Virus Induced RNA Silencing and Suppression:
Insights from Viral Infection

Speaker: Amrita Banerjee, Ph.D.

Post-transcriptional gene silencing (PTGS) or RNA interference (RNAi)

- **It is the manifestation of an evolutionary conserved process known as "RNA silencing".**

- **Over the last few years RNA silencing has become intensively studied biological system.**

- **Initially being discovered as a side effect of transgene expression in plants and a process by which transgenic virus resistance could be obtained, it has since been implicated in natural virus resistance and basic biological processes.**

- **In the plant cell RNA silencing, that act as antiviral defense during infection of virus and sub-viral pathogens, termed as virus induced gene silencing (VIGS).**

Development of Transgenic plant

Pathogen Derived Gene

Non Pathogen Derived Strategies

RNA interference (RNAi)
or
Post-Transcriptional Gene Silencing
(PTGS)

Manifestation of RNA Silencing

RNA Silencing regulate → **A wide range of biological processes**

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Virus resistance

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Antiviral RNA silencing

? **How viruses and related parasitic genetic elements induce RNA silencing**

? **How they suppress or evade this process**

? **What are the consequences of this for the host**

RNA Silencing

RNA silencing refers to
related homology dependent gene silencing mechanisms
in plants and animals
guided by small RNAs
such as microRNAs (miRNAs) and small interfering RNAs (siRNAs).

