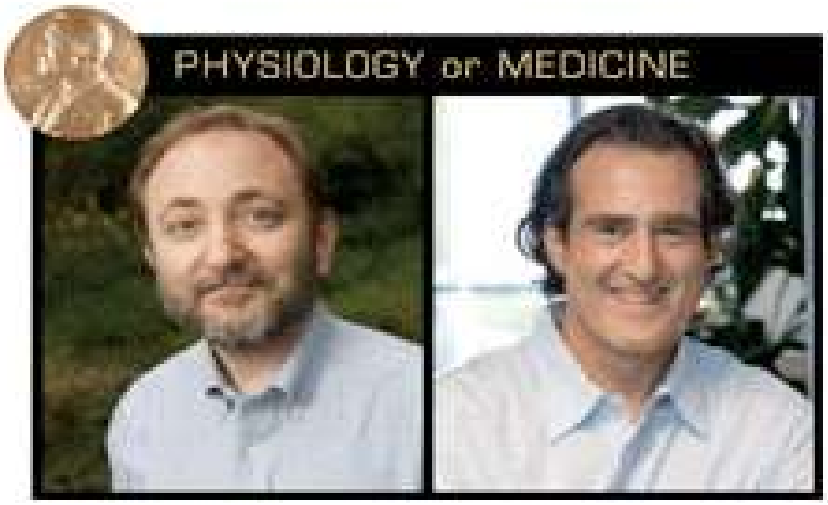
Post-transcriptional gene silencing: A viral defense response identified in plants



2006 Nobel Prize
Andrew Fire and Craig Mello

For discovery of RNAi's gene-quelling power

Mechanisms of Resistance

1. Immunity
2. Genetic Host Resistance
3. Systemic Acquired Resistance
4. Post-transcriptional Gene Silencing
5. Transgenic Resistance

Objectives:

- Be able to define and describe post transcriptional gene silencing (PTGS)
- Describe a basic model that explains PTGS
- Understand the concept that PTGS is a natural defense response of plants to infection of viruses
- Understand the role of PTGS in the production of virus-induced disease symptoms; cross protection; virus synergism; recovery

Gene Silencing

Definition:

Gene silencing is a general term that refers to the normal process of "switching off" a gene, which would be otherwise active.

Gene silencing describes epigenetic processes of gene regulation (epigenetic - means that the process is not controlled by chromosomal DNA sequences)

Gene Silencing

Two Types of Gene Silencing:

- **Transcriptional Gene Silencing (TGS)**

  sequence- specific methylation of genomic DNA in the nucleus

  ** DNA based, occurs in the nucleus

  ** Blocks transcription

- **Post–Transcriptional Gene Silencing (PTGS)**

  sequence-specific messenger RNA degradation mechanism in the cytoplasm of many life-forms

  ** RNA based, occurs in the cytosol

  ** Involves degradation of mRNA

  ** Represses translation

Post-transcriptional gene silencing (PTGS)

Functions of PTGS:

1. One part of a sophisticated network of inter-connected pathways for RNA surveillance and down regulation of genes involved in plant metabolism

2. Part of cellular defense system designed to limit foreign nucleic acids which could harm the cell's genetic integrity (foreign nucleic acids such as viroids, viruses, transposons, etc….)

   PTGS appears to be a type of immune system that operates at the nucleic acid level but the specificity is not genetically programmed (as it is in a true immune system**).**

Post-transcriptional gene silencing (PTGS)

Characteristics of PTGS:

Occurs in a wide range of eukaryotes (Bingham, 1997)

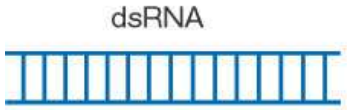
- In plants – its known as PTGS (Baulcombe, 2000)
- In animals – known as RNA interference (RNAi) (Montgomery et al., 1998)
- In fungi – known as quelling (Cogoni and Macino, 1999)

PTGS, RNAi and quelling are related pathways that use a conserved set of proteins.

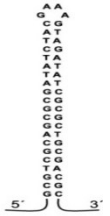
The Mechanism of PTGS

What turns on PTGS?

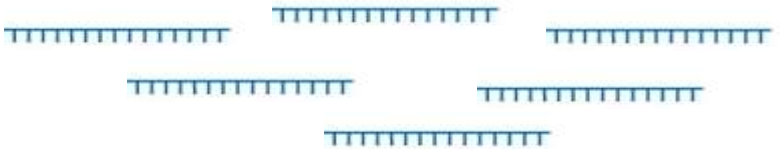
- **dsRNA (from mRNA, dsRNA viral genomes, replicative intermediates, viroids)**



- **mRNA from hairpin sequences of DNA**



- **Excess amounts of the same sequences of RNA**



https://www.youtube.com/watch?v=cK-OGB1_ELE

Best graphics
natureVideo

http://www.youtube.com/watch?v=UdwygnzIdVE&feature=related

Annoying music, But simpler with good details

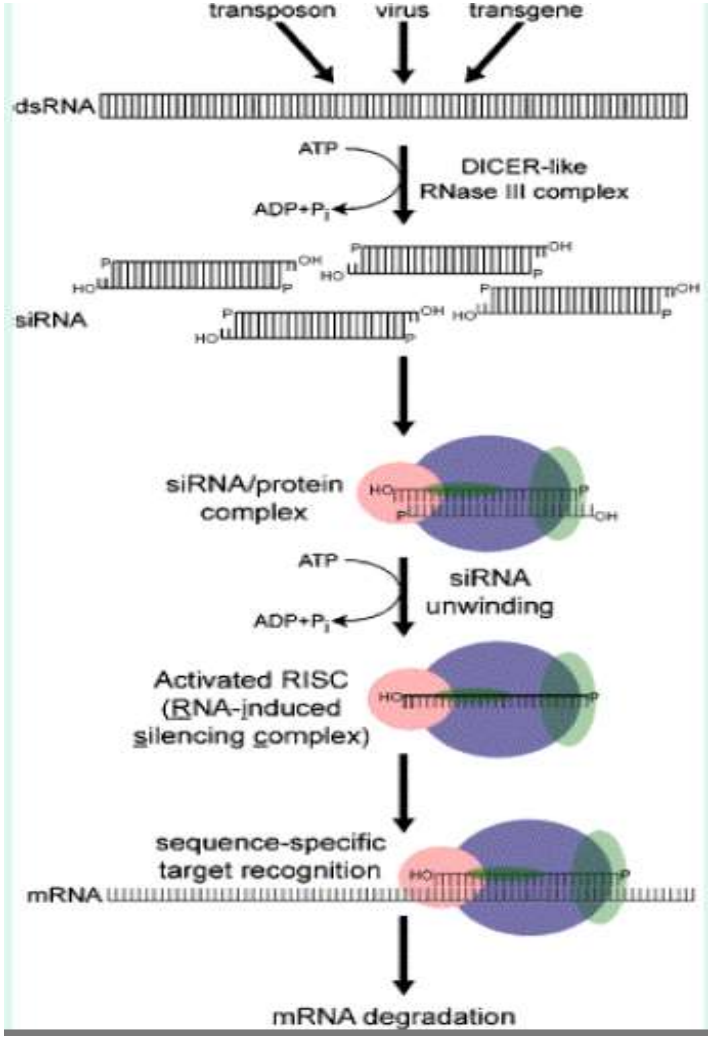
Many other good video are available on the internet

The Basic Mechanism of PTGS:

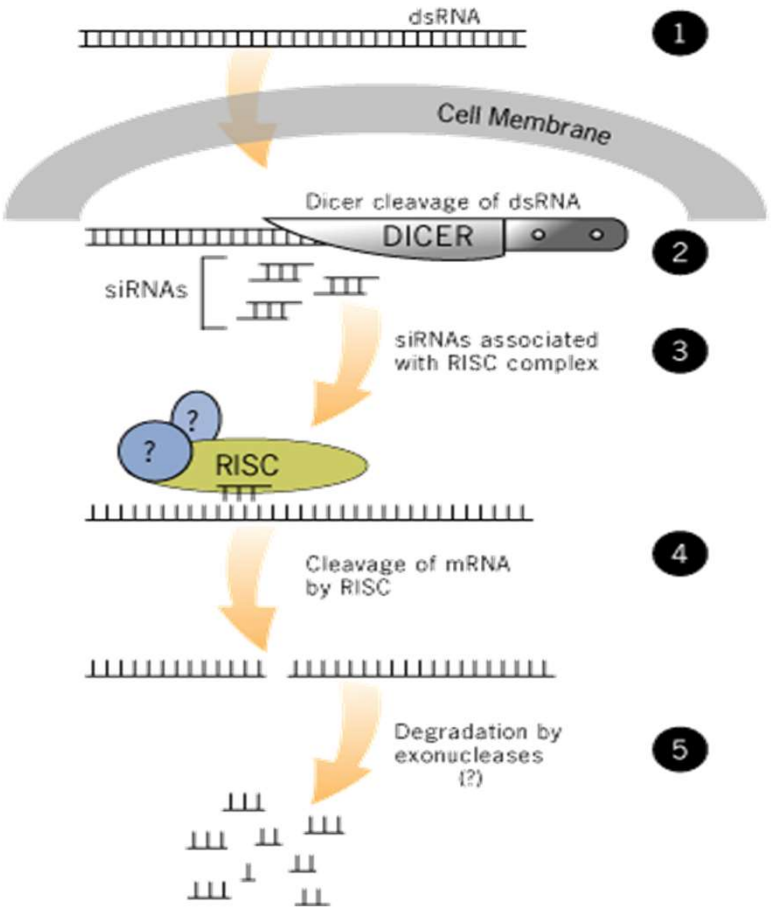
dsRNAs are processed into small interfering RNA (siRNAs, 21-23 nts) by Dicer (enzyme, RNase III-like).

