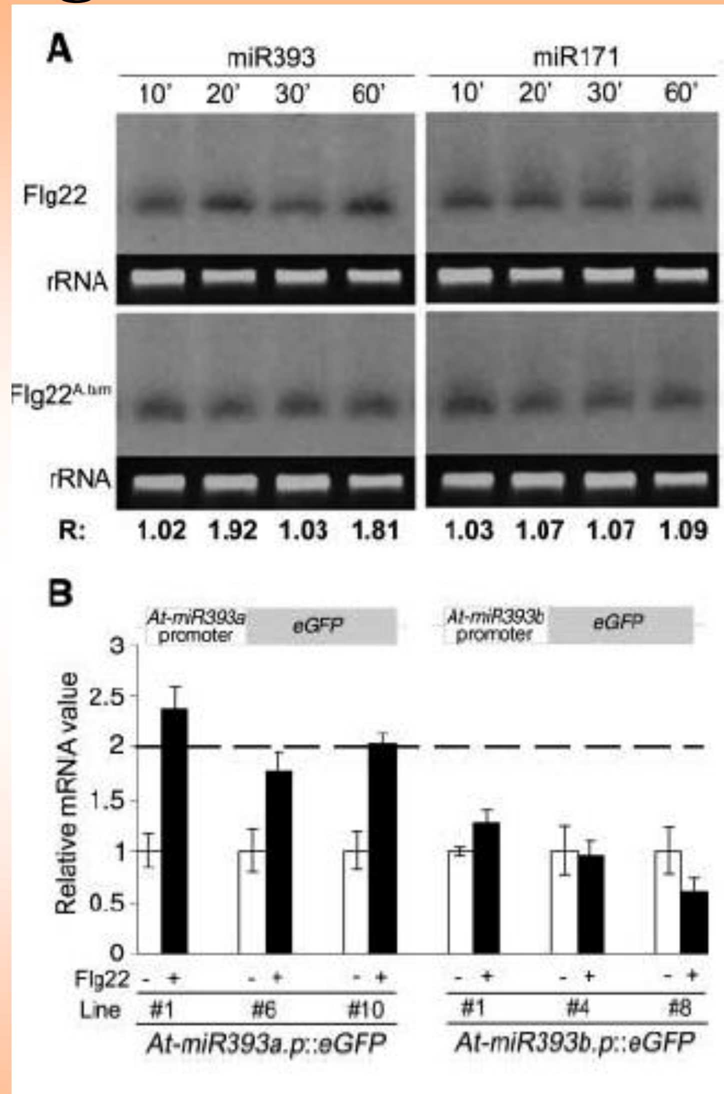
Science 2006

miR393a Is Induced By Flg22 Treatment



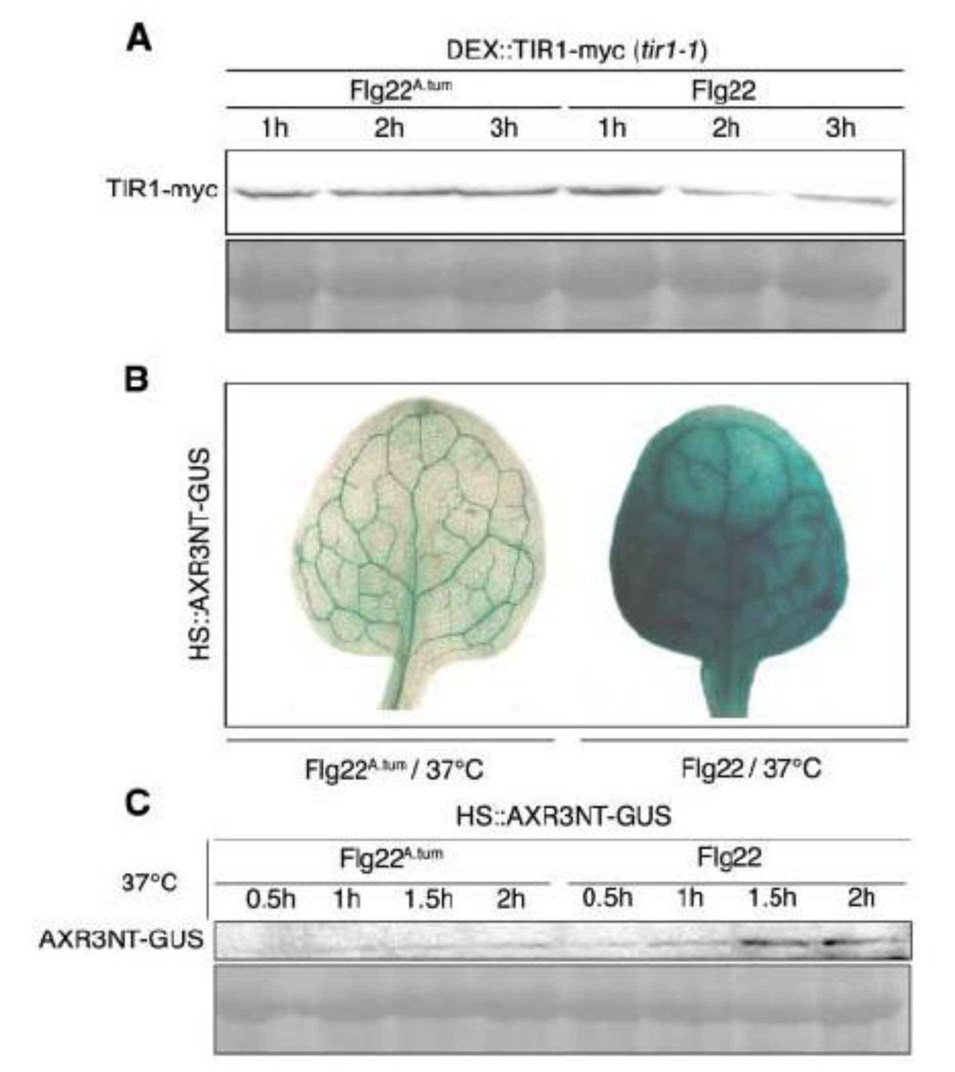
The F-box proteins TIR1, AFB1, AFB2, and AFB3 are receptors for the plant hormone auxin and the targets of miR393.

"AFB1 is partially resistant to miR393-directed cleavage."



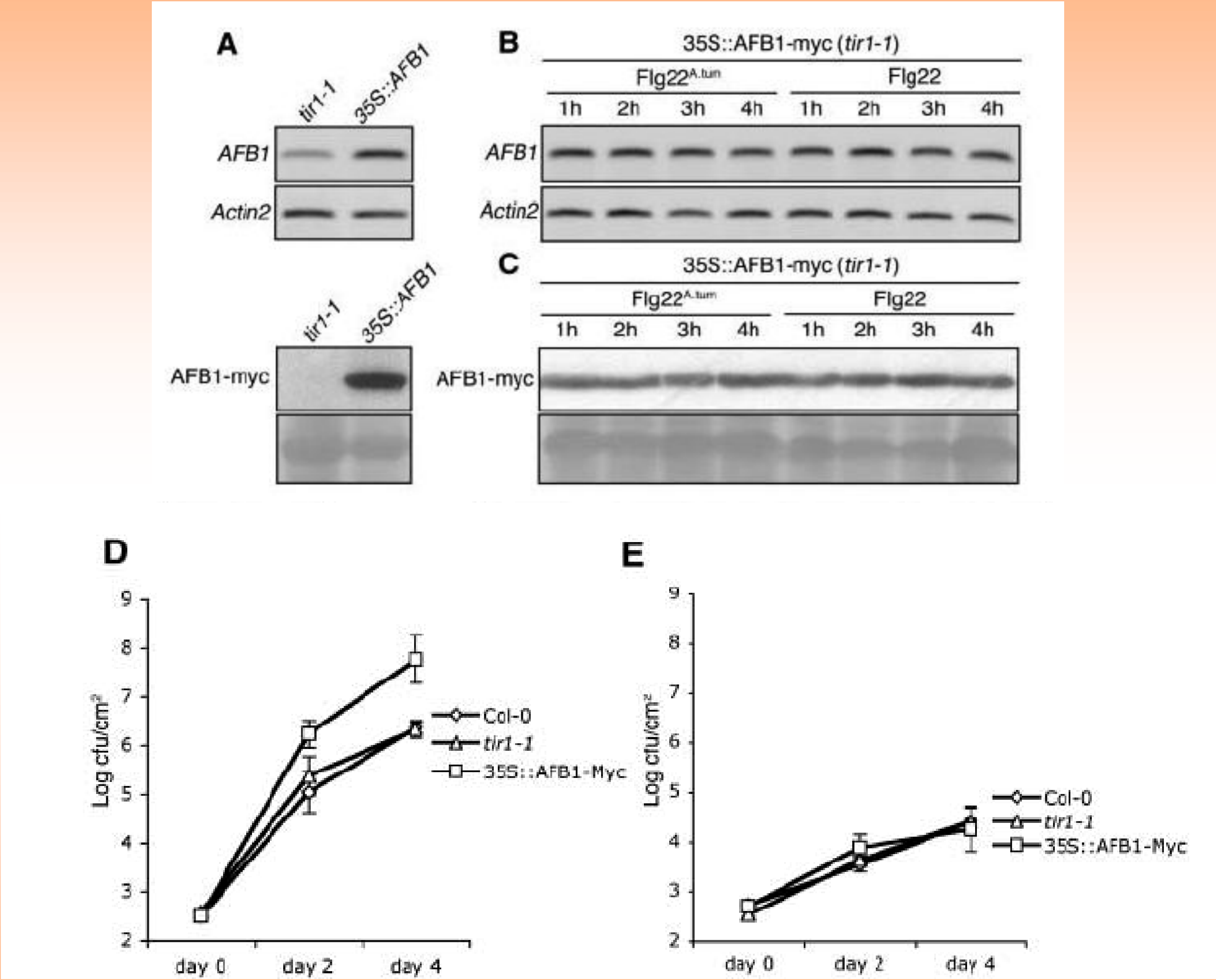
Flg22 → miR393 ⊣ TIR1, AFB2 and AFB3 but no AFB1