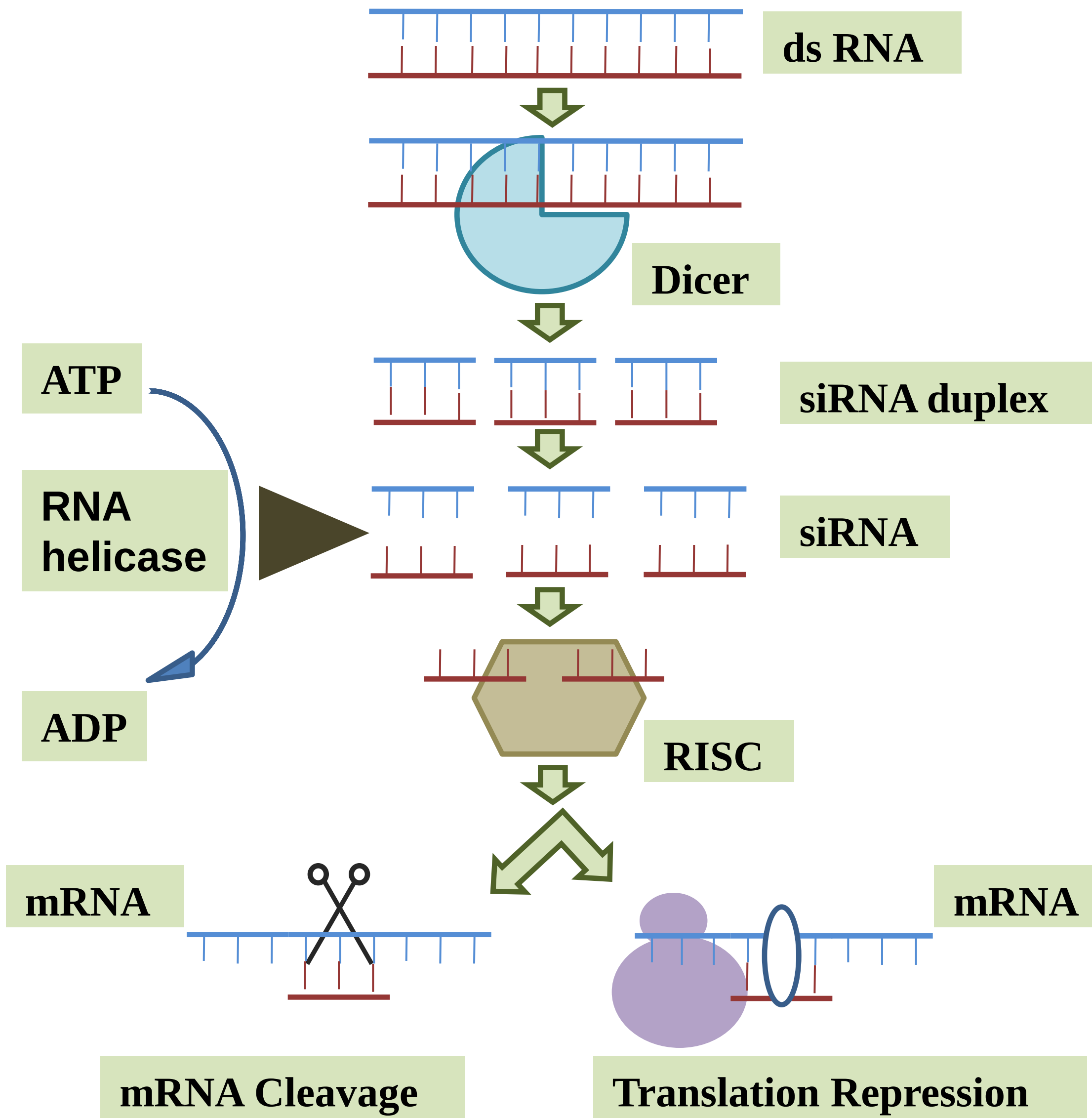
Basic Principle of RNA silencing

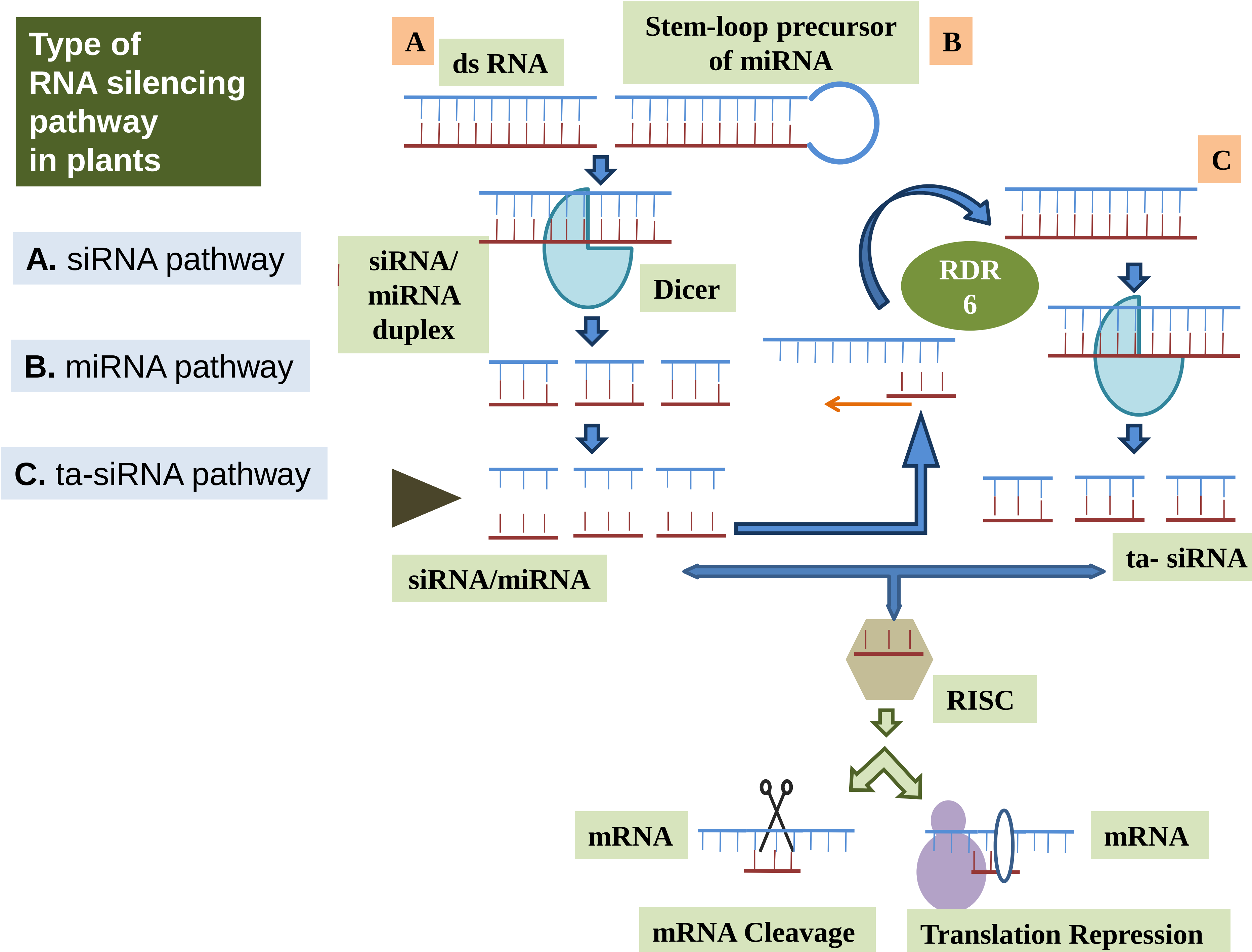
RNA silencing activated by

double stranded RNA (dsRNA)

21-24bp long RNA duplexes
– the small interfering (si)RNA –

by the RNase III enzyme “Dicer”




Type of RNA silencing pathway in plants
A. siRNA pathway
B. miRNA pathway
C. ta-siRNA pathway
A
ds RNA
Stem-loop precursor of miRNA
B
C
siRNA/ miRNA duplex
Dicer
RDR 6
siRNA/miRNA
ta- siRNA
RISC
mRNA
mRNA
mRNA Cleavage
Translation Repression

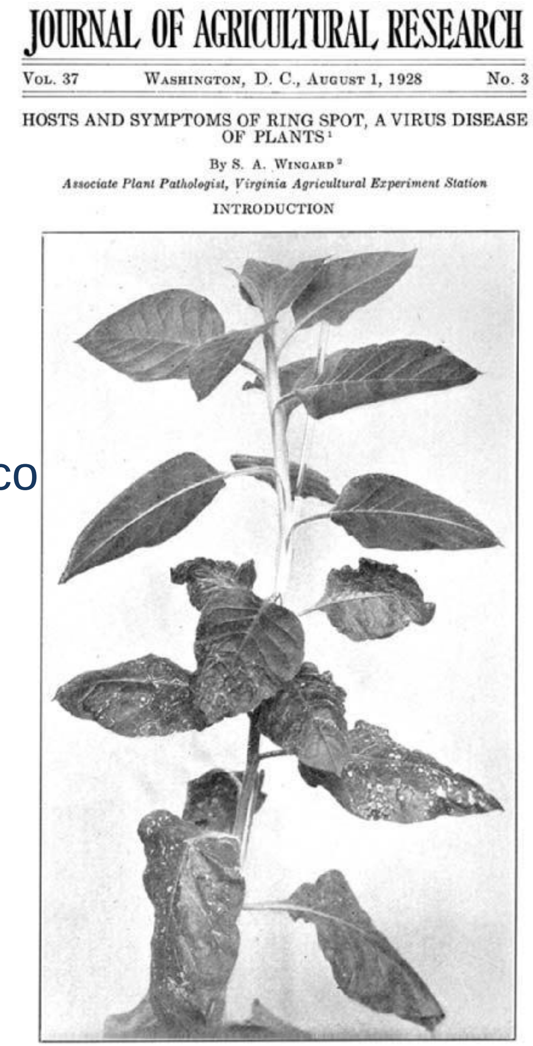
Discovery

- Initially tobacco ringspot virus infected leaves of tobacco were necrotic, but the upper leaves had somehow become immune to the virus and consequently were asymptomatic and resistant to secondary infection (Wingard, 1928).

At that time this "recovery" was a mystery.

- Not much later, McKinney during 1929 reported that tobacco plants infected with the "green" strain of tobacco mosaic virus (TMV) were protected against infection by a closely related second virus i.e. "yellow" strain of TMV (McKinney, 1929).

This phenomenon was later described as "cross protection".


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]

By S. A. Wingard[2]

Associate Plant Pathologist, Virginia Agricultural Experiment Station

INTRODUCTION

- The first recognized encounter with RNA silencing:
  petunia plants were transformed with petunia chalcone synthase (CHS) gene in order to obtain increased flower pigmentation due to overexpression of the CHS gene (Napoli, 1990; van der Krol et al., 1990).

  CHS mRNA levels were strongly reduced in the white sectors.

  This phenomenon was termed as **'co-suppression'.**

- Two years later, another encounter with RNA silencing was made in the field of virus resistance (de Haan et al., 1992; Lindbo and Dougherty, 1992; van der Viugt et al., 1992).

  virus resistance was correlated with reduction of transgene mRNA in the cytoplasm.

  Lindbo and co-workers (1993) proposed this phenomenon to be similar to co-suppression.

- The observation was that a silenced transgene could prevent virus accumulation of potato virus X (PVX) carrying same transgene sequences.

  That pointed toward a sequence specific antiviaral defense mechanism (English et al., 1996),

  what was then called **post-transcriptional gene silencing** (PTGS).

- PTGS also cross-protect the plant against other viruses carrying homologous sequences (Ratcliff et al., 1999).

- viral RNA-mediated cross protection was caused by the same mechanism as transgene induced PTGS.

- These phenomenons are now generally known as **virus-induced gene silencing** (**VIGS**) which explain the mystery of Wingard's finding.

Essential Components of RNA Silencing Pathway

The RNA silencing pathway is regulated by the following components:

Dicer: a RNase III like enzyme, required to produce siRNA and miRNA from perfect and near-perfect dsRNA respectively (Bartel, 2004 and Baulcombe, 2004).

RNA-induced silencing complex (RISC): Agronaute protein (AGO) is a core component and exhibits structural similarity to RNase H (Bartel, 2004; Hall, 2005; Tomari and Zamore, 2005).

dsRNA binding protein (DRB): required for loading of small RNA into RISC (Adenot et al, 2006; Nakazawa et al, 2007).