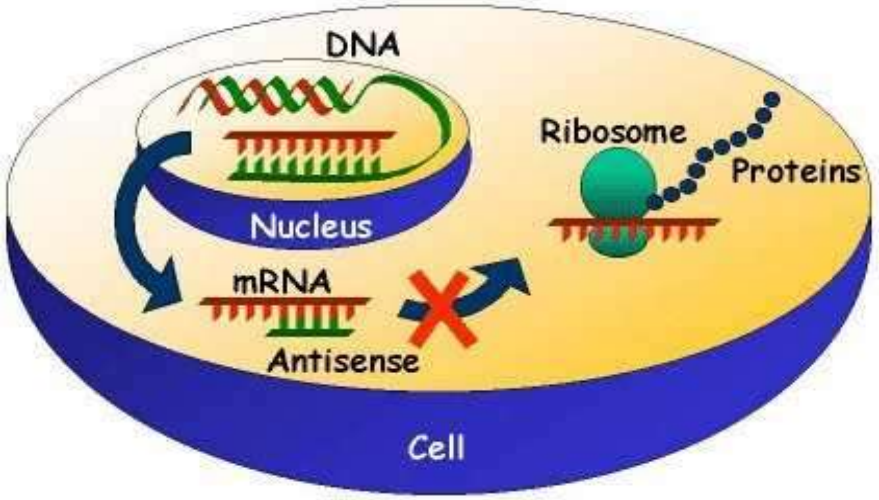
The siRNAs are incorporated into an RNA-induced silencing complex (RISC) to guide degradation of complementary mRNAs

RISC moves systemically throughout the plant and is associated with the accumulation of 21-23 nt RNAs



Gene Specific Silencing by RNAi





PTGS occurs in the cytoplasm

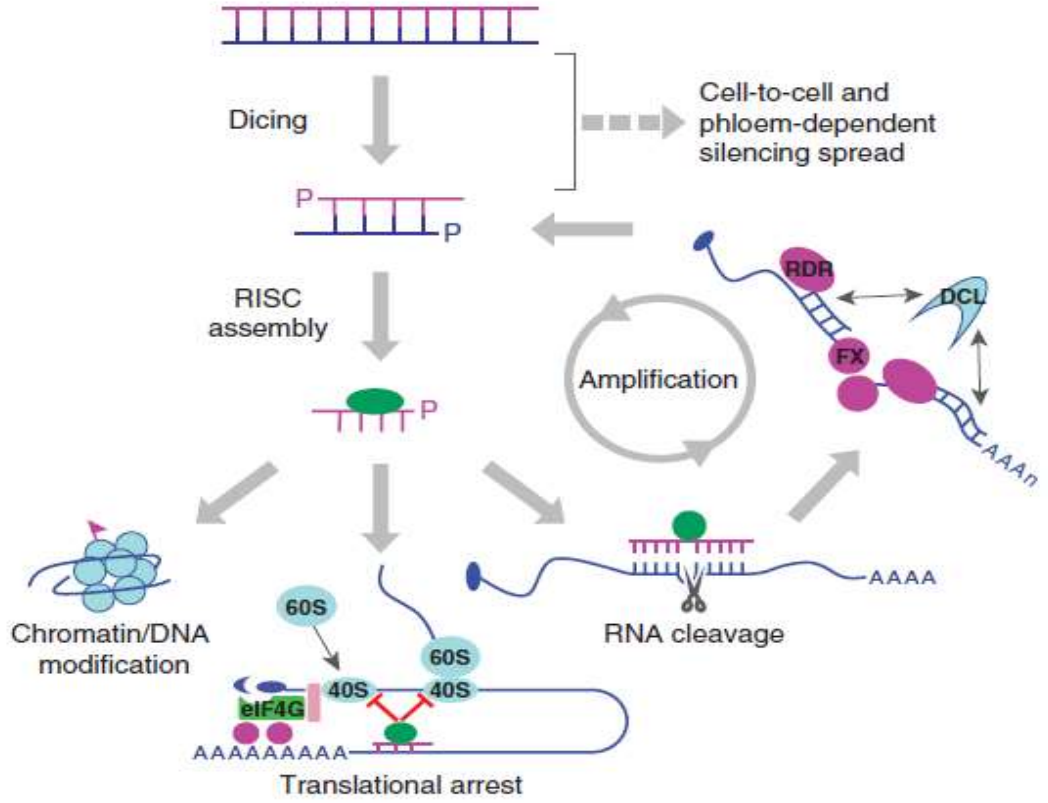


Figure 1

Key steps in RNA silencing. RNA silencing starts with dicing of dsRNA or pre-miRNAs. The resulting small RNAs are incorporated into distinct RISC complexes to direct chromatin modification, translational arrest, and mRNA cleavage. In organisms with RDR, secondary siRNA is synthesized from cleavage products. FX represents host factor implicated in stabilizing cleavage products similar to SGS3 (149). Dashed arrow represents cell-to-cell movement of 21 siRNAs and the potential long-distance movement of 24-nt siRNAs.

PTGS - regulates host genes:

Small RNAs are incorporated into distinct RISC complexes to direct
1.chromatin modification
2. translational arrest and
3. mRNA cleavage.

Li & Ding 2006
Ann. Rev. Microbiol. 60:503-531

There are at least 3 different classes of small RNAs involved in PTGS:

21 -23 nt siRNA

Dicer cleaves dsRNA to produce approximately 21nt-long small interfering RNAs (siRNAs), which guide RISC, to destroy specific target mRNAs based on sequence complementarity with the siRNA.

25 nt siRNA

Another class of siRNAs of 25nt-long is also produced from dsRNA by Dicer. These longer siRNAs are involved in systemic silencing during PTGS and guide methylation of both DNA and histones, and induce heterochromatinization and consequently transcriptional repression of the targeted gene.

21 nt micro RNAs (miRNAs)

Also produced by Dicer, these regulate the expression of endogenous (host) genes

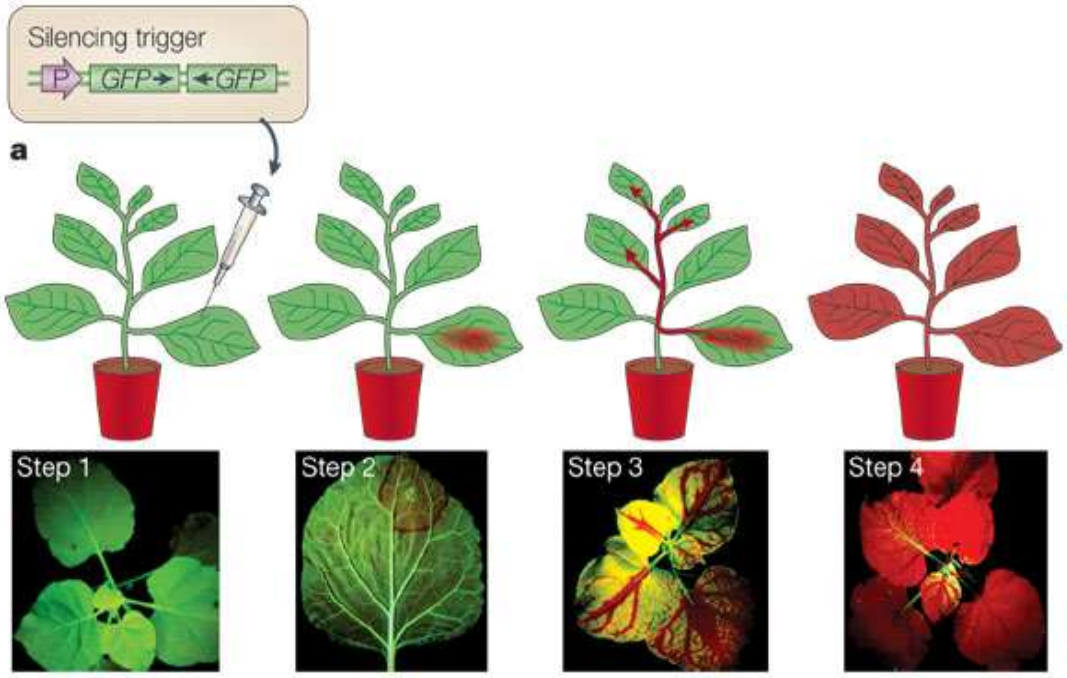
Pattanayak D, et al. 2005. **Small but mighty RNA-mediated interference in plants**. Indian J. of Exper. Biol. 43, 7 - 24

Demonstration of Systemic Nature of PTGS:

Tobacco plants are transformed with *GFP* and express GFP (green fluorescent protein):

A silencing trigger is created: an expression plasmid that contains a hairpin construct of 2 *GFP* genes: this results in the production a dsRNA of *GFP*

After injection of the expression plasmid: GFP is silenced at the site of introduction of the plasmid and later throughout the plant



Voinnet O. 2005. Induction and suppression of RNA silencing: insights from viral infections. Nat Rev Genet. Mar 6(3):206-20. Review.