Flg22 stabilizes AXR3IAA17



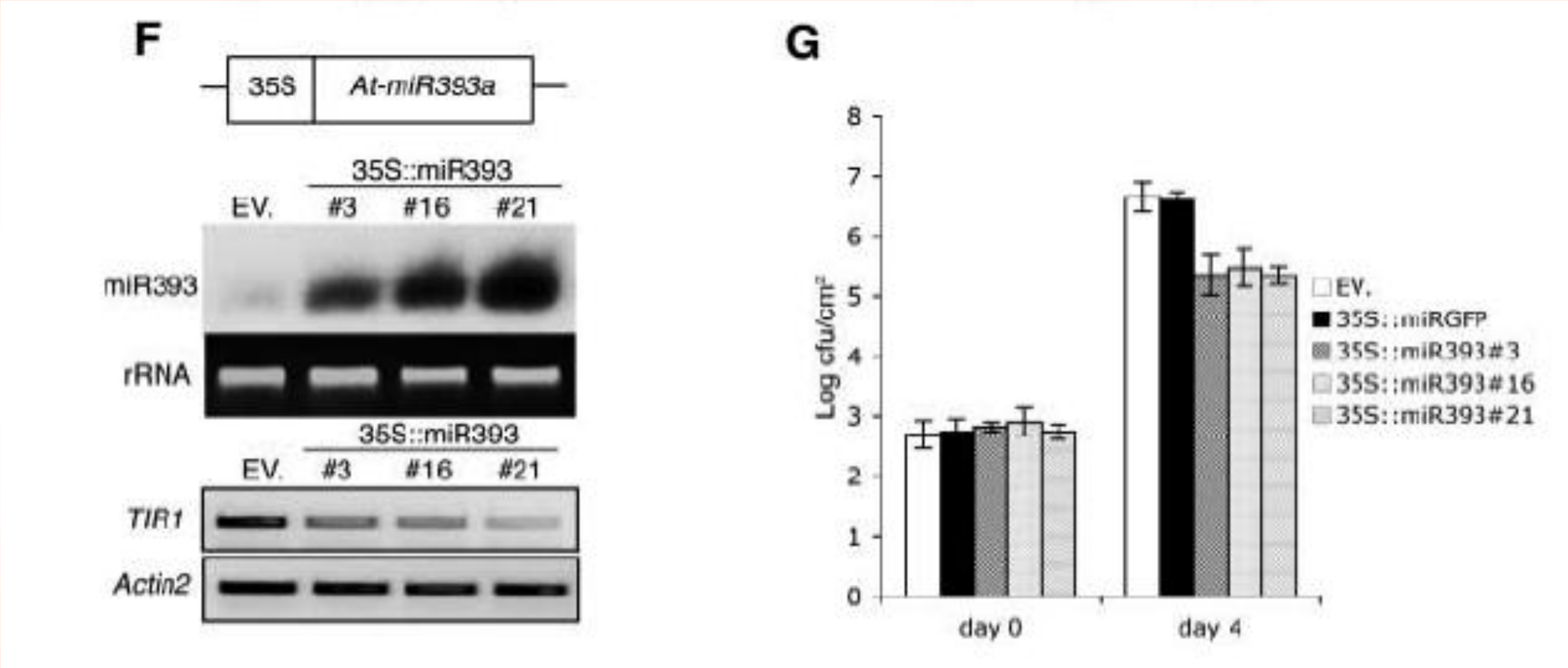
Flg22 → miR393 ⊣ TIR1, AFB2 and AFB3 but no AFB1 ⊣ **AXR3**

Overexpression of AFB1 Enhances Bacterial Susceptibility



Auxin promotes disease

Overexpression of miR393 Increases Resistance



Flg22 → miR393 ⊣ TIR1, AFB2 and AFB3 but no AFB1 ⊣**Resistance**

Regulation Model

Flg22

↓

miR393

⊥

TIR1, AFB2 and AFB3 but no AFB1

↓ ⊥

Disease Resistance

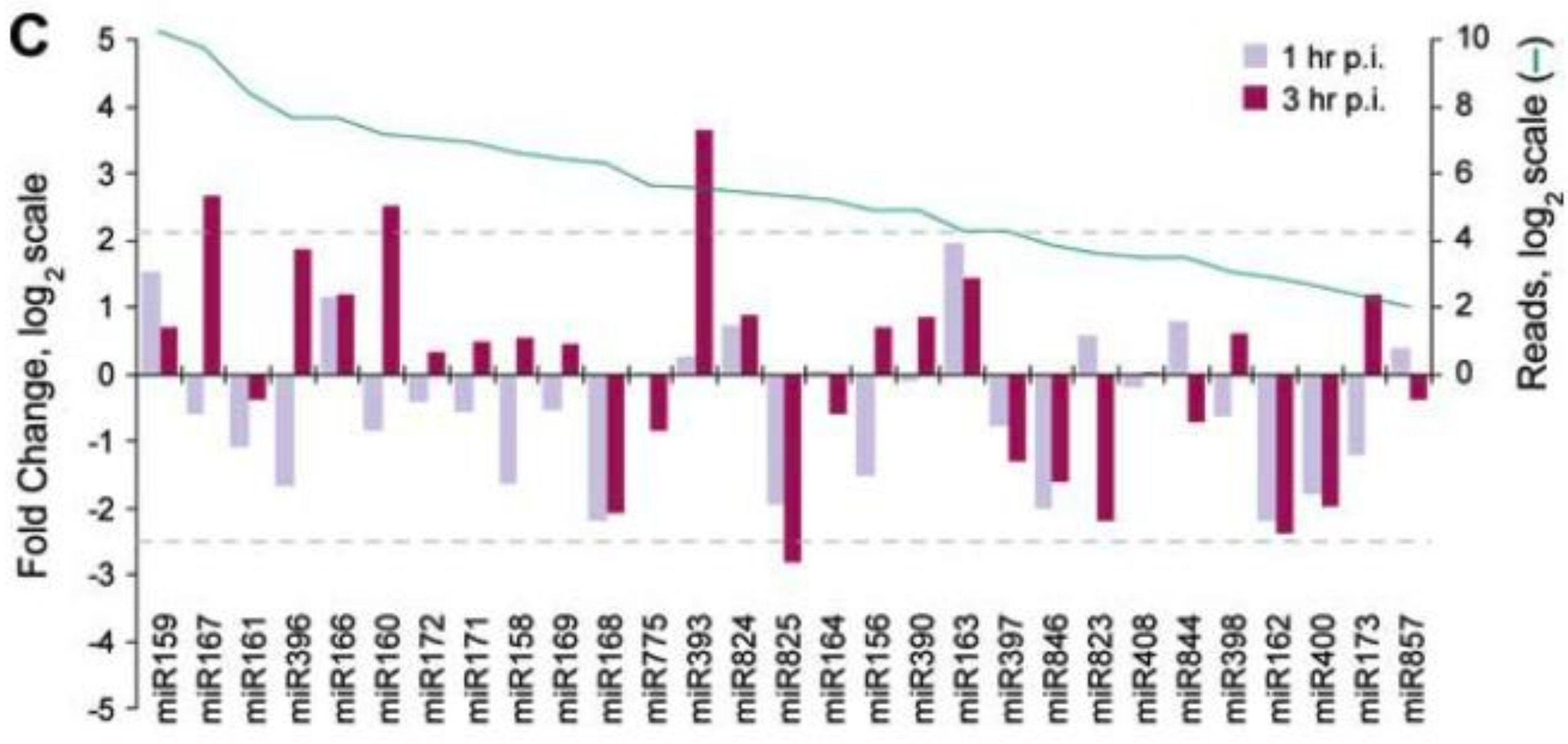
Increase Auxin Signaling ------ Disease
Repress Auxin Signaling -------Resistance

Most P.syringae strains can produce IAA
Pto DC3000 infection triggers increased IAA levels in Arabidopsis ----Enhances disease

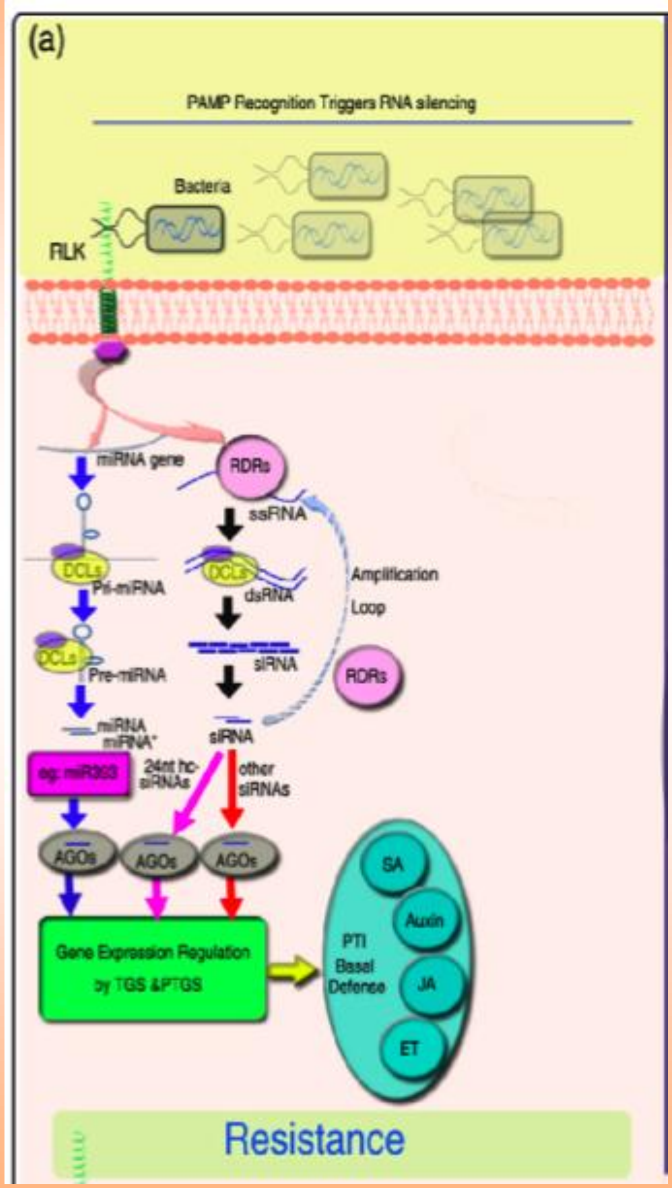
OPEN ACCESS Freely available online PLoS one

High-Throughput Sequencing of *Arabidopsis* microRNAs: Evidence for Frequent Birth and Death of *MIRNA* Genes

Noah Fahlgren[1,2], Miya D. Howell[1], Kristin D. Kasschau[1], Elisabeth J. Chapman[1,2], Christopher M. Sullivan[1], Jason S. Cumbie[1], Scott A. Givan[1], Theresa F. Law[3], Sarah R. Grant[3], Jeffery L. Dangl[3], James C. Carrington[1]*



Plant miRNAs contribute to PTI



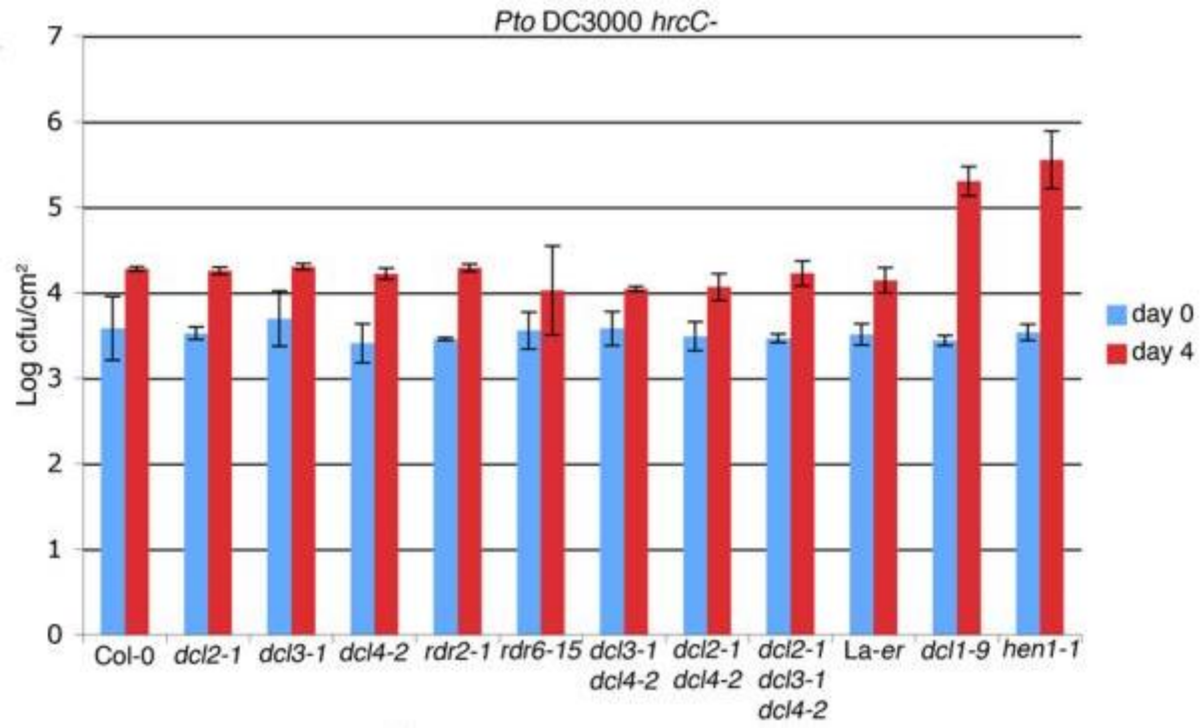
Padmanabhan C, Zhang X, Jin H.
Curr Opin Plant Biol. 2009