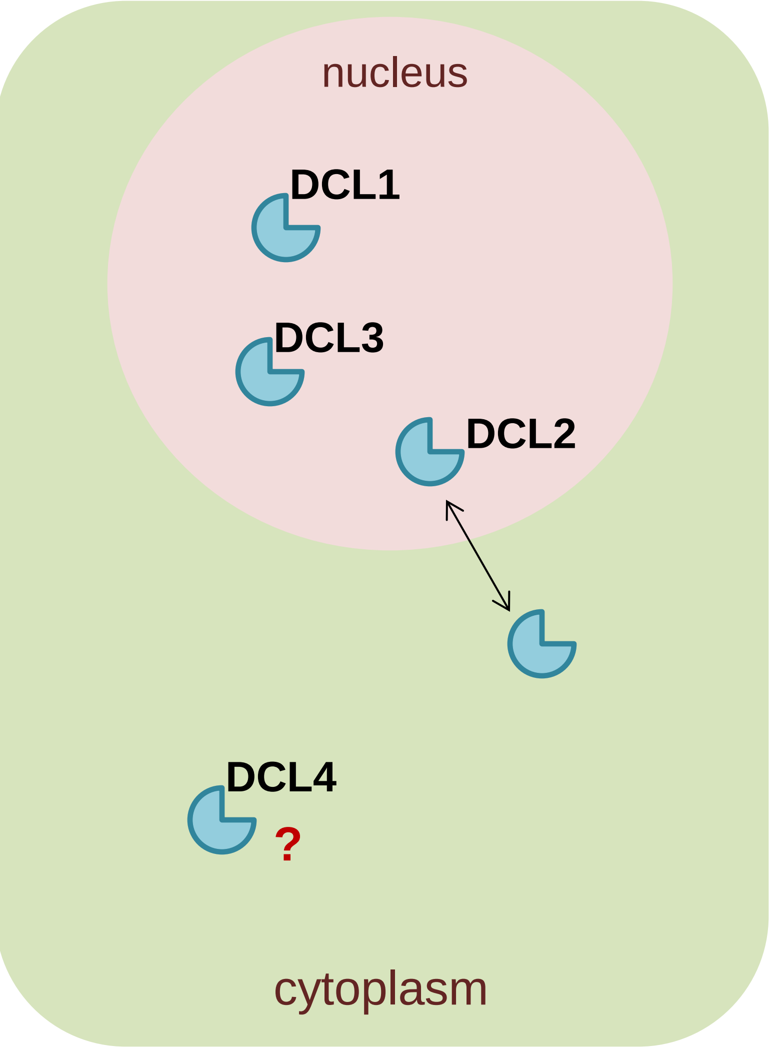
RNA-dependent RNA polymerase (RDR): Unlike miRNA, siRNAs are amplified in plants in a process that requires hostRDR (Baulcombe, 2004).

Previously mentioned proteins are often encoded by **multigene** families in several organisms

Arabidopsis thaliana encodes :

4 Dicer-like proteins (DCLs)
10 Agronautes (AGOs)
5 DRBs
6 RDRs (Juan et al., 2008).

There is functional redundancy among DCLs



RNA Silencing Pathway: A Viral perspective

In the plant cell RNA silencing, that act as antiviral defence during infection of virus and sub-viral pathogens, termed as virus induced gene silencing (VIGS).

The accumulation of virus derived siRNAs – the hallmark of gene silencing – in virus infected tissues → indicate the activation of VIGS

High levels of siRNA correlate with the activity of VIGS

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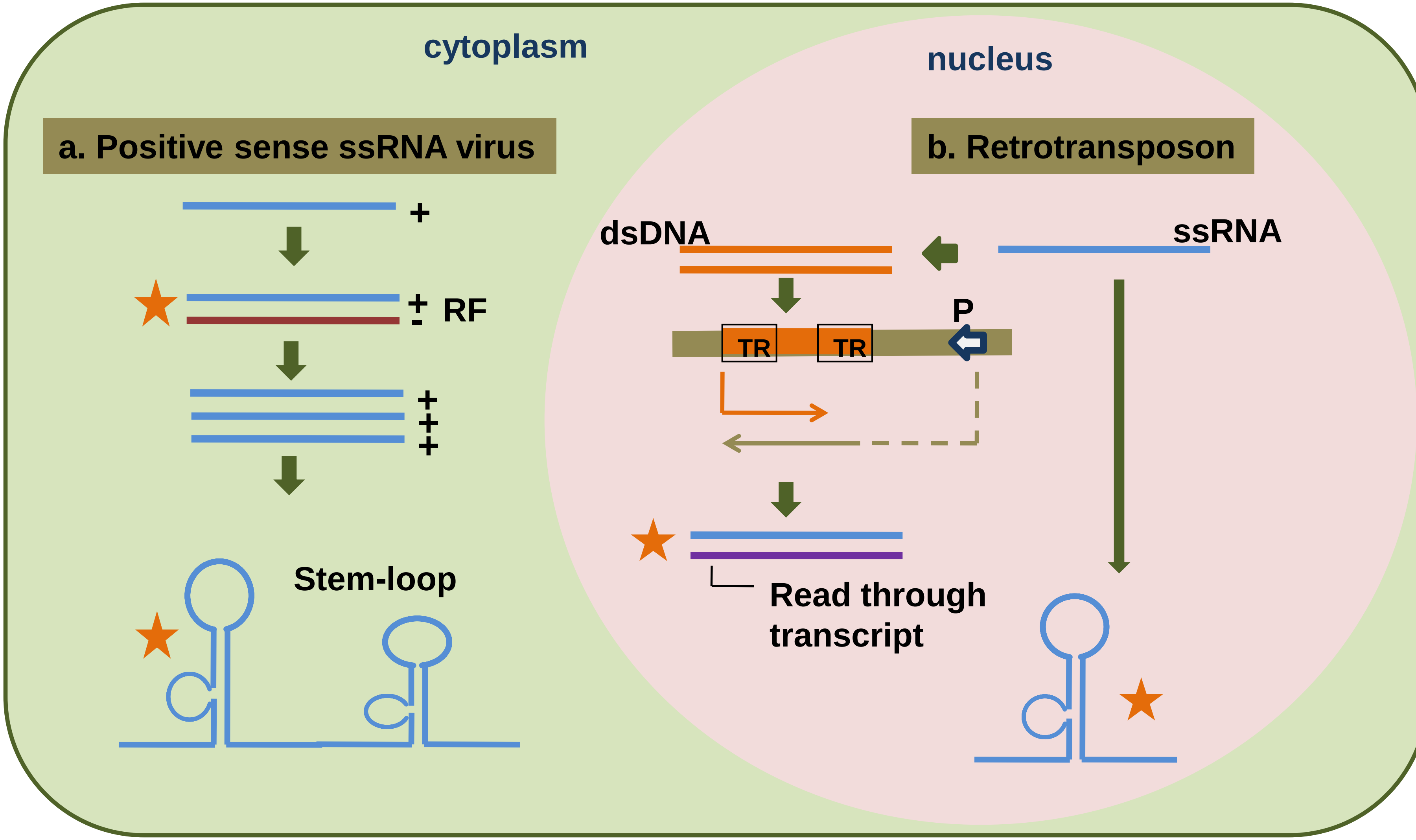
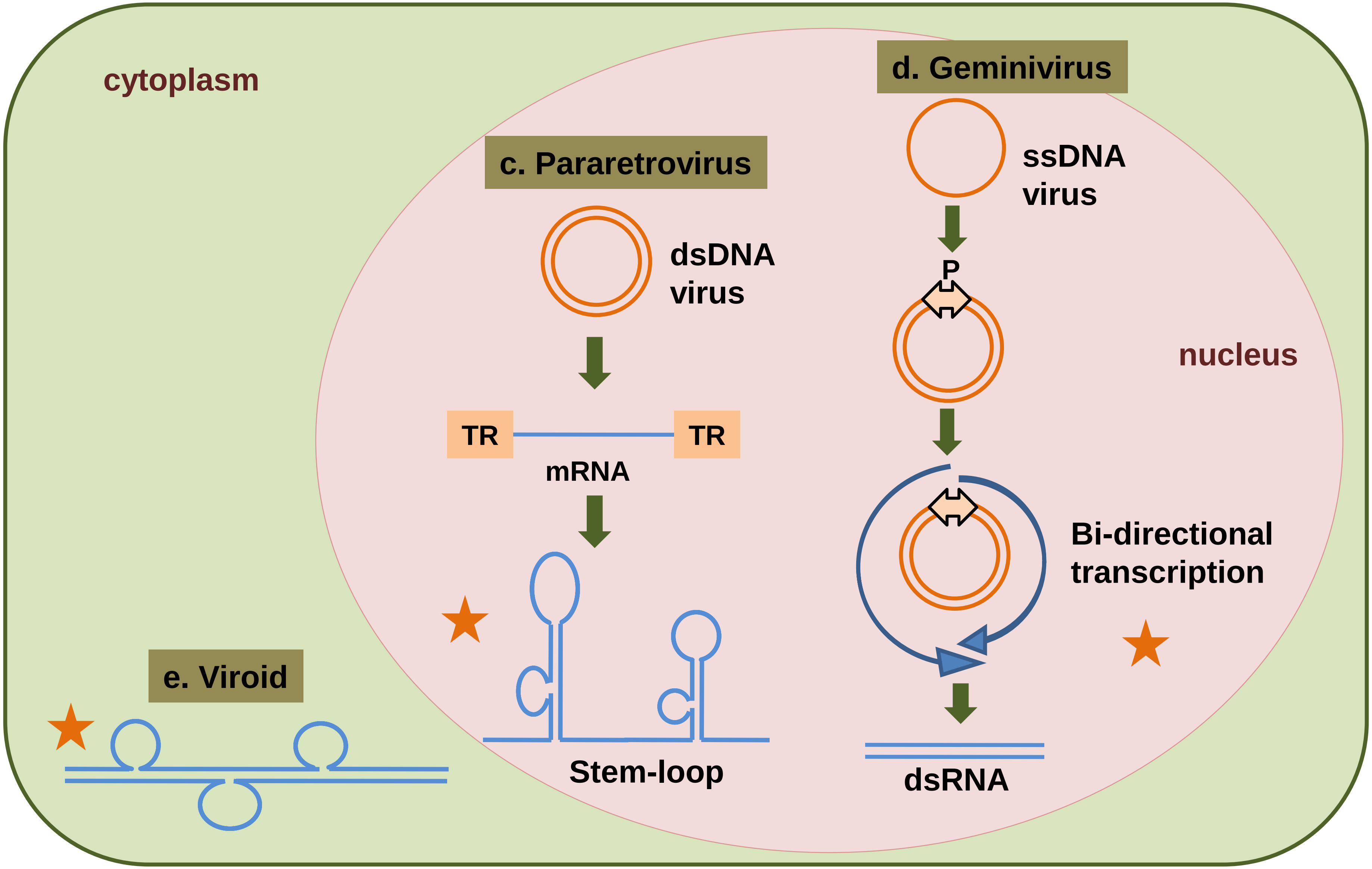
lower viral titre and in some cases, immunity or recovery in upper non-inoculated leaves (Ratcliff et al., 1997; Szittya et al., 2002)