First recognition of the association of "PTGS" with virus resistance in plants was the mid 1980's

PTGS and Plant Viruses:

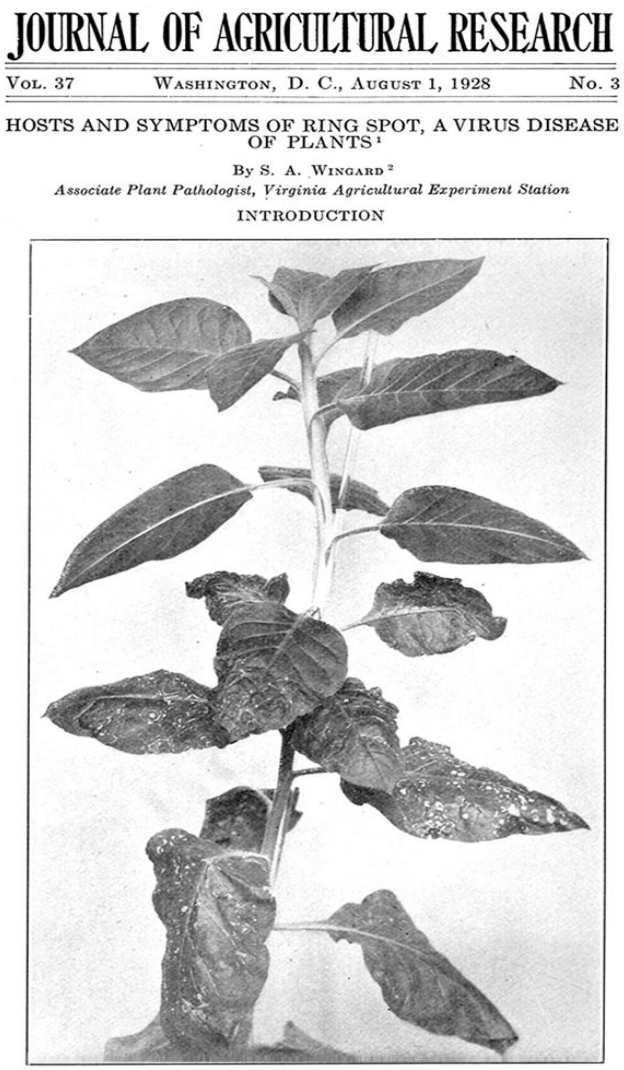
- PTGS is thought to be an important component of a general defense system against plant viruses and other mobile genetic agents, known as non-host resistance (and innate immunity).

- “Most plants are resistant against most viruses”.

- Host resistance based on PTGS is characterized by:

  No symptoms

  Or “Recovery” from initial symptoms



JOURNAL OF AGRICULTURAL RESEARCH

VOL. 37 WASHINGTON, D. C., AUGUST 1, 1928 NO. 3

HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS[1]

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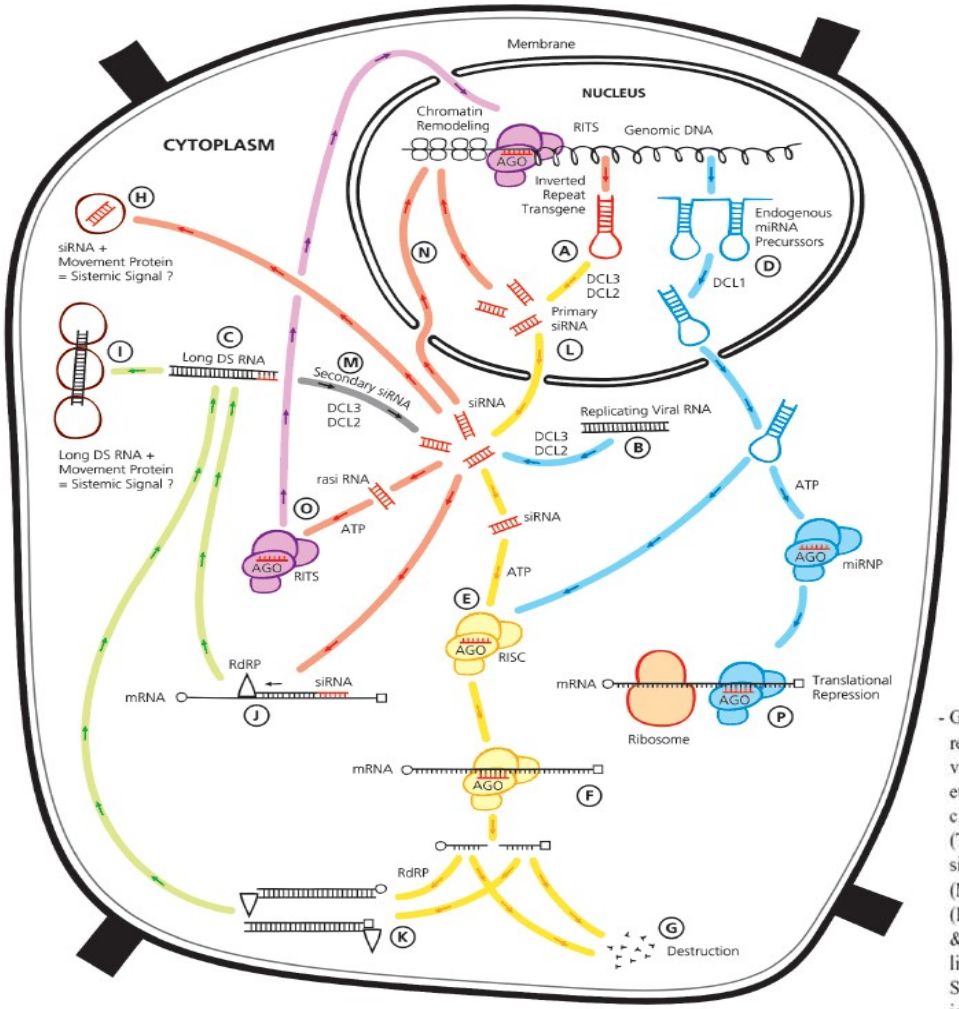
INTRODUCTION

Recovery from virus infections:

Known for a long time, however the mechanism that explains the recovery was not know until the 1990's

Recovery from virus infections:

- Many plant viral hosts show a “recovery” from virus-induced symptoms 1-3 weeks after onset of symptoms.
- The new symptomless growth may be free of virus and resistant to new virus inoculations.
- This acquired resistance is due to the activation of host defense against viruses and can be due to PTGS.



PTGS is a complicated process Involving many host proteins and pathways

Some of the pathways occur in the nucleus

Some of the pathways occur in the cytoplasm

- Gene Silencing pathways in plant cells. (A) Dicer-like protein (DCL2, DCL3) processing of transcripts containing inverted repeats (Meister & Tuschl, 2004); (B) Dicer-like protein (DCL2, DCL3) processing of intermediates formed during RNA virus replication (Hannon, 2002); (C) Long dsRNA; (D) Dicer-like protein (DCL1) processing of miRNAs precursors (Xie et al., 2004); (E) RNA-induced silencing complex (RISC) (Hammond et al., 2000; Nykänen et al., 2001); (F) Targeting and cleavage of sequence specific mRNA by RISC (Martinez & Tuschl, 2004); (G) mRNA destruction after RISC processing (Tolia & Joshua-Tor, 2006); (H) Possible systemic signal composed by siRNA + movement proteins; (I) Possible systemic signal composed by long dsRNA + movement proteins (Waterhouse et al., 2001); (J) Primer dependent RdRP amplification (Matzke et al., 2001; Ceruti, 2003; Baulcombe, 2004); (K) Primer-independent (aberrant RNA features) RdRP amplification (Baulcombe, 2004); (L) Primary siRNA (Pak & Fire, 2007); (M) Secondary siRNA processing by Dicer-like enzymes (Pak & Fire, 2007); (N) RNA-directed DNA methylation (RdDM) signal transmitted from the cytoplasm to the nucleus is most likely siRNA (Xie et al., 2004); (O) RNAi effector complex termed RITS (RNA-induced Initiation of Transcriptional gene Silencing) required for heterochromatin assembly in fission yeast (*Schizosaccharomyces pombe*) (Verdel et al., 2004). RITS is composed by repeat-associated short interfering RNA (rasiRNA) (Meister & Tuschl, 2004); (P) Translational repression of mRNA by miRNP (Meister & Tuschl, 2004).