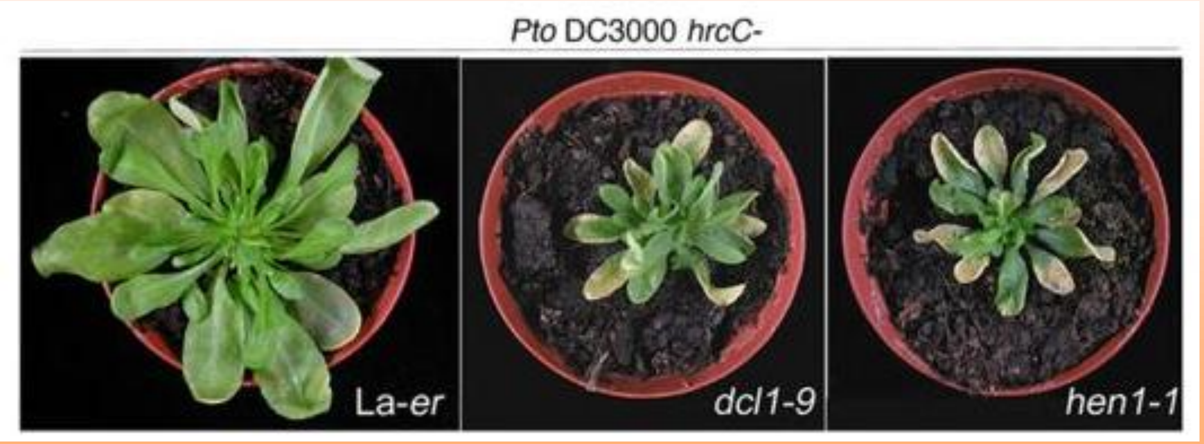
Suppression of the MicroRNA Pathway by Bacterial Effector Proteins

Lionel Navarro,[1] Florence Jay,[1] Kinya Nomura,[2] Sheng Yang He,[2] Olivier Voinnet[1]*

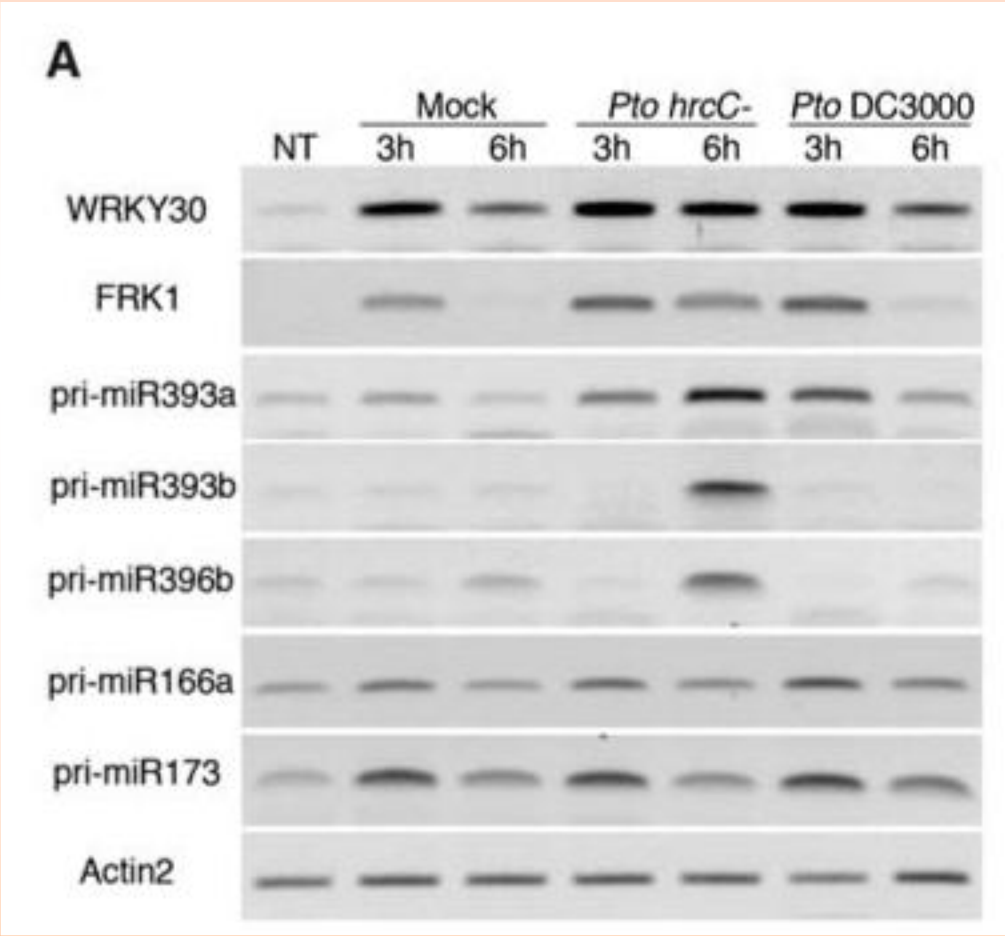
Science. 2008 Aug 15;321(5891):964-7.

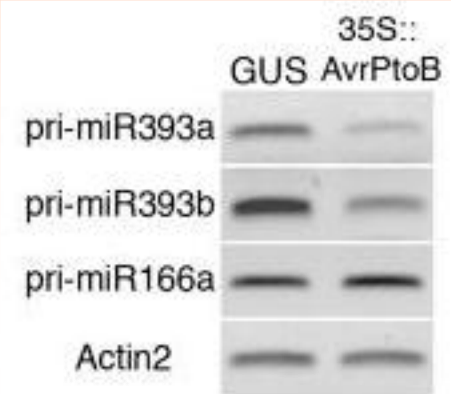
The Arabidopsis miRNA pathway promotes basal resistance to bacteria

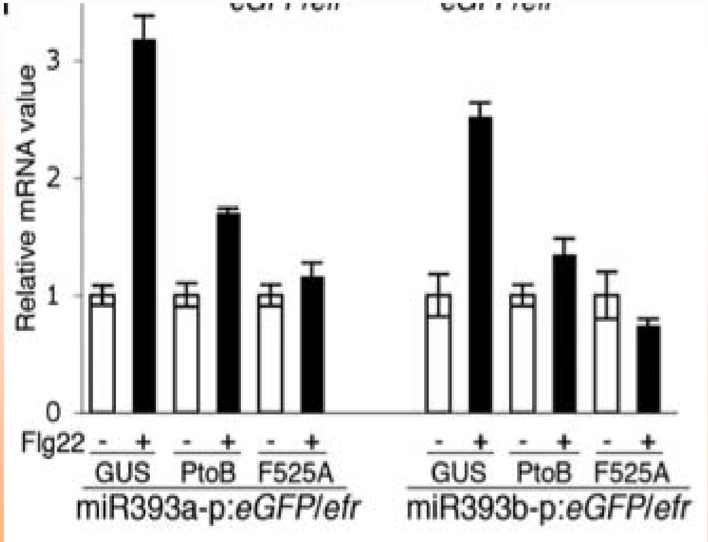




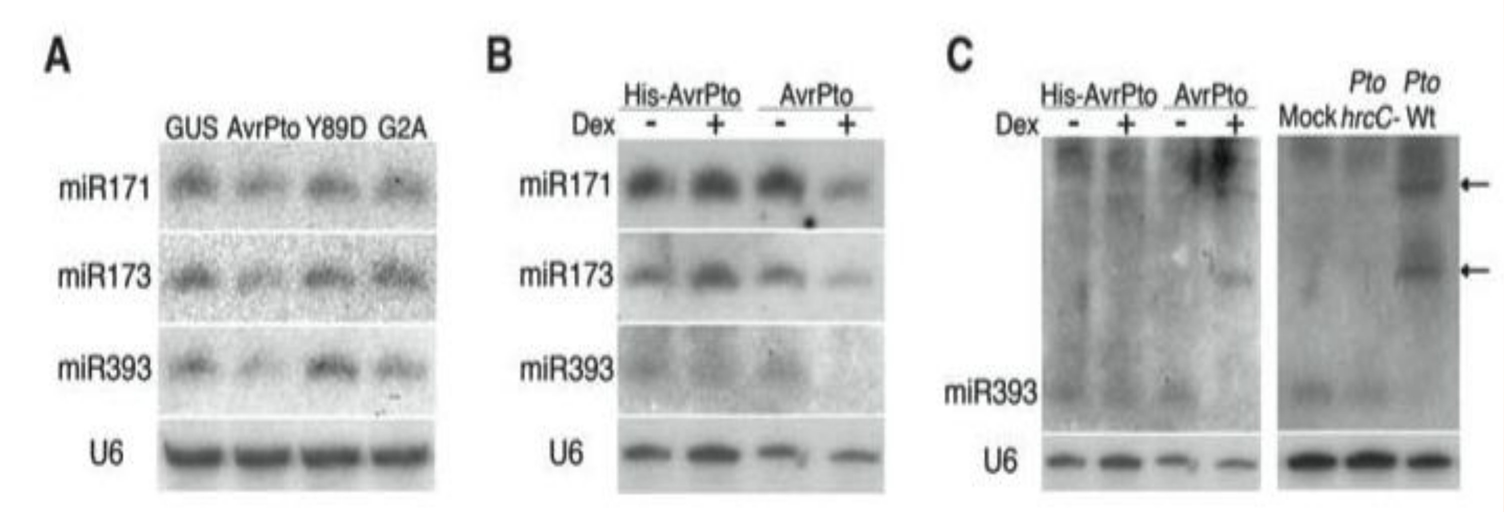
Transcriptional repression of PAMP- responsive miRNAs by Pto DC3000 and AvrPtoB