Possible Primary Source of dsRNA: Inducer of VIGS in Virus Infected Plants



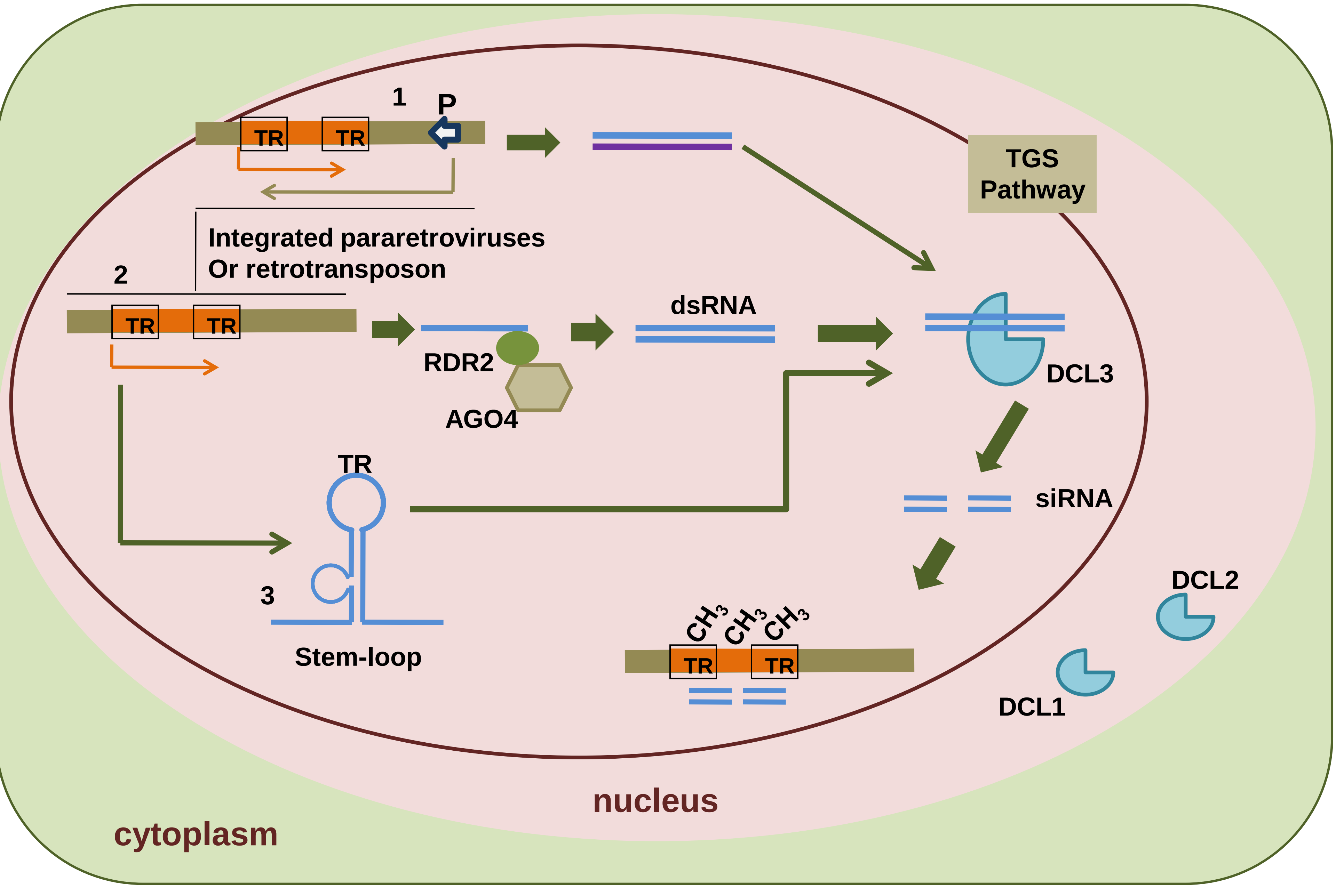
Indicates the formation of dsRNA during viral replication cycle