So PTGS is one reason that most plants are resistant to most viruses.

- If PTGS is a normal defense response in plants to foreign nucleic acids how does a virus ever cause disease?
- A virus that is able to infect a plant must have some way to overcome PTGS in that plant
- Viruses have gene(s) that suppress PTGS – viral silencing suppressors.
- Plant virus infection is one of balance and counter-balance between the virus replication (viral PTGS suppressors) and host response (PTGS) to viral genomic nucleic acids.

PTGS in plants can be suppressed by virus-encoded proteins.

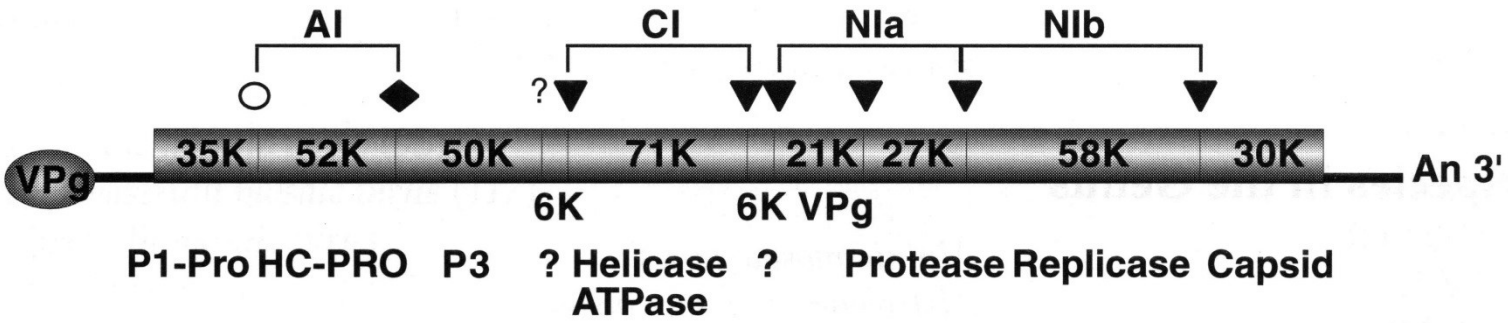


FIGURE 2
Generic genomic map of a member of the genus *Potyvirus*. The RNA genome is represented

Potyvirus HC-PRO =

helper factor (vector), protease, suppressor of host defense

Partial list of virus-encoded PTGS suppressor proteins

Table 1. Virus-encoded suppressor proteins

Virus genus	Virus	Suppressor	RNA binding	Other viral function
Positive-strand RNA viruses				
Carmovirus	Turnip crinkle virus	P38	Yes	Coat protein
Cucumovirus	Cucumber mosaic virus	2b	Yes	Movement
	Tomato aspermy virus	2b	ND	
Closterovirus	Beet yellows virus	P21	ND	Replicational enhancer
Hordeivirus	Barley yellow mosaic virus	γb	ND	Movement, virulence
Pecluvirus	Peanut clump virus	P15	ND	Movement
Poleovirus	Beet Western yellows virus	P0	ND	Pathogenesis related
Potexvirus	Potato virus X	P25	Yes	Movement
Potyvirus	Potato virus Y	HC-Pro	Yes	Long-distance movement
	Tobacco etch virus	HC-Pro	ND	Polyprotein processing
	Turnip mosaic virus	HC-Pro	ND	Aphid transmission
Sobemovirus	Rice yellow mosaic virus	P1	Yes	Movement
	Cocksfoot mottle virus	P1	ND	Movement
Tombusvirus	Artichoke mottled crinkle virus	P19	Yes	Pathogenesis determinant
	Carnation Italian ringspot virus	P19	Yes	Movement
	Cymbidium ringspot virus	P19	Yes	
	Tomato bushy stunt virus	P19	Yes	
Tobamovirus	Tomato mosaic virus	P130	ND	Replication protein
Negative-strand RNA viruses				
Tospovirus	Tomato spotted wilt virus	NSs	ND	Virulence
Tenuivirus	Rice hoja blanca virus	NS3	ND	None known
DNA viruses				
Begomovirus	African cassava mosaic virus	AC2	ND	Transcriptional activator
	Tomato yellow leaf curl virus	C2	ND	Transcriptional activator

Abbreviation: ND, no data available.

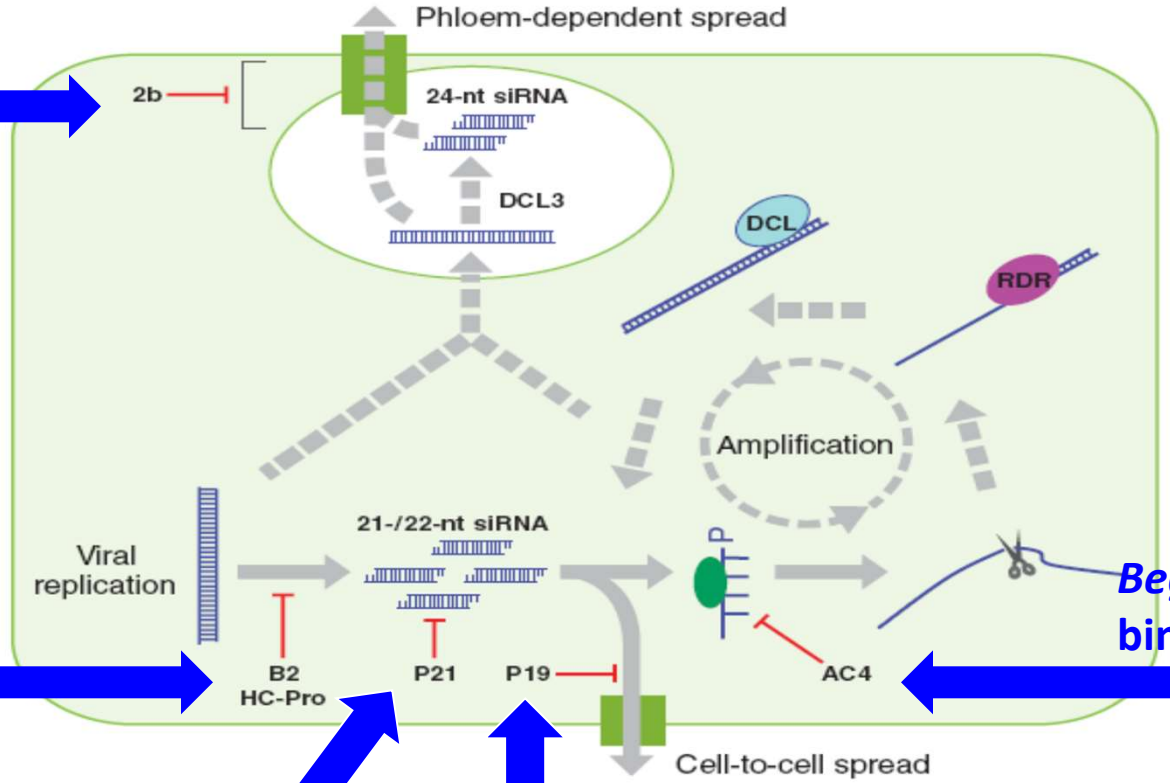
Silhavy and Burgyan 2004 Trends in Plant Science 9: 76-83

Viral Silencing Suppressors:

- Different genes in different virus genera
- Mechanism of how and where each gene suppresses silencing varies among viruses in different genera or families
- Effectiveness (or “strength”) of the suppressors varies