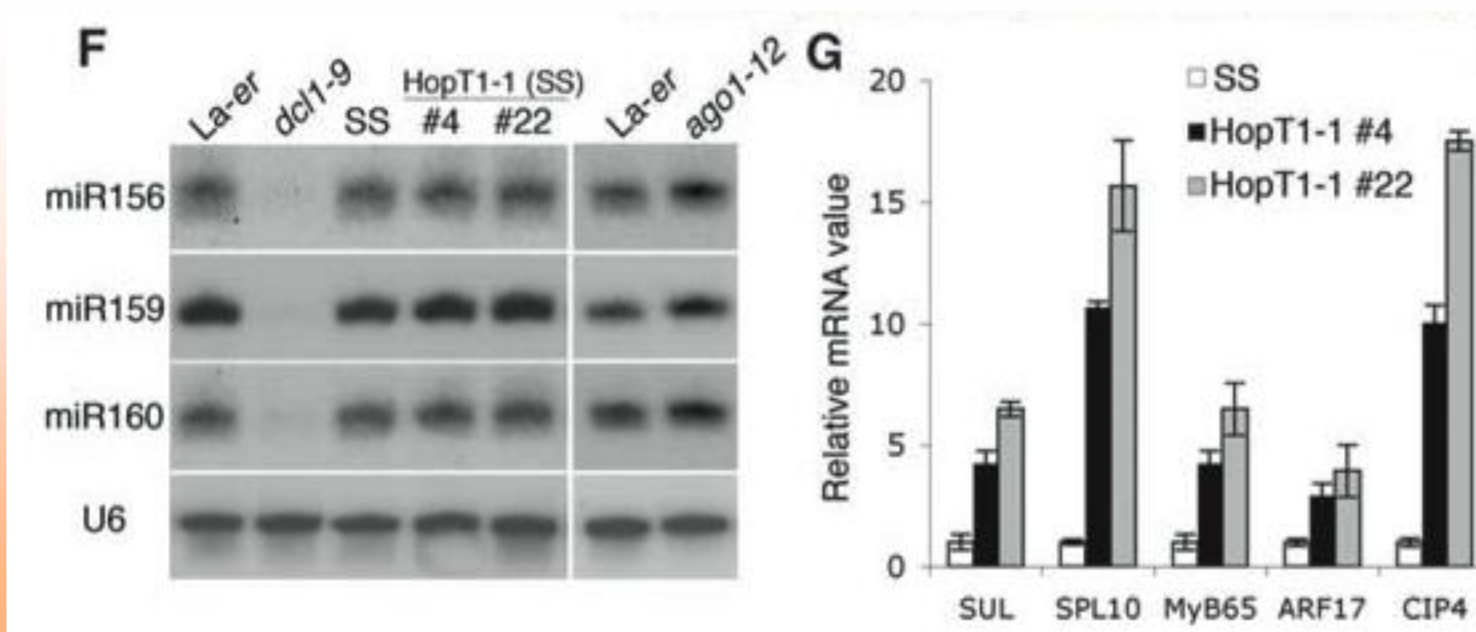




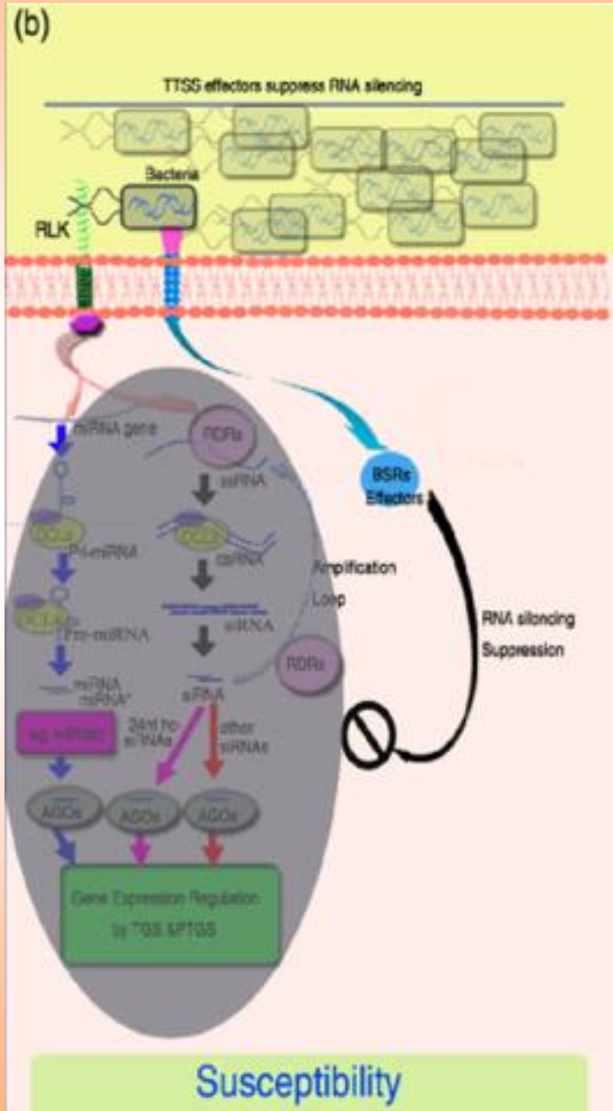
Suppression of miRNA accumulation by bacterial effectors