In Case of DNA Virus and Viroids

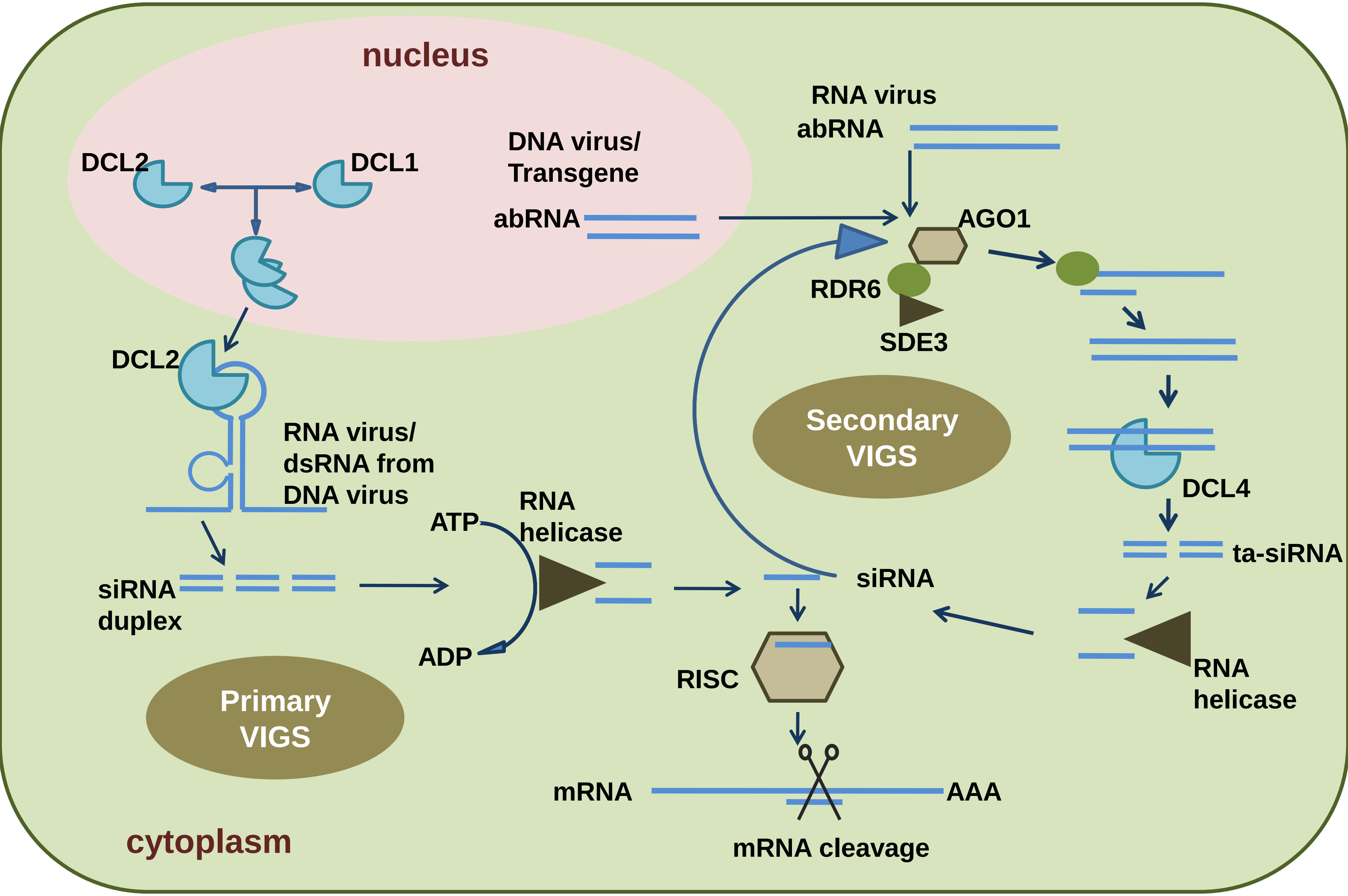


Mechanism of Antiviral Silencing in Plants

Antiviral VIGS Pathway in Nucleus



Antiviral VIGS Pathway in Cytoplasm



Movement of silencing Signal

In plants, indirect evidence indicates

- cell to cell signalling through **plasmodesmata**
- long distances signalling through the **phloem**

Voinnet et al., 1998

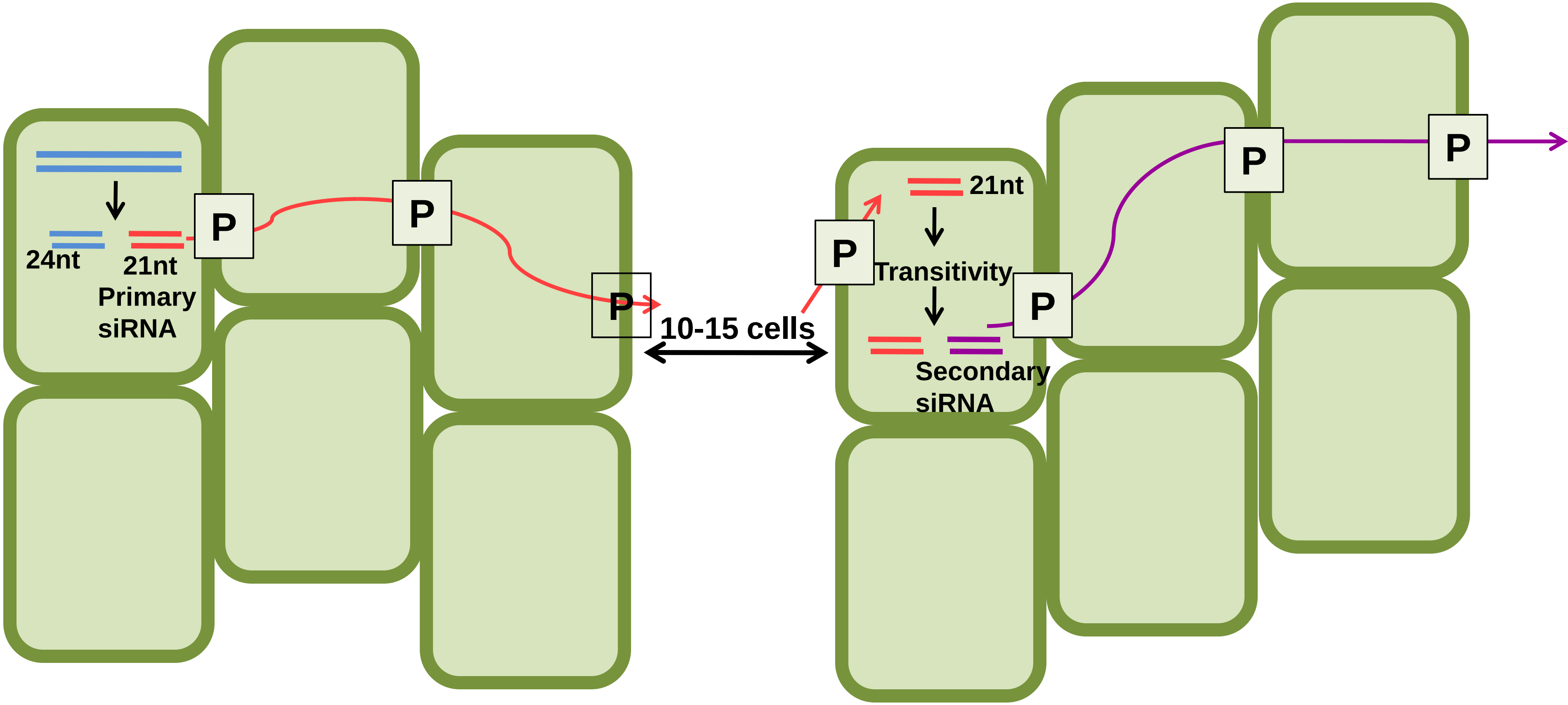
Plant silencing machinery has the unique ability to produce **24 nt siRNA** — **correlated with the long-distance spread of RNA silencing.**

Hamilton et al., 2002

But recent genetic study revealed that **long range cell-to-cell** communication of the silencing signal proceeds through the **relay amplification of short distance signalling events**, which require *de novo* synthesis of secondary **21 nt siRNAs** produced by **transitivity** .