Viral Silencing Suppressors

CMV 2b blocks systemic spread



Begomovirus binds ss si RNAs

Potyvirus HC-Pro blocks RISC activity

BYV (*Closterovirus*) Bind ds siRNAs

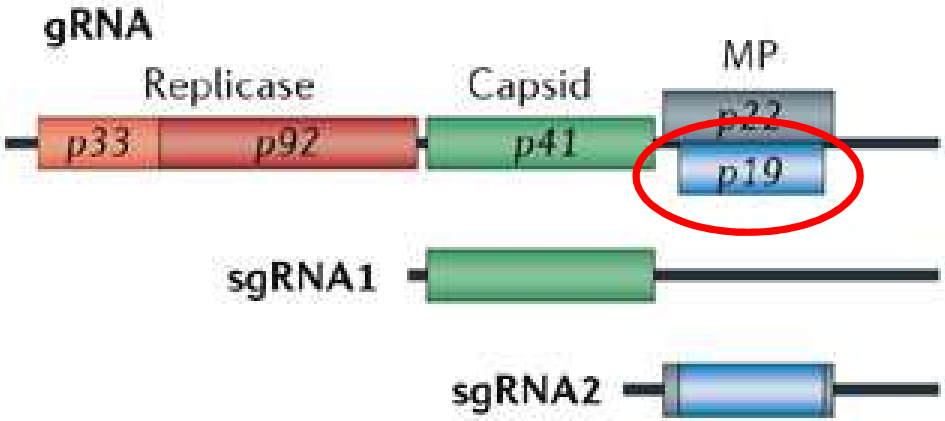
TBSV (*Tombusvirus*) Bind ds siRNAs

Li & Ding 2006 Ann. Rev. Microbiol. 60:503-531

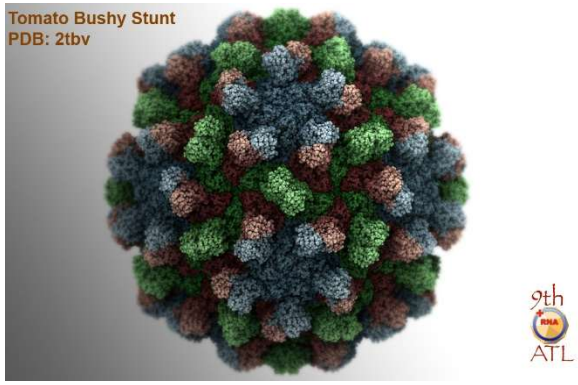
Tombusvirus P19

+ ssRNA genome

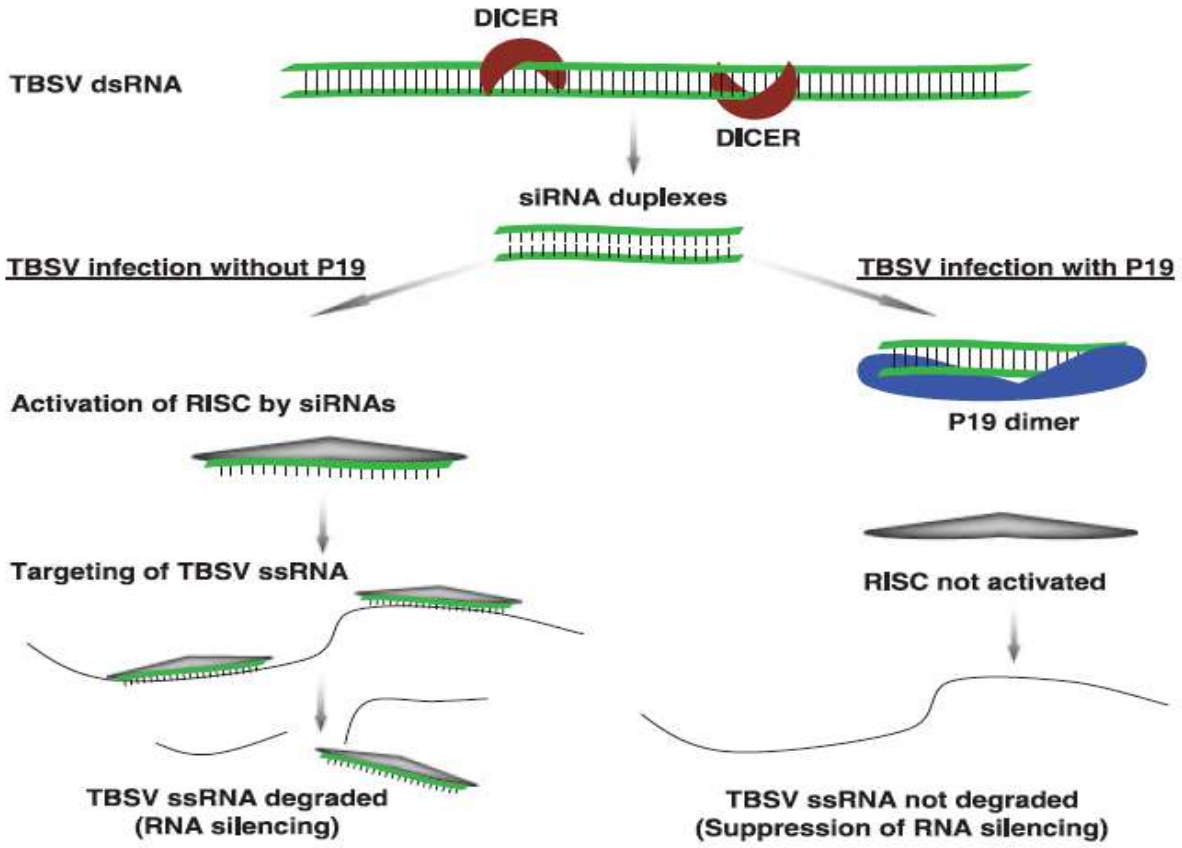
Genome organization:



Tomato bushy stunt virus



Mechanisms of PTGS suppression



P19 sequesters 21-22 nt siRNAs

Prevents the formation of RISC

Fig. 4 A model of how P19 mediates the RNA silencing pathway. Left, in the absence of P19, DICER cleaves TBSV double-stranded RNA (dsRNA) followed by loading of the RNA-induced silencing complex (RISC) with single-stranded (ss) short interfering RNAs (siRNAs) and subsequent targeting of TBSV ssRNA for degradation. Right, the P19 homodimers bind the ds-siRNA DICER cleavage products, to prevent loading of RISC. Consequently, the RISC cannot be activated by the siRNAs, and the TBSV RNA is not targeted for degradation.

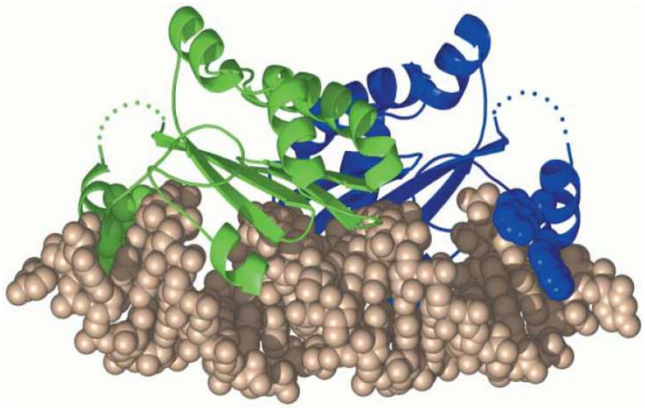
MOLECULAR PLANT PATHOLOGY (2005) **6**(5), 491–502

Mechanisms of PTGS suppression

A representation of the X-ray crystallographic structure of the P19–siRNA complex

The green and blue ribbon structures represent the individual P19 monomers arranged in a tail-to-tail dimer, and the helical complex is the siRNA duplex.

P19 suppresses PTGS in both plant and animal systems



Vargason et al., 2003; Ye et al., 2003; Yamamura and H. B. Scholthof.
Molecular Plant Pathology (2005) **6** (5) , 491–502

PTGS explains several biological phenomenon of plant viruses:

A. Symptoms

B. Cross protection

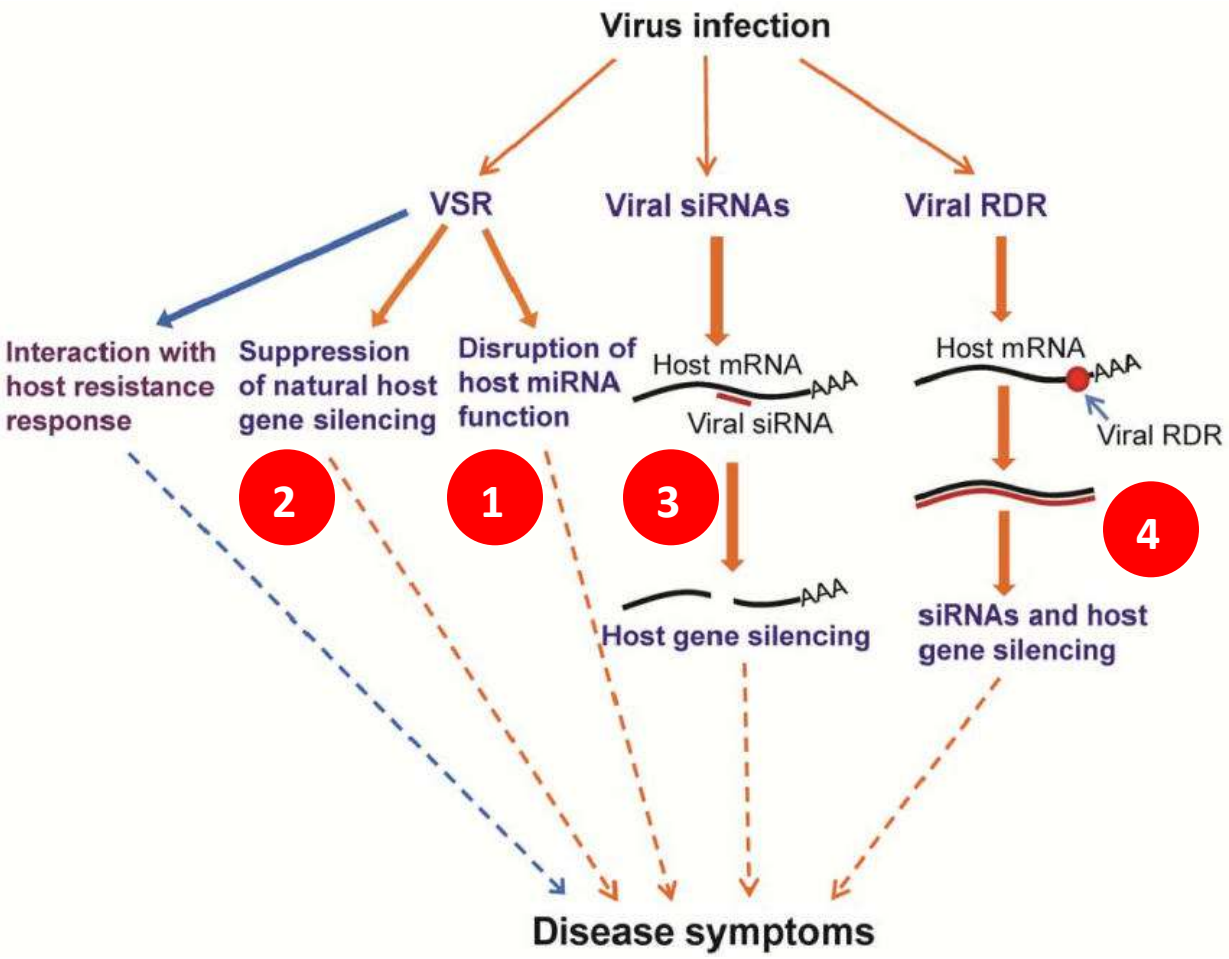
C. Recovery from infection

D. Synergism

A. **Systemic infection by plant viruses often results in disease symptoms that resemble:**

- Developmental defects
- Loss of leaf polarity
- Loss of proper control of cell division
- And loss of reproductive functions

These phenotypes have been genetically mapped as viral-encoded pathogenicity factors – these are the same genes that were later identified as suppressors of PTGS



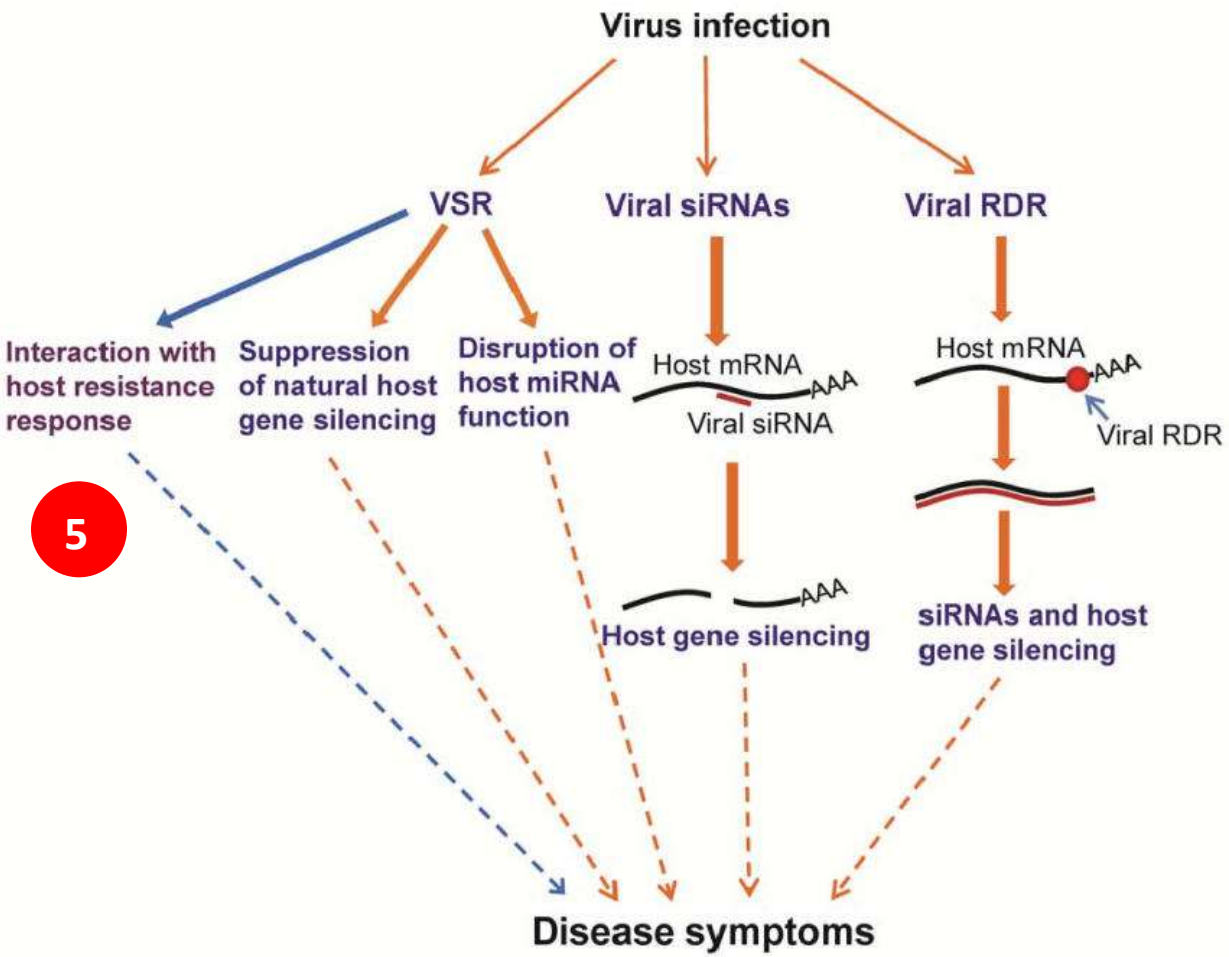
Viral suppressors of RNA silencing (VSR) interfere with:

1) Host micro (mi)RNA function, causing developmental defects or disease symptoms

2) VSR can repress naturally occurring silencing of host genes, resulting in phenotypic changes

3) Viral small interfering (si)RNAs can direct silencing of host genes due to fortuitous sequence homology between the viral genome and host genes, causing disease symptoms.

4) Host gene silencing could also be induced by viral RdRP-mediated ds RNA synthesis of host mRNA due to existence of sequence motifs in the mRNA that resemble viral origin of replication.

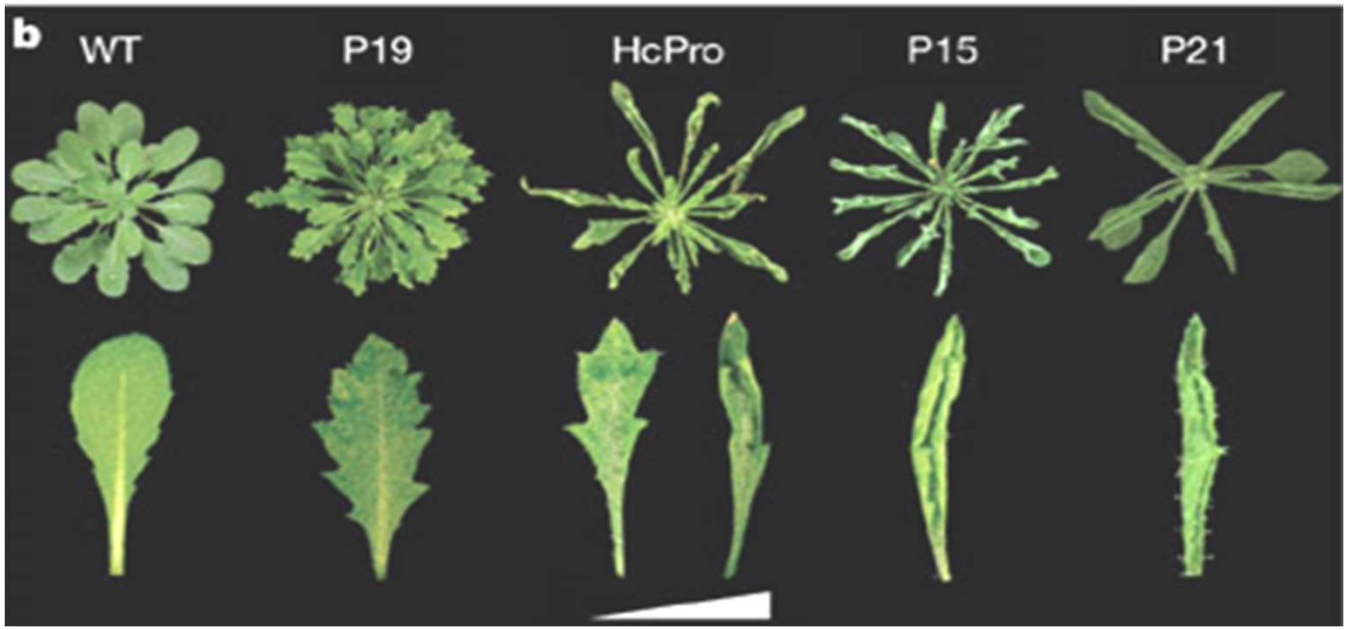


Viral suppressors of RNA silencing (VSR) interfere with:

5) VSR can function as avirulence proteins to trigger resistance gene–mediated defense response (blue arrow), which could also result in viral-like symptoms.