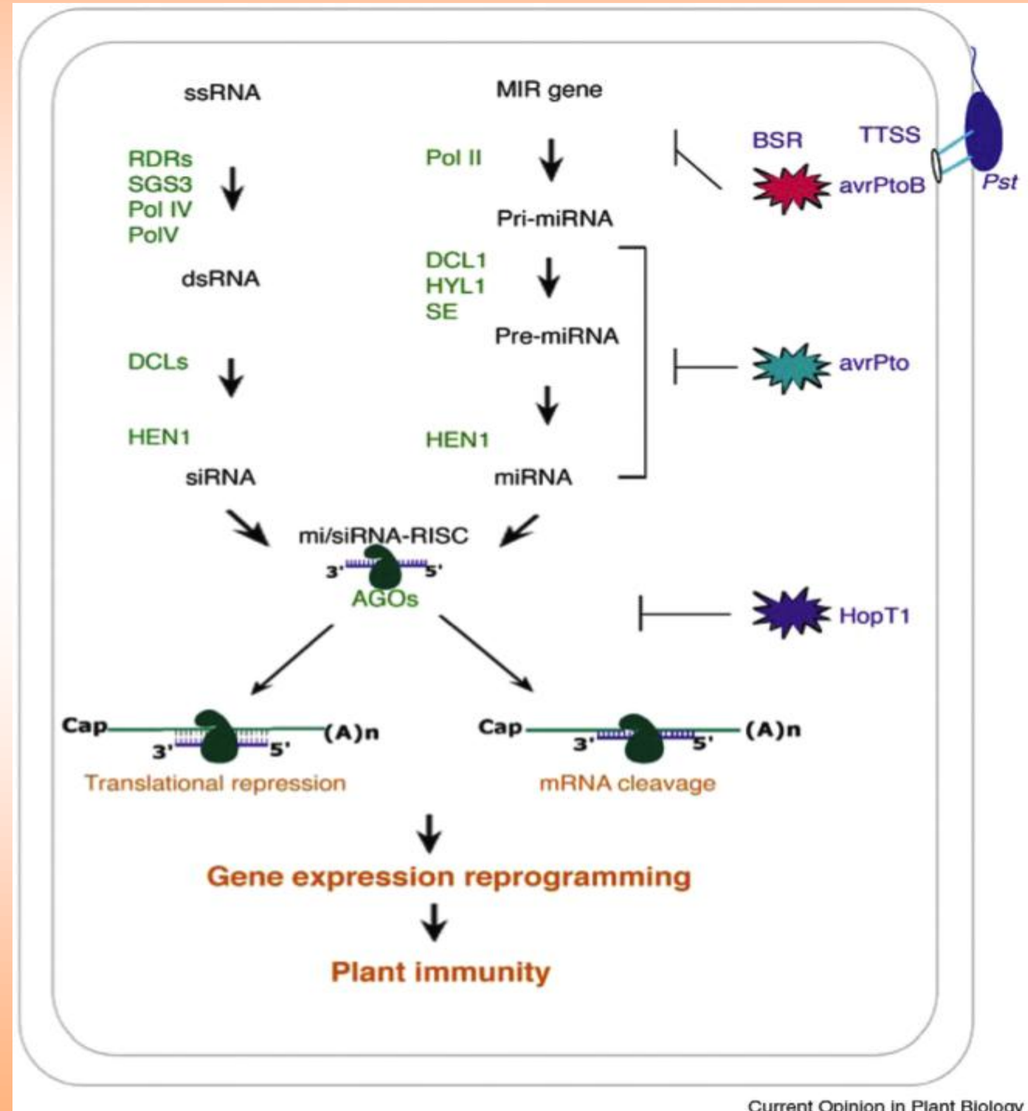


Suppression of miRNA activity by bacterial effectors



Effector Proteins Act As Silencing Suppressors





Gene Regulation and Plant Immunity

Plants response to pathogen attacks

Recognition

R & Avr

RLP & PAMPs

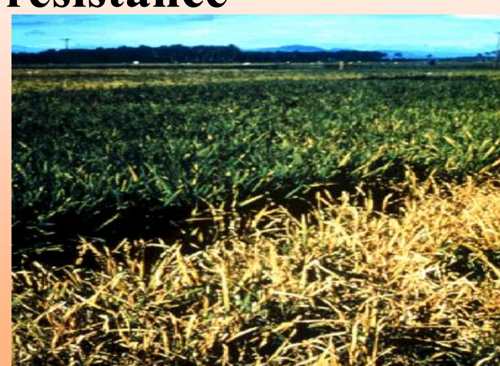
siRNA

Gene Expression Reprogramming

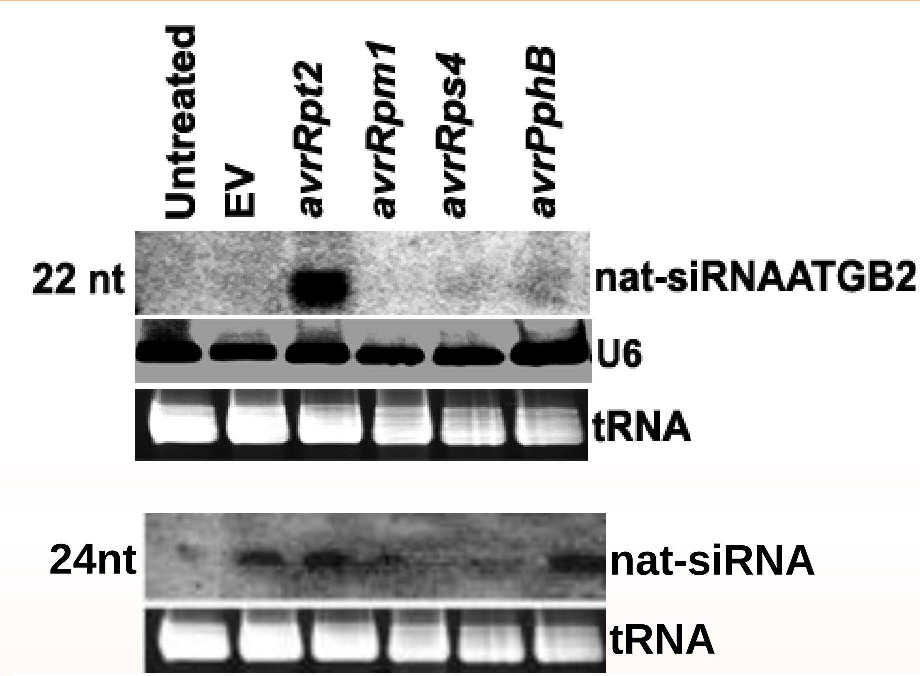
miRNA

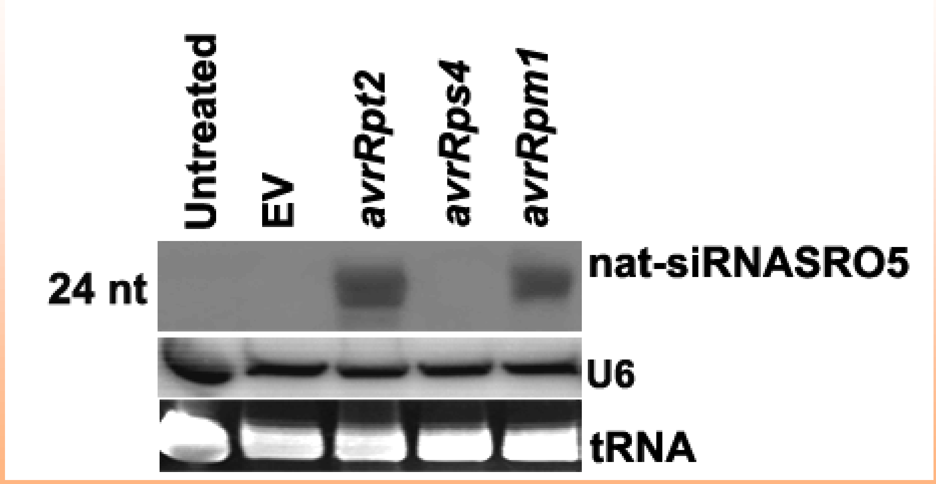
Race-specific Disease resistance

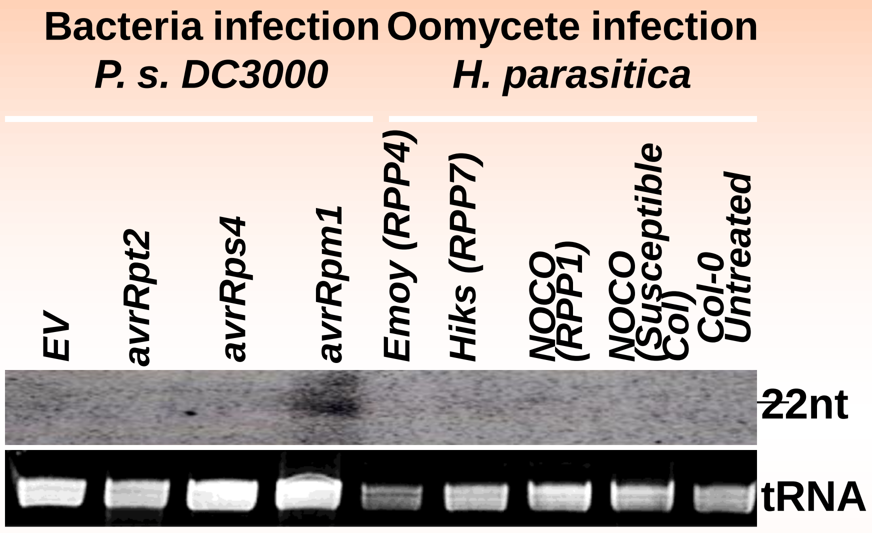
Basal defense

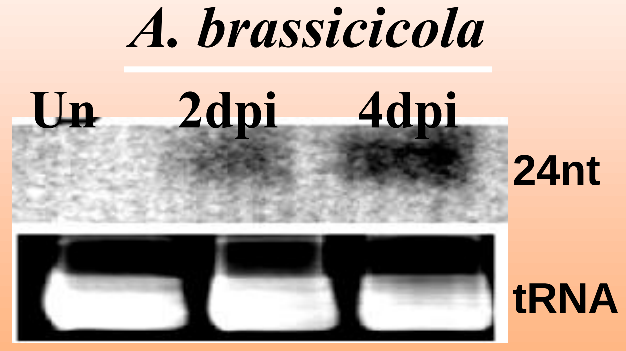


Pathogen-induced endogenous siRNAs in Arabidopsis







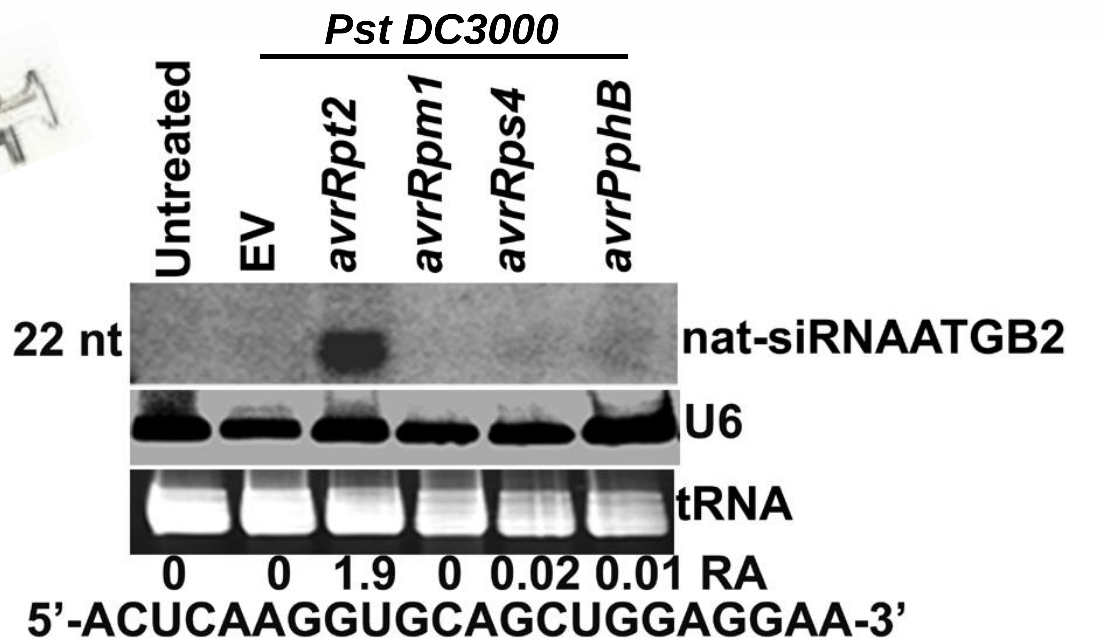


A pathogen-inducible endogenous siRNA in plant immunity

Surekha Katiyar-Agarwal*, Rebekah Morgan*, Douglas Dahlbeck†, Omar Borsani‡, Andy Villegas, Jr.*, Jian-Kang Zhu‡, Brian J. Staskawicz†§, and Hailing Jin*§

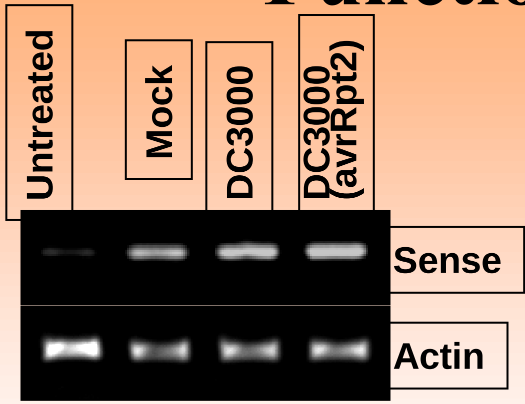
Departments of *Plant Pathology and ‡Botany and Plant Sciences, Center for Plant Cell Biology and Institute for Integrative Genome Biology, University of California, Riverside, CA 92521; and †Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