Himber et al., 2003

Model of cell-to-cell Movement of RNA Silencing



P Plasmodesmata

Due to highly adaptive, specific and systemic nature,
RNA silencing can therefore be seen as a form of Genetic Immunity System

Viral Suppression of RNA Silencing

The discovery of viral RNA silencing suppressor
gave a first hint on how viruses could counteract the plant defence.

Initial work showed that the potyvirus-encoded HcPro enhances the replication of many unrelated viruses (Pruss et a., 1997; Kasschau et al., 1997).

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HcPro inhibits RNA silencing

Over 30 VSRs have been identified from different RNA and DNA viruses (Li et al., 2002).

- **VSR function is conserved among homologous viral group**
- **do not share any sequence homology among different viral groups**
- **have different other functions in the virus life cycle**
- **evolved independently in different groups (Burgyan, 2006)**

RNA silencing suppressors encoded by plant viruses

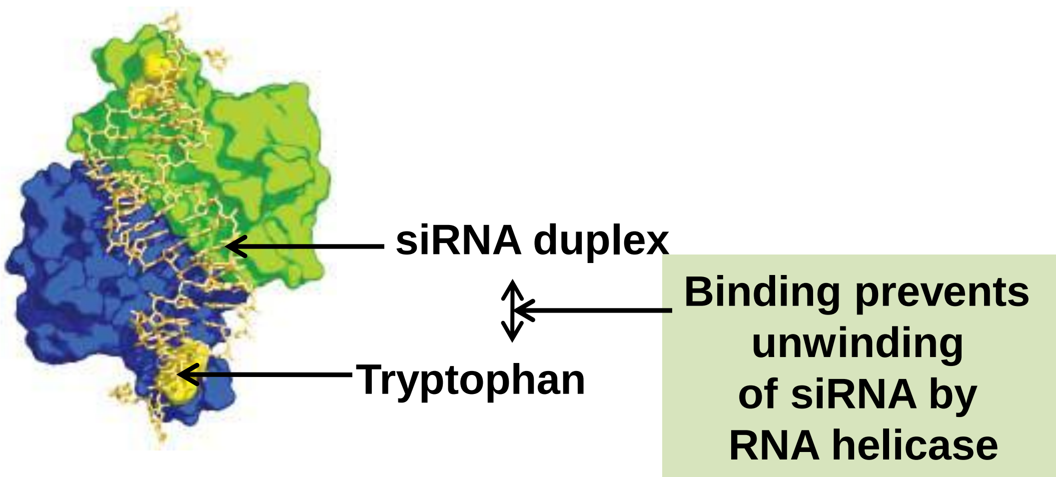
Viral Family	Virus	Suppressors	Other Functions
Positive-strand RNA viruses			
Carmovirus	Turnip crinkle virus	P38	Coat protein
Cucumovirus	Cucumber mosaic virus Tomato aspermy virus	2b	Host-specific movement
Closterovirus	Beet yellows virus Citrus tristeza virus	P21 P20 P23 CP	Replication enhancer Replication enhancer Nucleic-acid binding Coat protein
Comovirus	Cowpea mosaic virus	S protein	Small coat protein
Polerovirus	Beet western yellows virus; Cucurbit aphid-borne yellos virus	P0	pathogenicity determinant
Potexvirus	Potato virus X	P25	Movement
Potyvirus	Potato virus Y	HcPro	Movement; polyprotein processing; aphid transmission; pathogenicity determinant
Sobemovirus	Rice yellow mottle virus	P1	Movement; pathogenicity determinant
Tombusvirus	Tomato bushy stunt virus; Cymbidium ringspot virus; Carnation Italian ringspot virus	P19	Movement; pathogenicity determinant
Tobamovirus	Tobaccomosaic virus; Tomato mosaic virus	P30	Replication
Tymovirus	Turnip yellow mosaic virus	P69	Movement; pathogenicity determinant

RNA silencing suppressors encoded by plant viruses

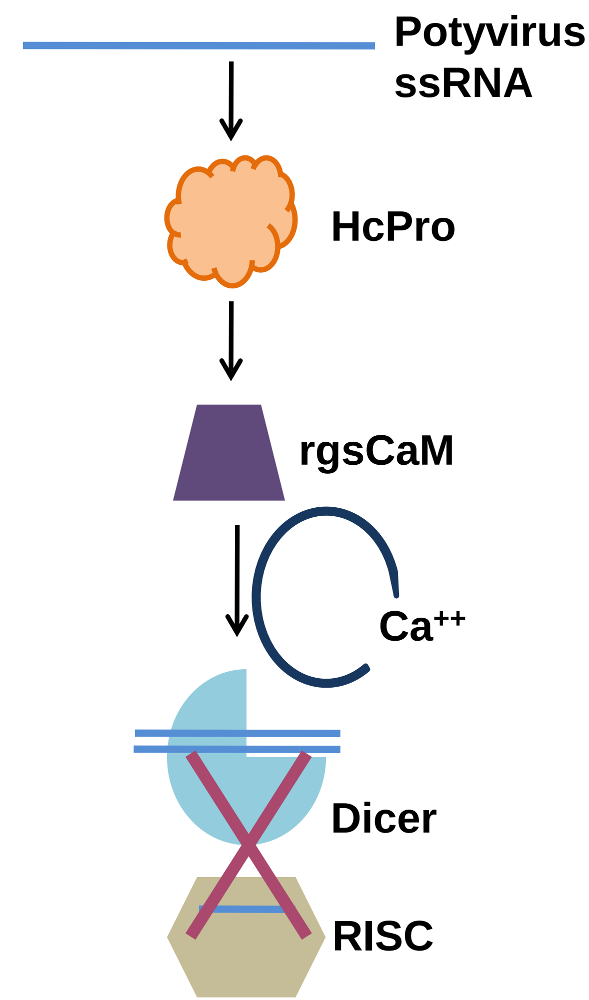
Negative-strand RNA virus			
Tospovirus	Tomato spotted wilt virus	NSs	pathogenicity determinant
Tenuivirus	Rice hoja blanca virus	NS3	Unknown
Double stranded RNA virus			
Phytoreovirus	Rice dwarf virus	Pns10	Unknwn
DNA virus			
Geminivirus	African cassava mosaic virus Tomato yellow leaf curl virus Mungbean yellow mosaic virus	AC2 C2 C2	Transcriptional Activator Protein (TrAP)