Viral symptoms and silencing suppression

Developmental symptoms in leaves of *Arabidopsis thaliana* that constitutively express silencing suppressors.



WT – empty vector

P19 - TBSV

P15 - Peculuvirus

P21 - Beet yellows virus (*Closterovirus*)

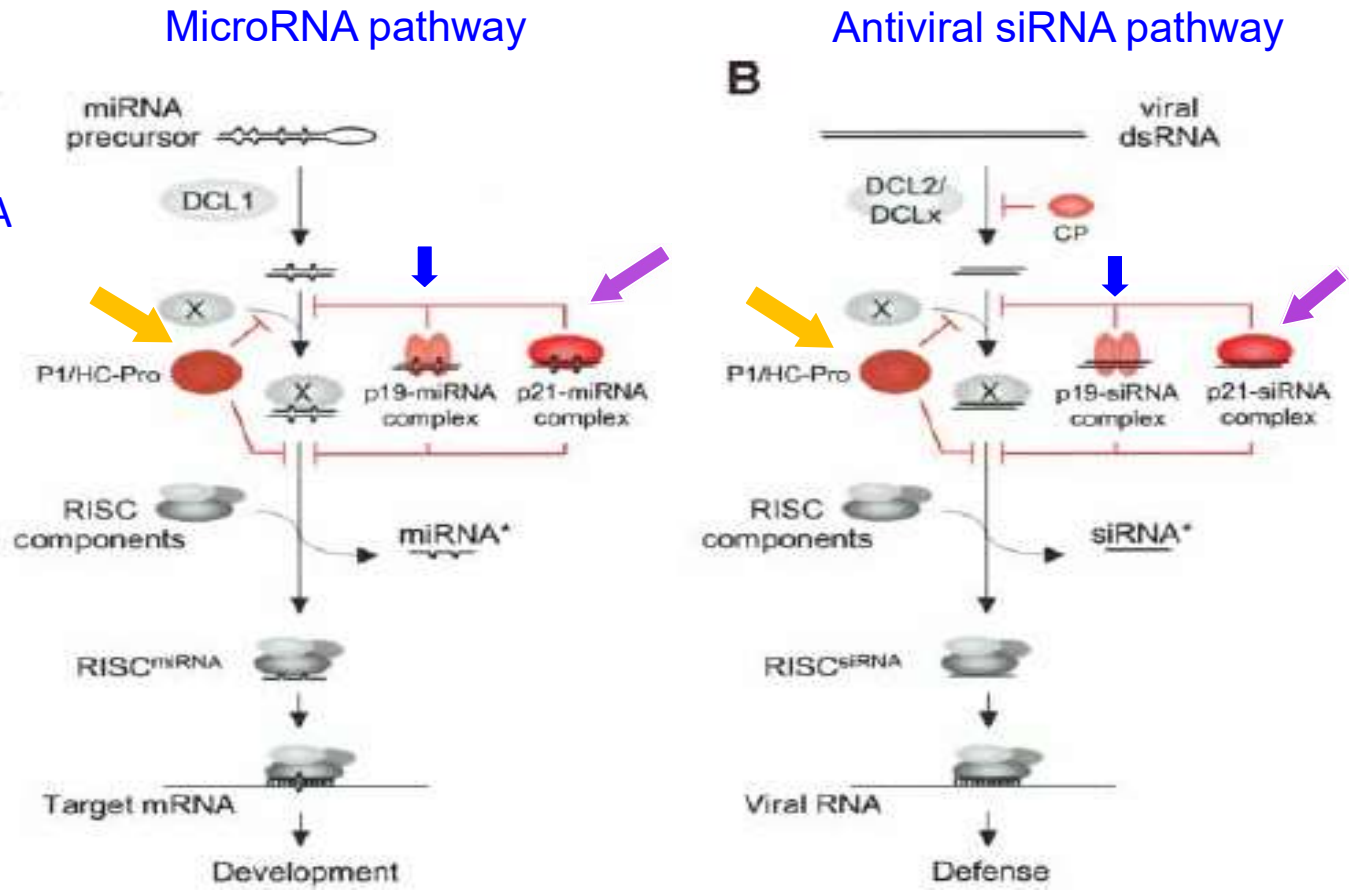
HC-Pro - Potyvirus

Voinnet O. 2005. Induction and suppression of RNA silencing: insights from viral infections. Nat Rev Genet. Mar;6(3):206-20. Review.

Suppression of miRNA and siRNA pathways by viral silencing suppressors

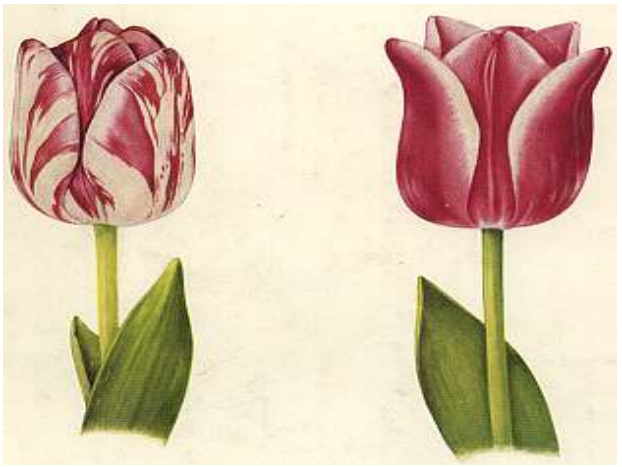
HC-Pro, **p19**, and **p21** prevents RISC assembly and subsequent target RNA degradation in both siRNA and miRNA pathways.

The CMV **2b** protein functions to prevent the systemic spread of RNA silencing signals in both siRNA and miRNA pathways.



Some symptoms are due to PTGS

Color breaking in flowers in infected plants
(Tobacco mild green mosaic virus in tobacco)



Tulip on the left infected with *Tulip breaking virus* (*Potyvirus*)

Foliar mottles and deformation can be due to PTGS

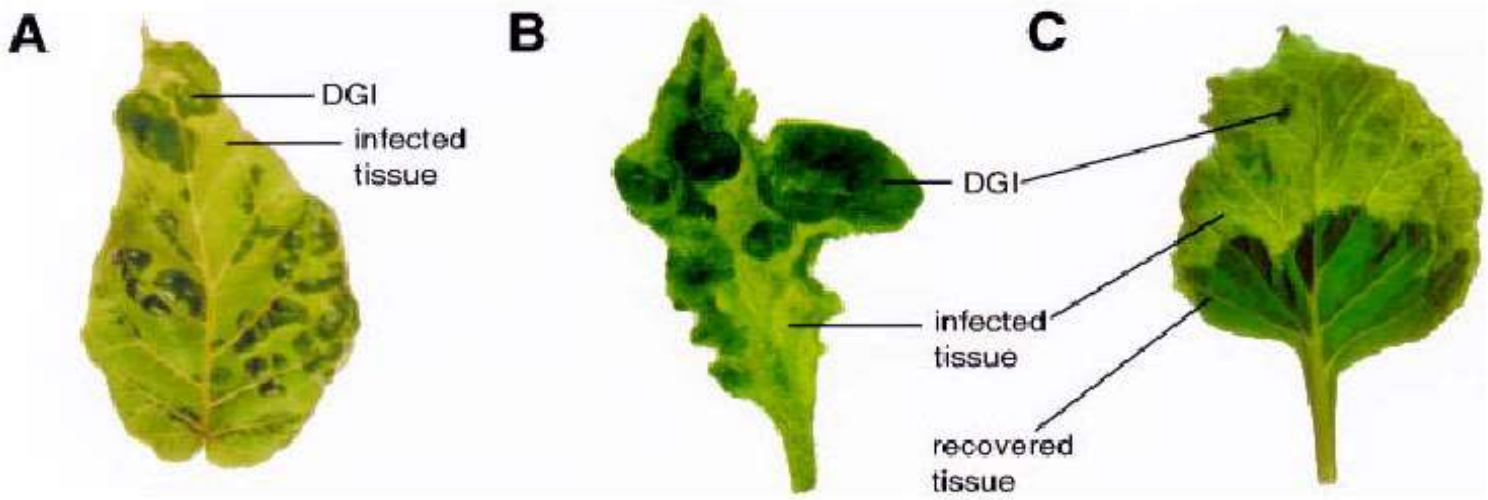


Fig. 1. Characteristic symptoms of *Tamarillo mosaic potyvirus* (TaMV) infection. **A,** Tamarillo leaf displaying the prominent dark green islands (DGIs), which led to the isolation of the virus. Leaf length is 25 cm. **B,** Wild-type *Nicotiana benthamiana* leaf infected with TaMV. Leaf length is 5 cm. **C,** PS26 transgenic *N. benthamiana* leaf showing DGIs and the beginning of the recovery phenotype in one leaf (bizonal). Leaf length is 7 cm.