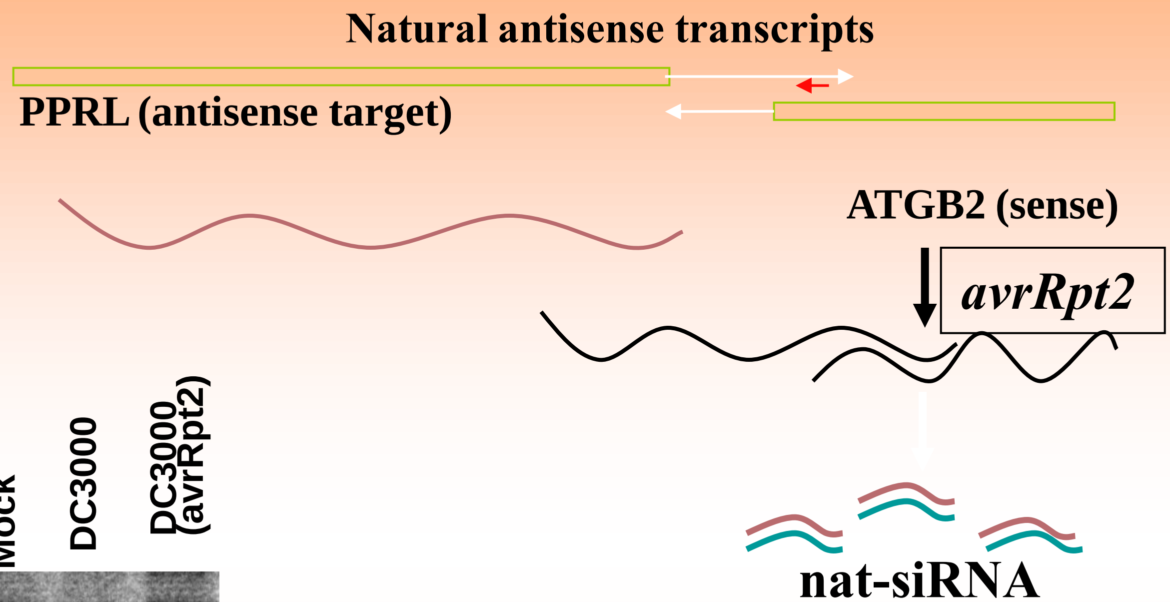
3-4 week old *A. thaliana*



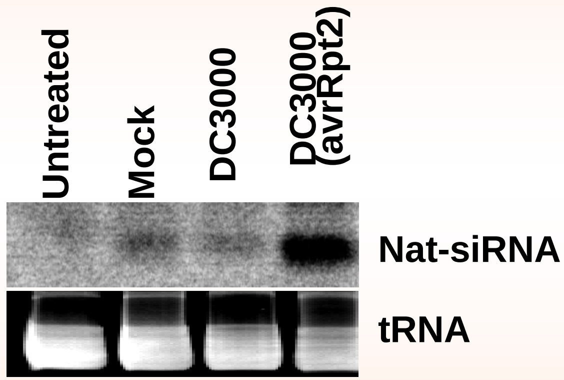
PNAS, 2006

Function Of The nat-siRNAATGB2

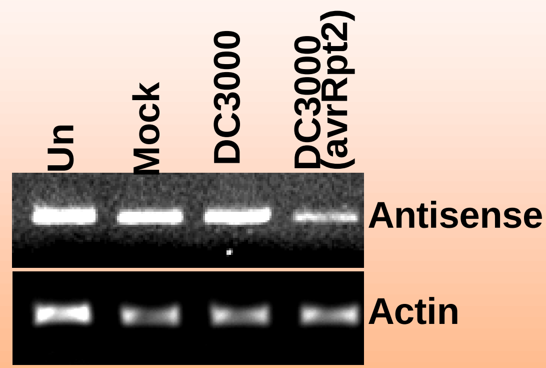


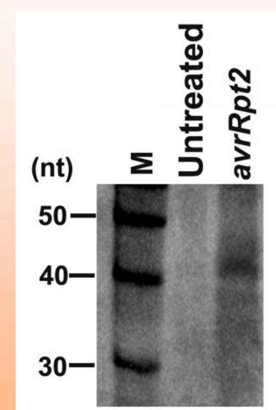


Induction of sense gene



dsRNA pairing nat-siRNA forming

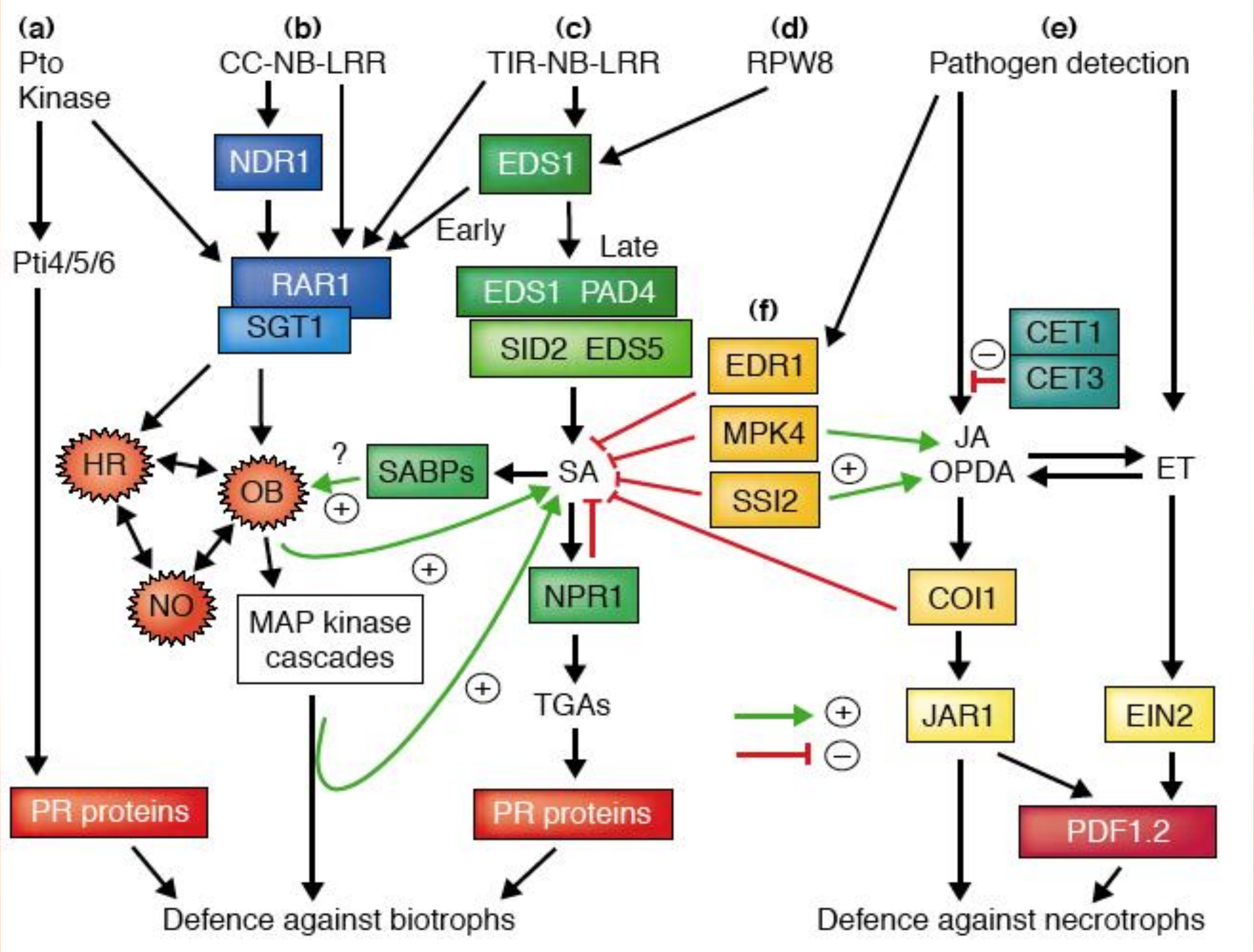




Silencing of the antisense gene

	Pathogen	
	-Avr	**+AvrRpt2**
Plants -R	Disease	Disease
Plants +Rps2	Disease	Resistance

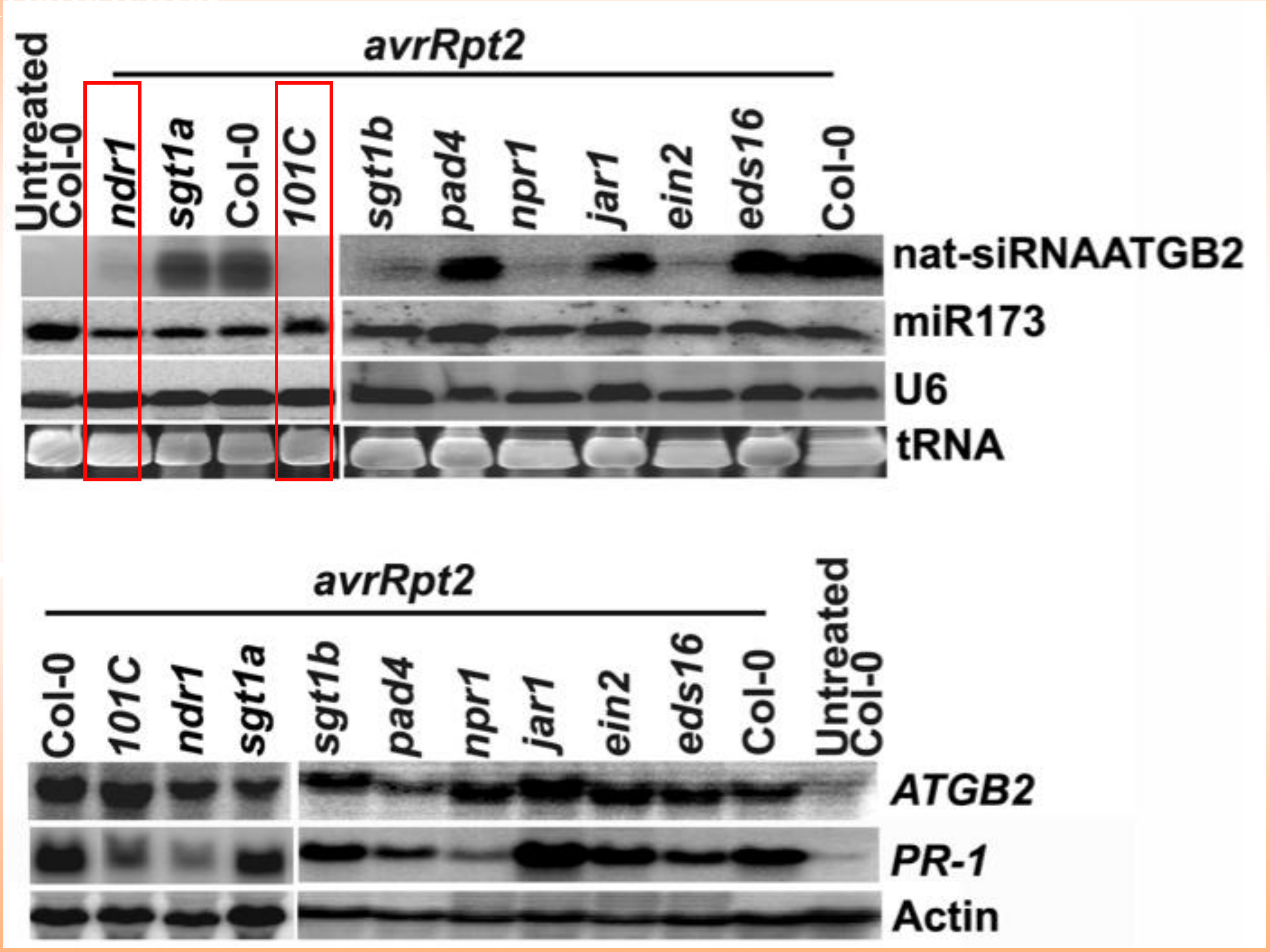
Disease Resistance Signaling Network



Hammond-Kosack and Parker, COBiotech, 2003, 14:177

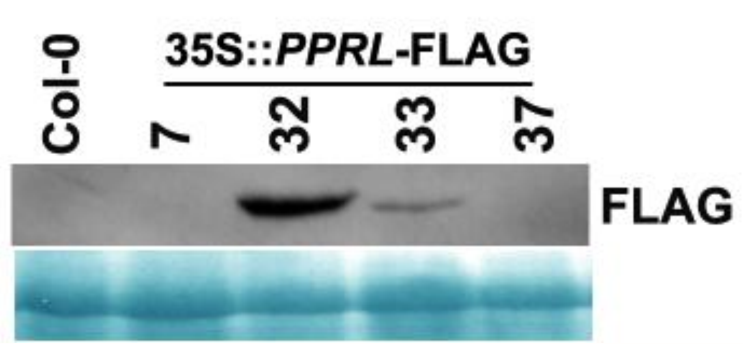
The Induction Of nat-siRNAATGB2 Requires Functional RPS2 And NDR1