Molecular Basis of Silencing Suppression

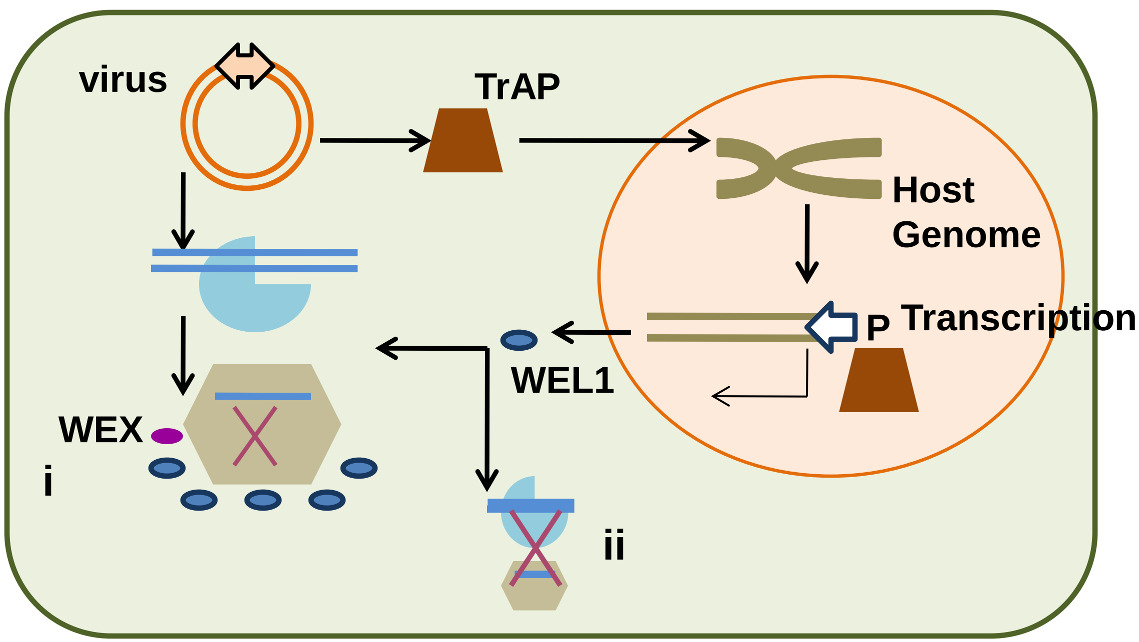
a. Tombusviral P19 protein



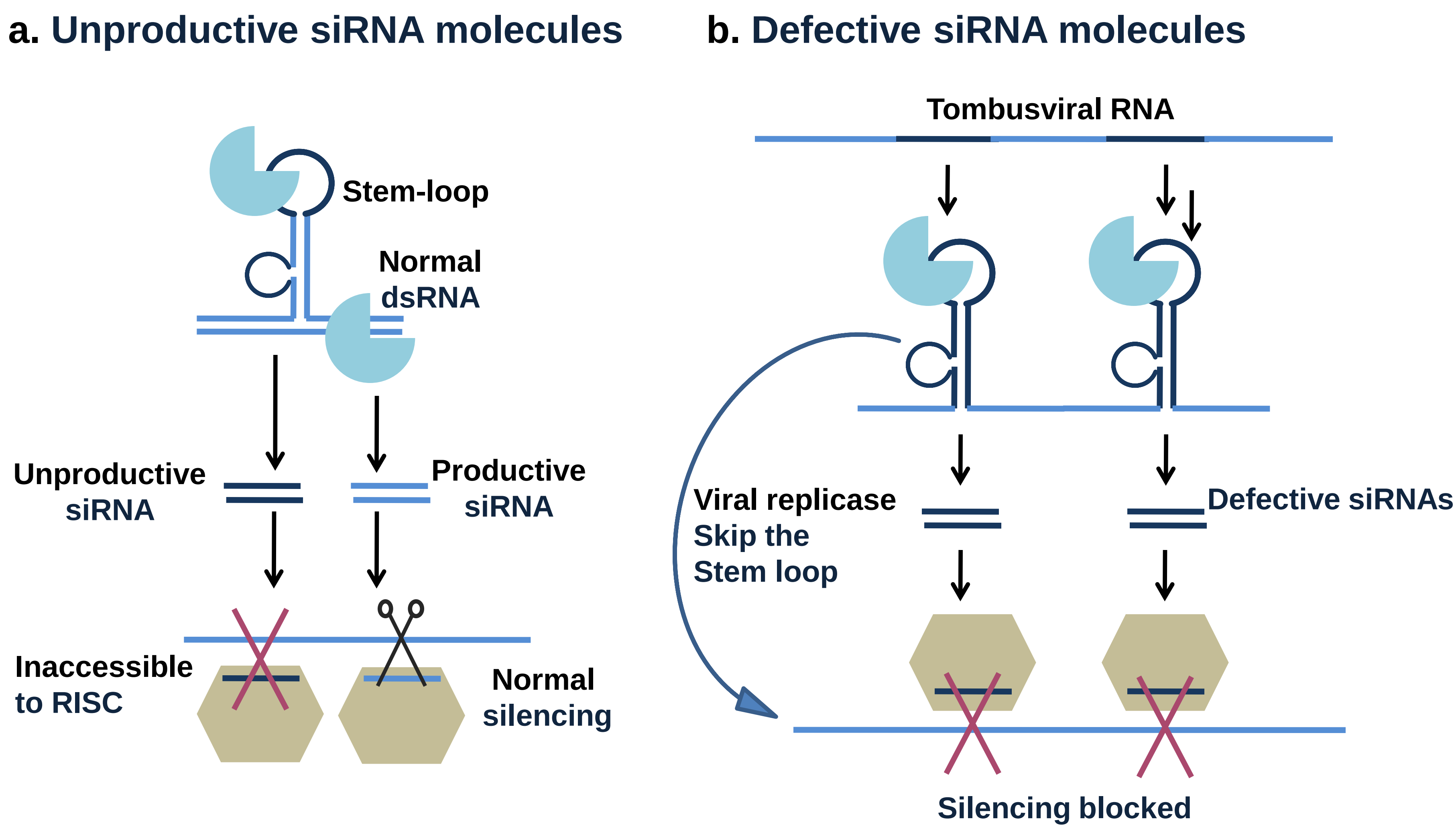
b. Potyviral HcPro protein



c. Geminiviral TrAP (Transcription Activator Protein)



RNA-Mediated Silencing Suppression



VSRs and Induction of plant viral disease symptoms

It is well established that the antiviral and endogenous silencing pathways share common elements
(e.g.: endogenous small regulatory RNAs such as
si-, tasi- and ds miRNA intermediates)
and silencing suppressors often interact with these common elements.