MPMI Vol. 14, No. 8, 2001, pp. 939–946. Publication no. M-2001-0427-01R.

Dark Green Islands in Plant Virus Infection are the Result of Posttranscriptional Gene Silencing

Carolyn J. Moore,[1,2] Paul W. Sutherland,[1] Richard L. S. Forster,[1] Richard C. Gardner,[2] and Robin M. MacDiarmid[1]

Viral symptoms and silencing suppression

Mottling in soybean seeds: results from the infection of the mother plant by potyviruses and cucumoviruses.

Production of silencing suppressors by these viruses reverses silencing of chalcone synthase (an enzyme that is involved in pigment synthesis, which is naturally silenced in most soybean varieties) resulting in a pale seed color.

[So in virus infected cells – the viral silencing suppressors prevent the silencing of chalcone synthase and the pigment is produced]

Voinnet O. 2005. Induction and suppression of RNA silencing: insights from viral infections. Nat Rev Genet. Mar;6(3):206-20. Review.

PTGS also implicated in induction of some of the symptoms caused by viroids

Fig. 1. Healthy avocado leaf (A) as compared with sunblotch infected leaves displaying bleached (B), variegated (C) and symptomless carrier (D) responses. (Arrowhead indicates typical bleached area)

Markarian et al 2004 Arch Virol 149, 397-406

B. CROSS-PROTECTION

Cross protection: prior infection with a mild virus strain may modify or prevent symptoms in a subsequent infection by a severe strain of the mild virus.

Prior mild virus infection activates a host gene silencing system that targets virus genomic RNA - thus interferes with the replication of subsequent infection by a more severe but closely related virus.

C. SYNERGISM

- Synergism is the accentuation of symptoms of one virus caused by co-infection of a second, unrelated virus. (often symptom severity is directly related to virus accumulation)

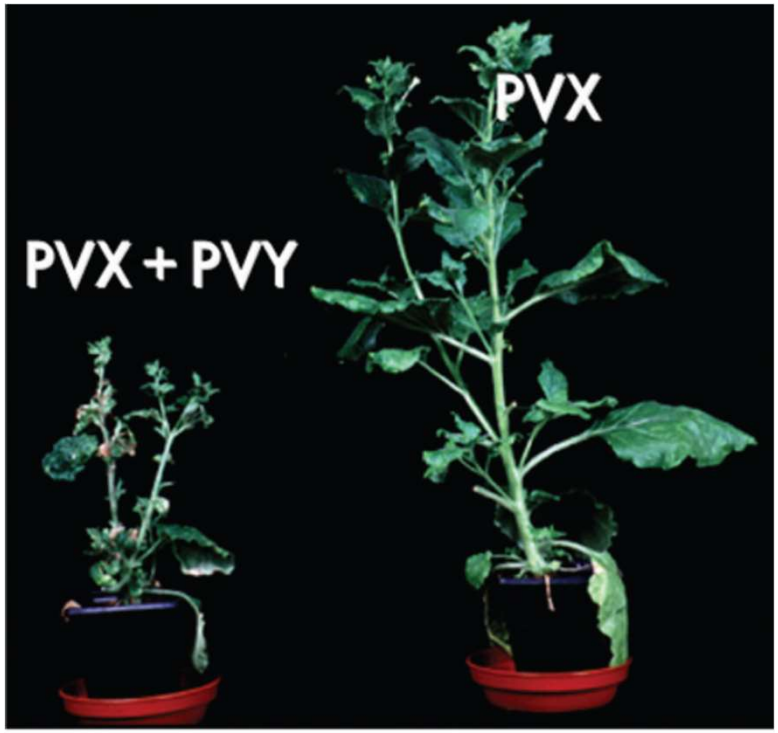
- Some (not all) examples of synergism may be due to PTGS:

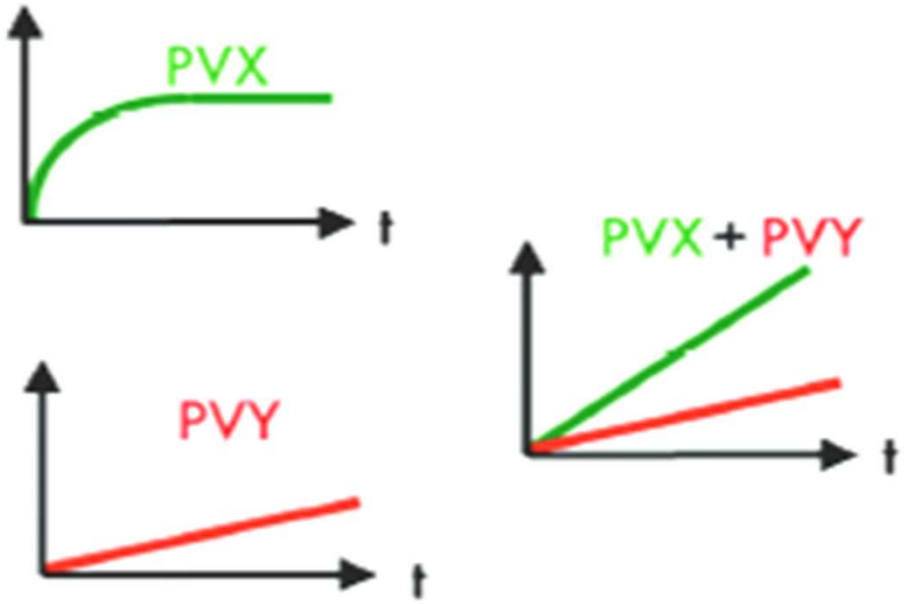
  The molecular basis of synergism of an “enhancer” virus is associated with a more effective suppression of anti-viral host gene silencing by that virus.

Moissiard and Voinnet, 2004. Molecular Plant Pathology 5, 71-82

Synergy and virus-encoded silencing suppressors

Ex. PVY enhances the accumulation of PVX

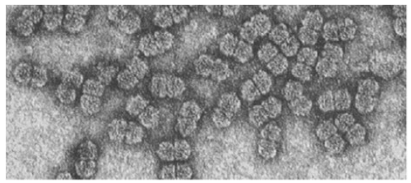


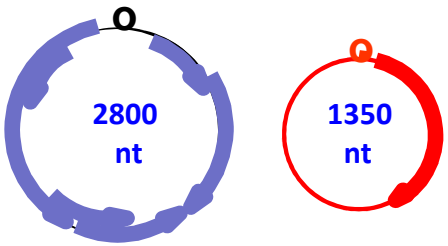


Lecellier C. H. and Voinnet O. 2004. **RNA silencing: no mercy for viruses?** *Immunological Reviews Vol. 198: 285–303*

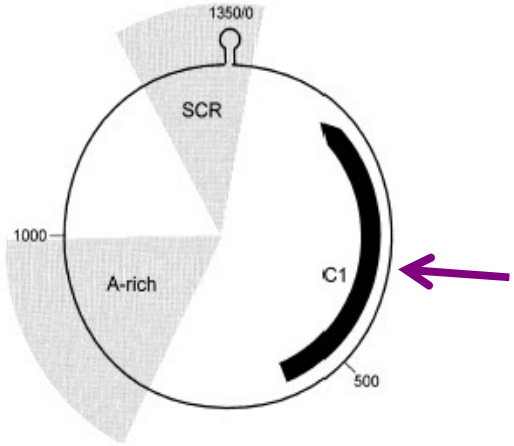
Beta Satellites

- ssDNA satellites of monopartite begomoviruses
- Encode one replication initiator protein (C1)
- Implicated in the suppression host defense (gene silencing)
- β satellites affect host range and symptom expression of helper virus





genome map of DNA β



C1 – silencing suppressor

TYLCCNV **TYLCCNV + β**

Applications of PTGS:

- Cross protection for virus management

- PTGS is used as a reverse genetics tool to knock out specific genes.

- PTGS may be used to silence specific plant genes and thereby create new horticultural traits.

- PTGS is often the mechanism that explains the creation of virus resistance by genetic engineering: transformation of plants with viral genes (transgenes) (also known as Pathogen-derived resistance)