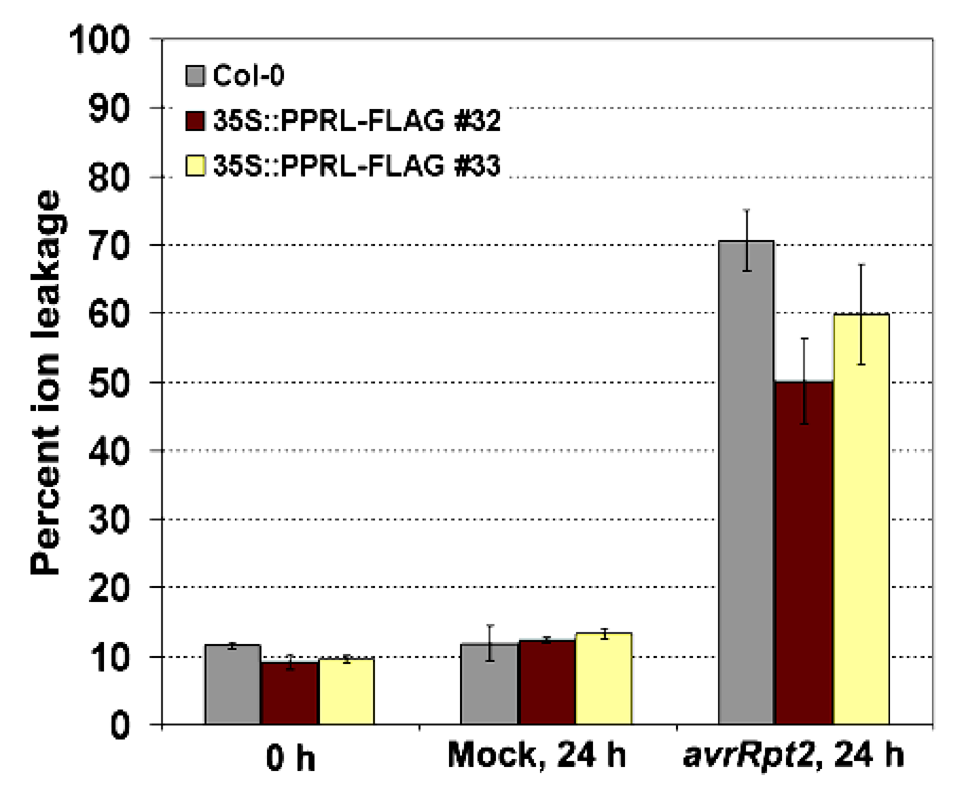
Low Mol



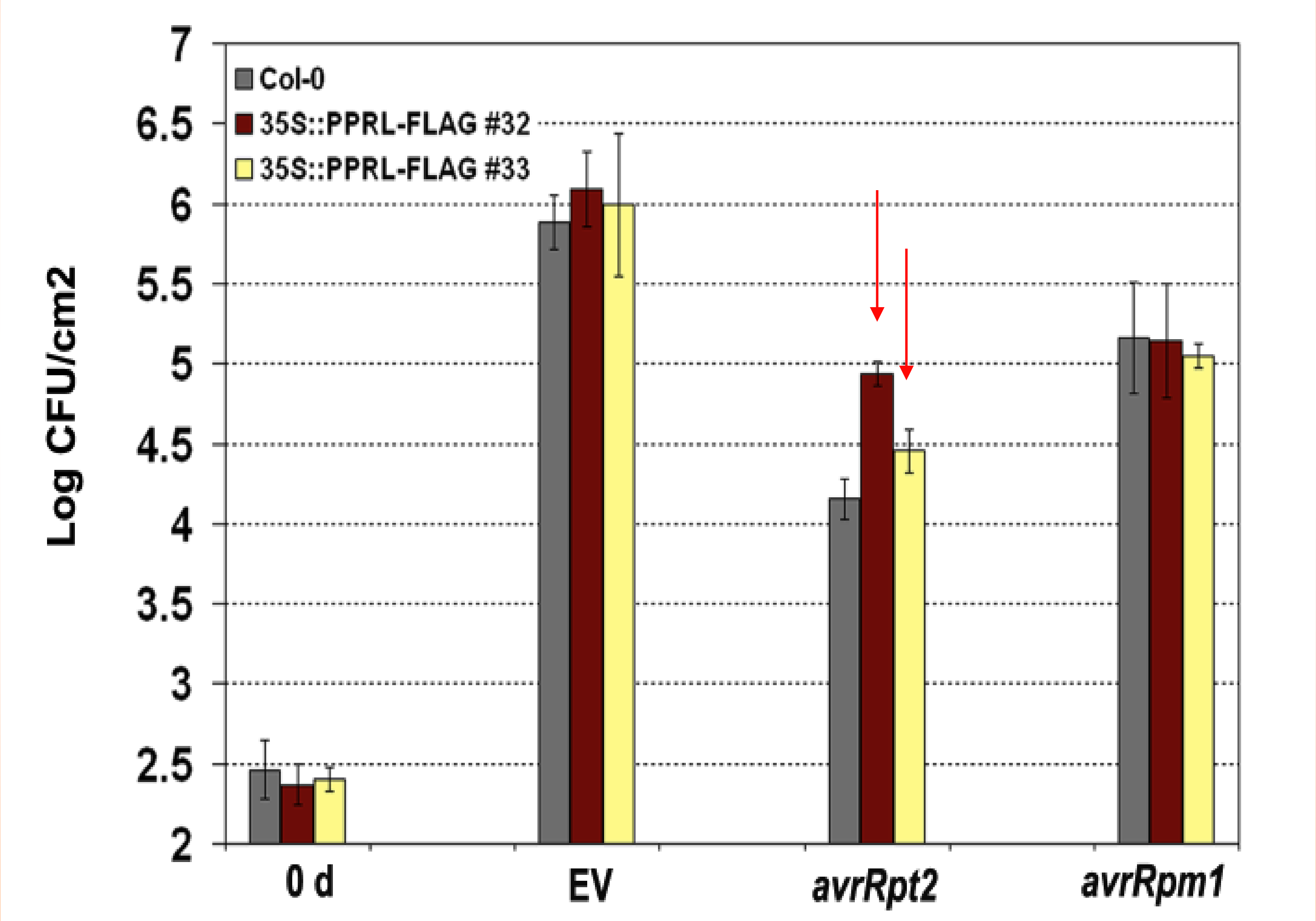
High Mo

Overexpression of The nat-siRNA Target---*PPRL* Reduces The Hypersensitive Response To *avrRpt2*

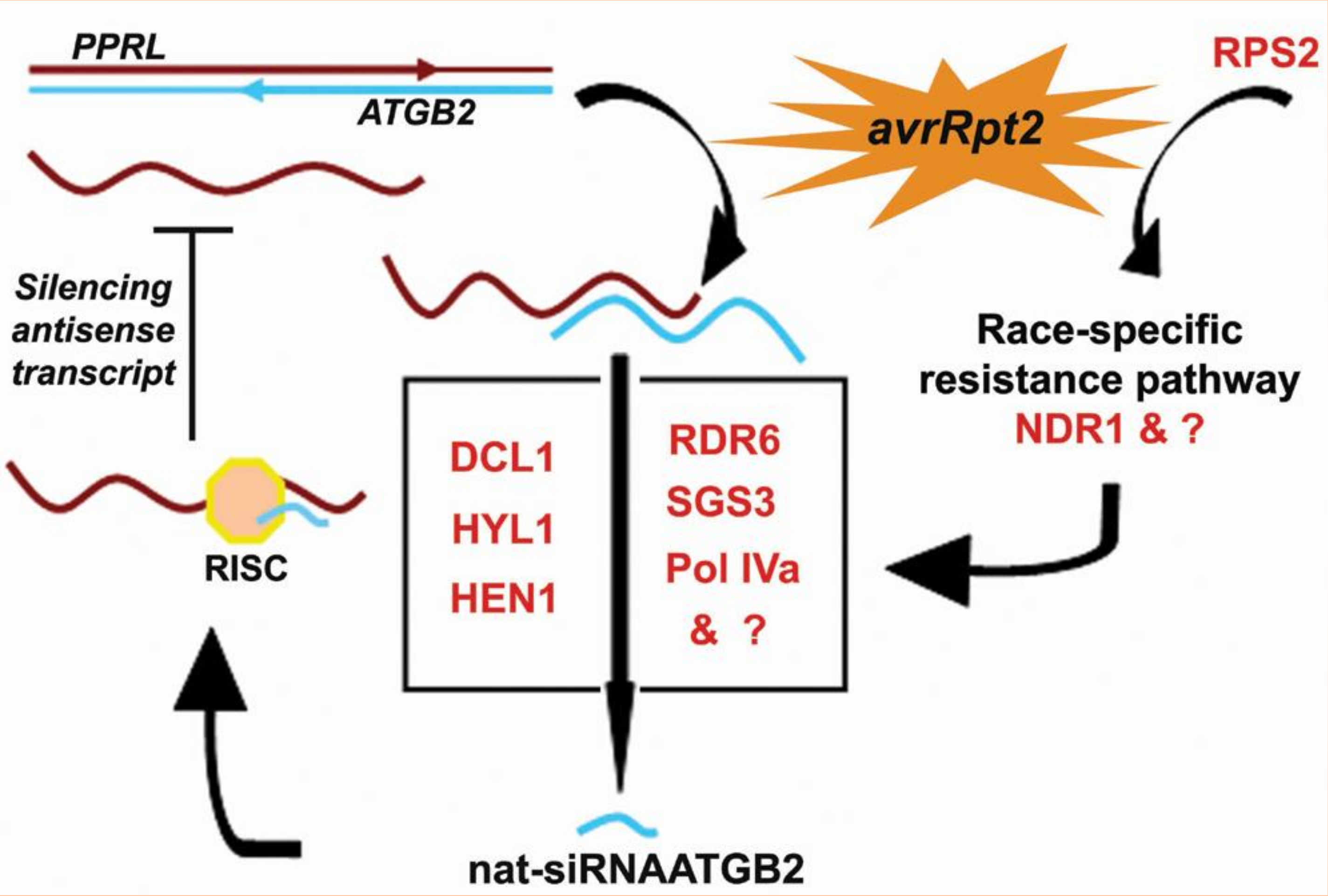




Overexpression of *PPRL* Enhances The Susceptibility To *Bacteria* Carrying *avrRpt2*