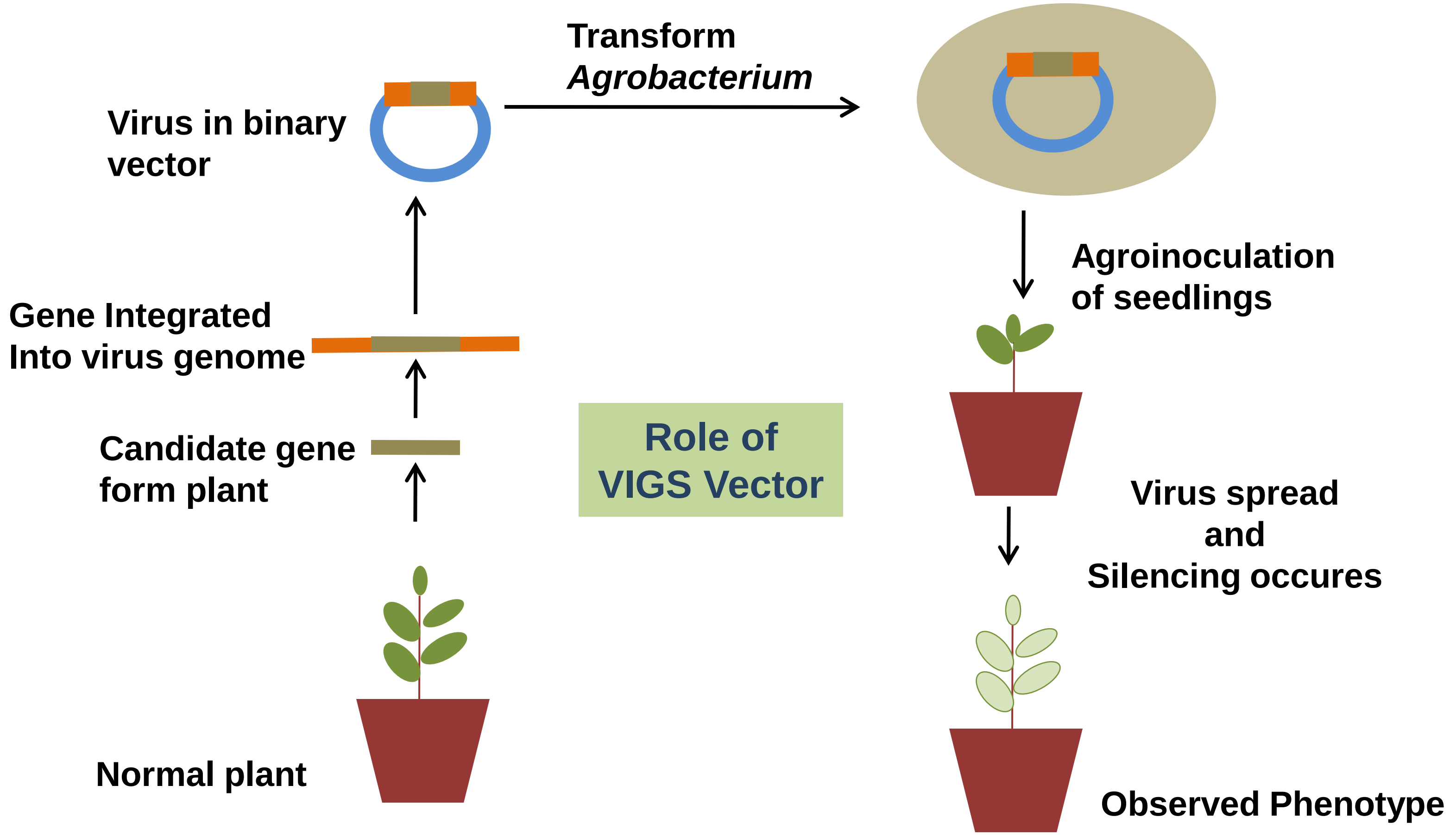
virus-induced symptoms are the consequences of the interaction of silencing suppressors and endogenous RNA silencing-mediated developmental pathways

presence of the silencing suppressor is essential for the development of systemic virus infection.

VSRs do not always play a direct role in eliciting the disease symptoms.
(Deleris et al., 2006)

Application of VIGS

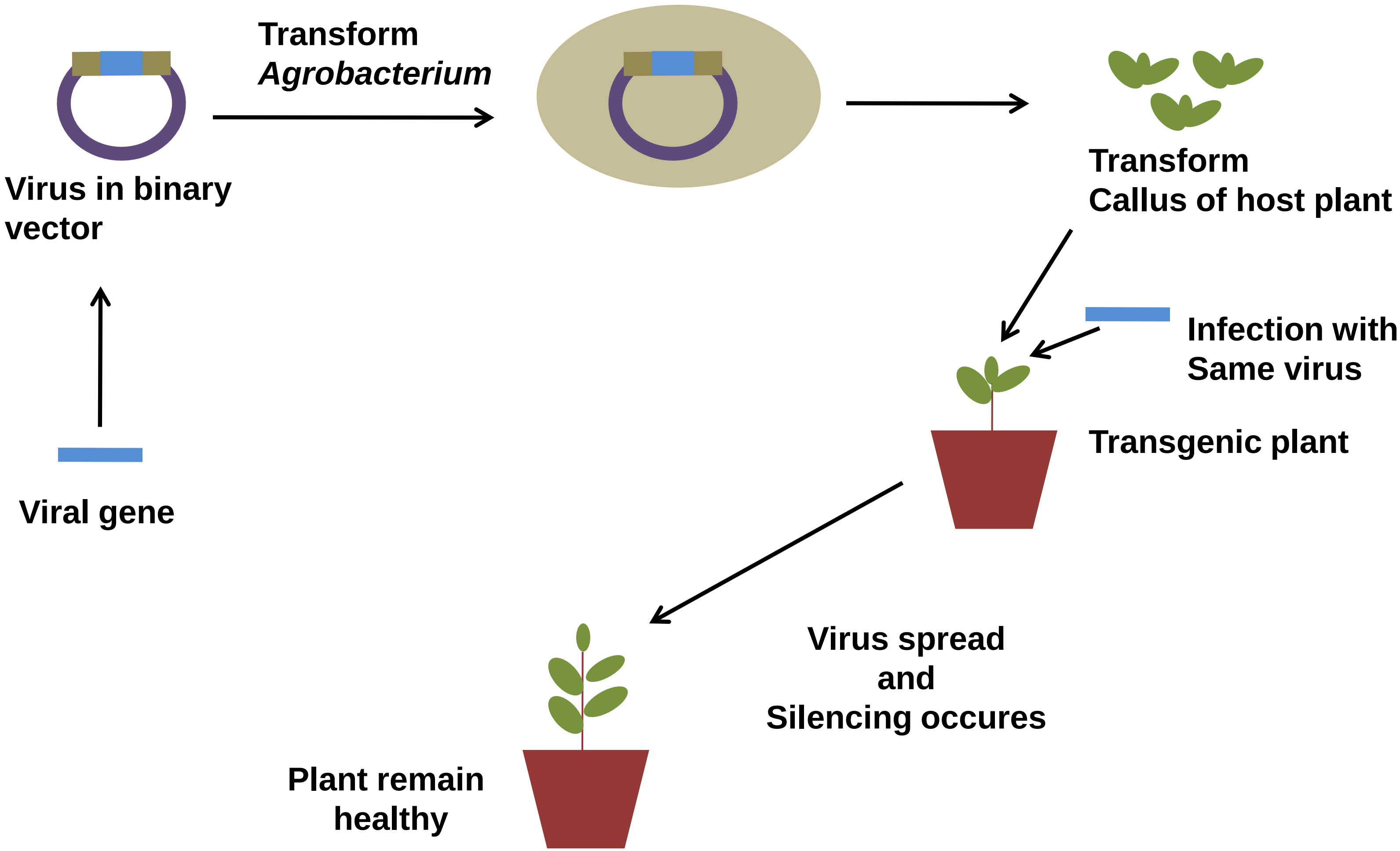
Used as a technology for functional genomics



The most often used VIGS vectors

Vectors	Plant species	Vector induced symptoms	Developed for large scale analysis
Tobacco mosaic virus	*Nicotiana benthamiana*	Variable	Yes
Potato virus X	*Nicotiana benthamiana*	Variable	Yes
Tobacco rattle virus	*Nicotiana benthamiana* *Arabidopsis*, Tomato	Mild	Yes
TMV satellite virus-induced silencing system	Tobacco	Mild	No
Barley stripe mosaic virus	Barley	Moderate	Yes
Cabbage leaf curl virus	*Arabidopsis*	Variable	No
Tomato golden mosaic virus	*Nicotiana benthamiana*	Variable	No

Used as a new approach for transgenic plant development



Transgenic Developed

Transgenic plant	Virus	Developed by
Tobacco	PVY	Waterhouse et al. ,1998
Tobacco	African cassava mosaic virus	Vanitharani et al., 2003
Tobacco	Pepper mild mottle virus Plum pox virus	Tenllado et al., 2003
Tobacco Tomato	Tomato yellow leaf curl virus	Abhary et al., 2006
Rice (PB1)	RTBV	Tyagi et al., 2008

Thank You…