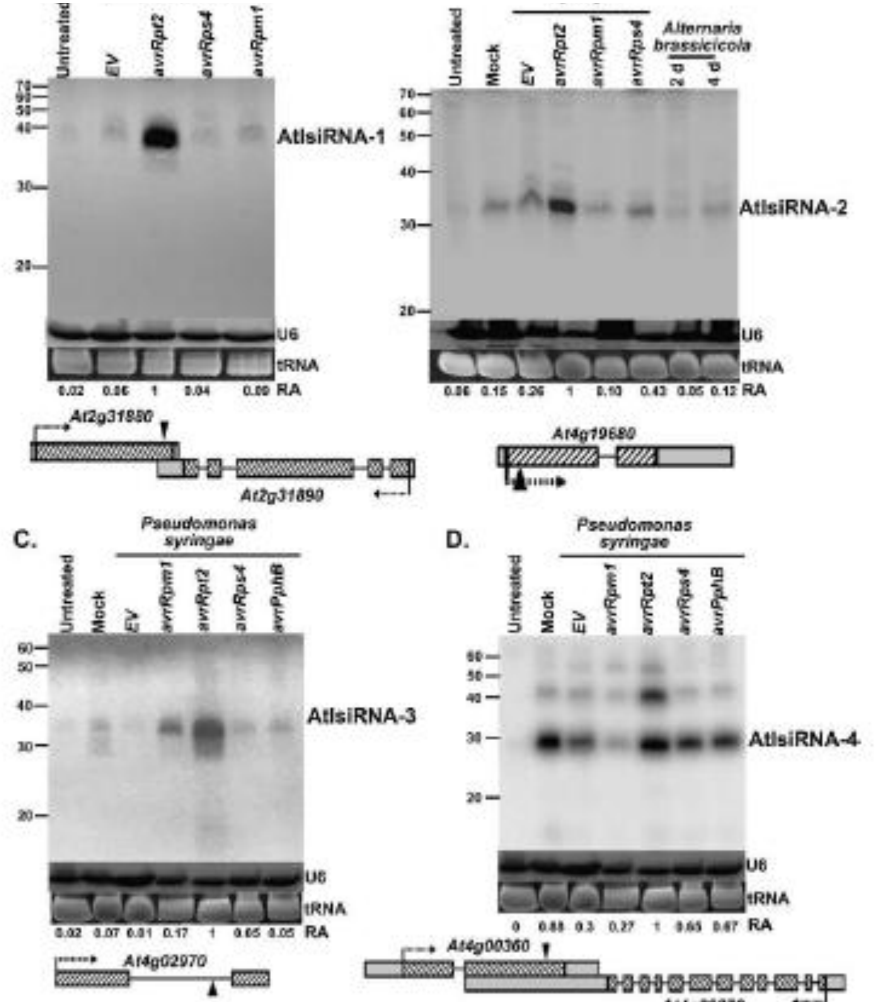
nat-siRNAATGB2 Working Model



A novel class of bacteria-induced small RNAs in *Arabidopsis*

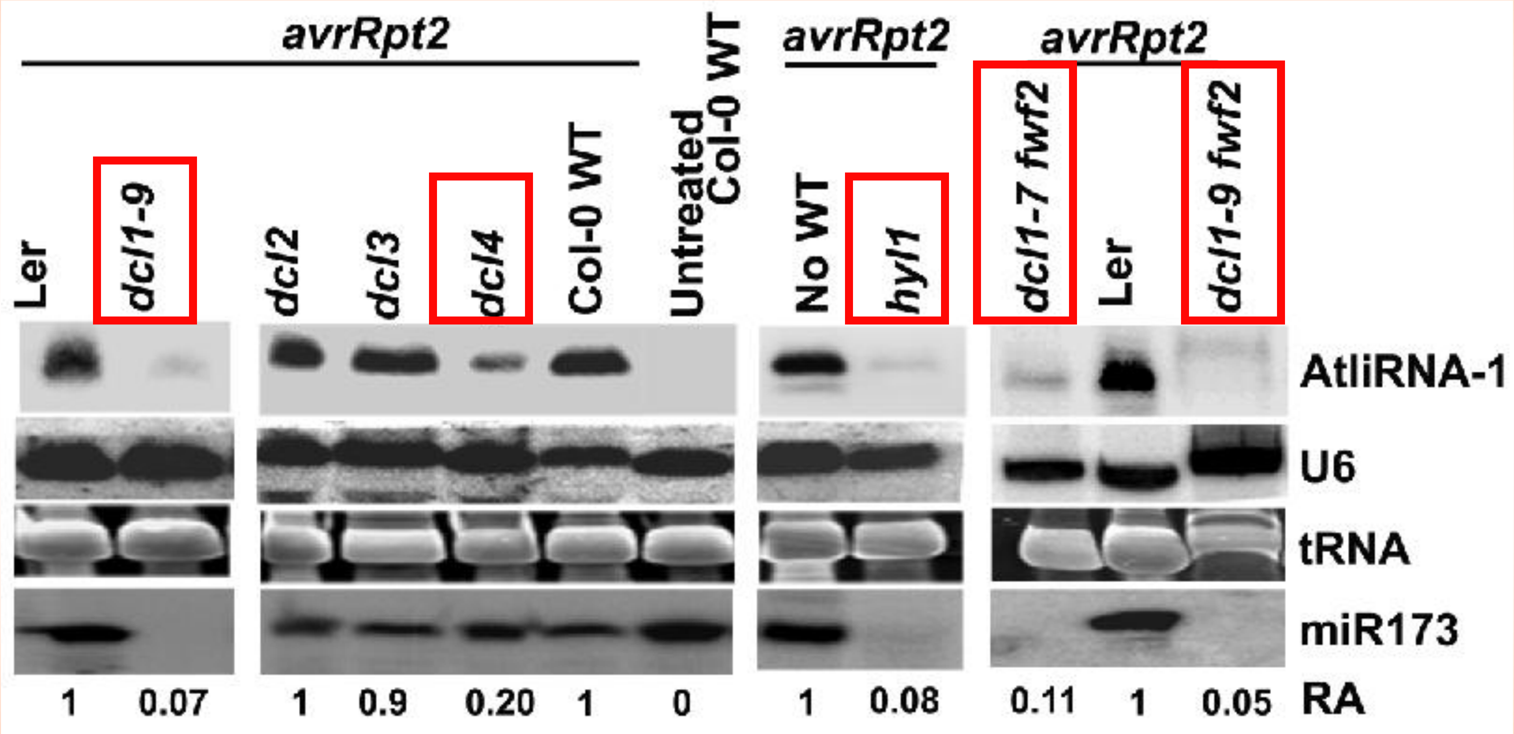
Surekha Katiyar-Agarwal,[1,3] Shang Gao,[1] Adam Vivian-Smith,[2] and Hailing Jin[1,4]

[1]Department of Plant Pathology and Microbiology, Center for Plant Cell Biology and Institute for Integrative Genome Biology, University of California at Riverside, Riverside, California 92521, USA; [2]Institute of Biology, Leiden University, 2332 AL Leiden, The Netherlands



Genes & Development, 2007

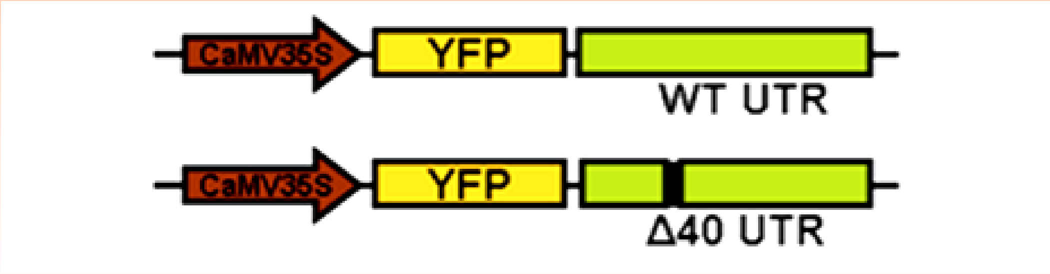
The Genetic Requirement For AtlsiRNA-1

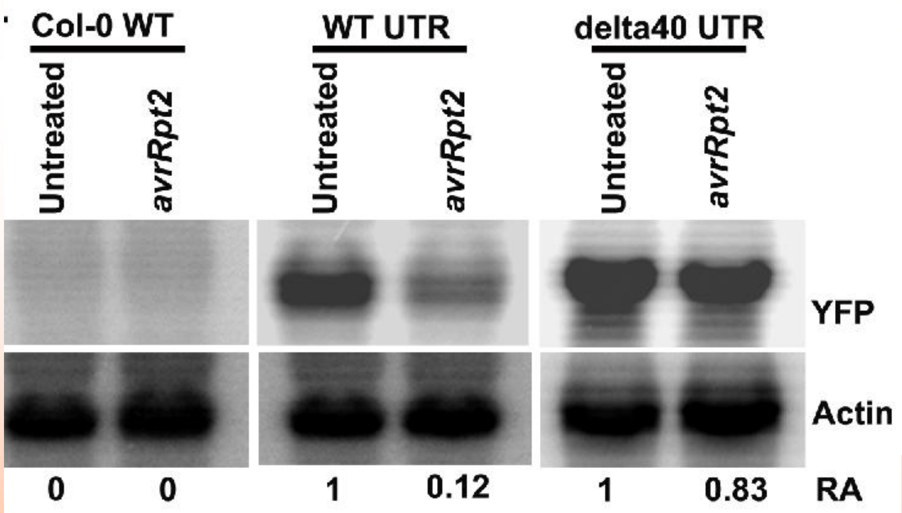


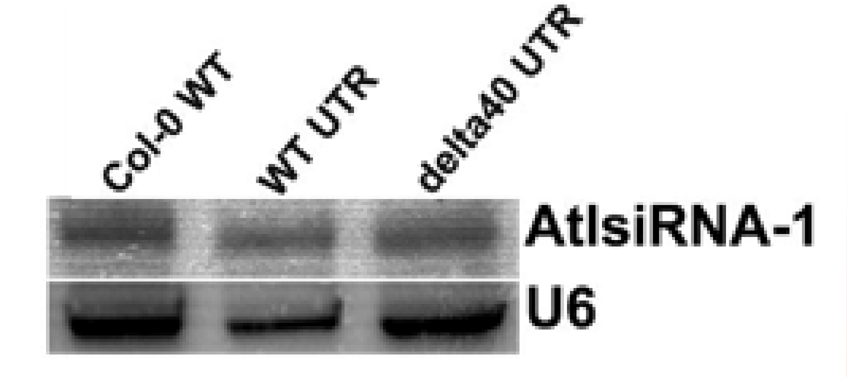
Depends on DCL1, DCL4 and HYL1

Katiyar-Agarwal et al., Genes & Dev. 2007

Induction Of AtliRNA-1 Down-Regulates The Antisense Target

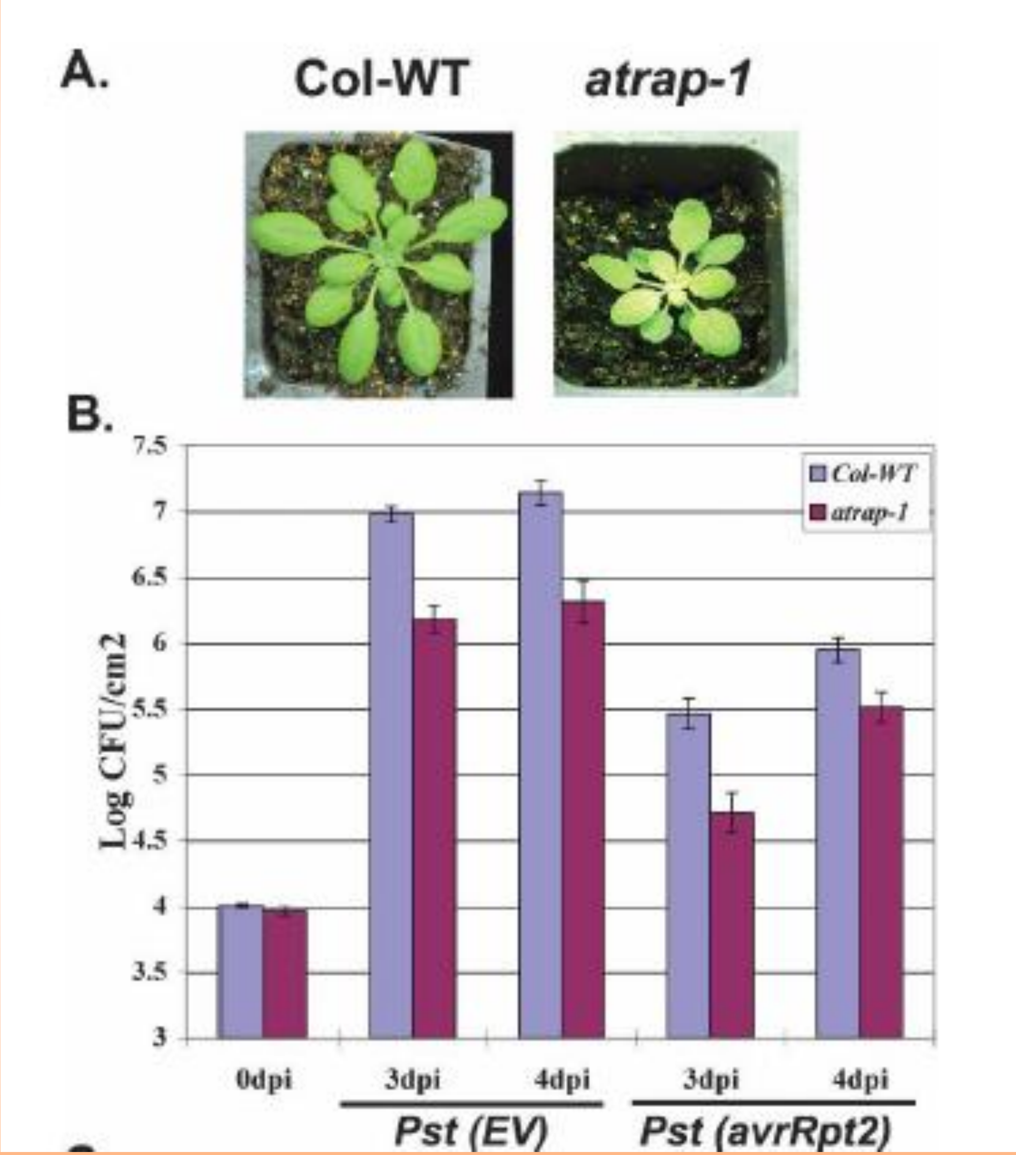






Katiyar-Agarwal et al., Genes & Dev. 2007

AtRAP protein acts as a negative regulator of plant immunity



Katiyar-Agarwal et al., 2007

Small RNAs Play An Important Role In Plant Immunity