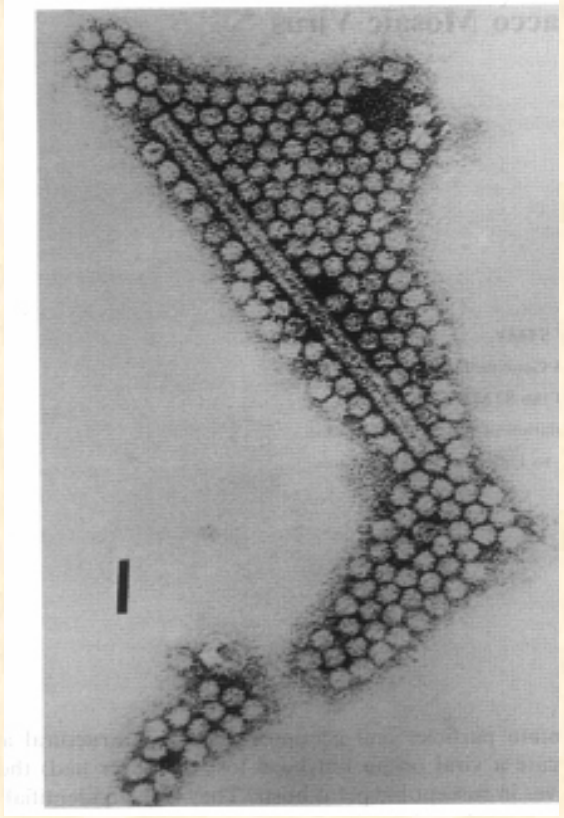
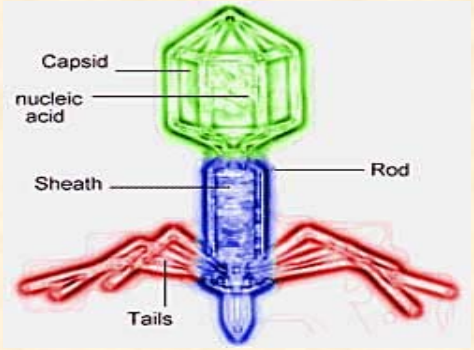
Subviral Agents

"Great fleas have little fleas,
Upon their backs to bite 'em,
And little fleas have lesser fleas, and
so, ad infinitum."

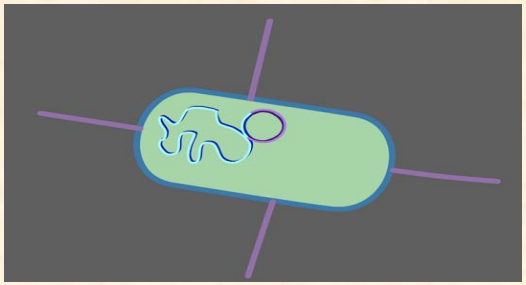
Augustus DeMorgan,
Mathemetician 1806-1871



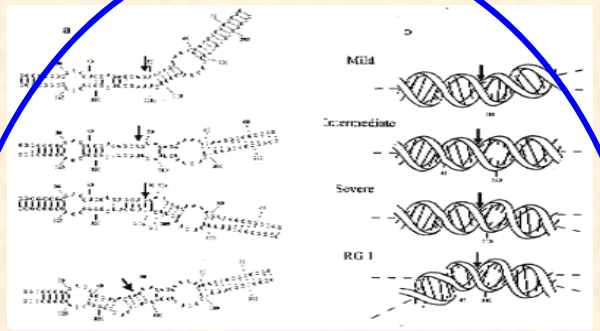
Rich diversity of parasites at the molecular level:
Known collectively as Mobile Genetic Elements



Viruses

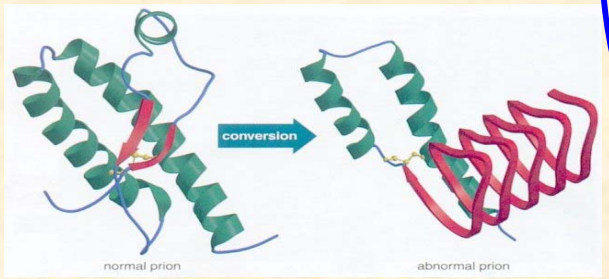


Plasmids

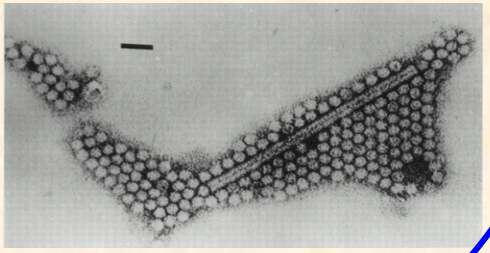


Viroids

Transposons



Prions



Satellites

TOPICS COVERED:

1. Differences between viruses and subviral agents (satellites, viroids)
2. Different types of subviral agents and how they are distinguished
3. Different roles played by subviral agents in disease
4. Characteristics of Viroids
5. Taxonomy of Viroids

Subviral Agents

There are 4 types of subviral agents that replicate in plants:

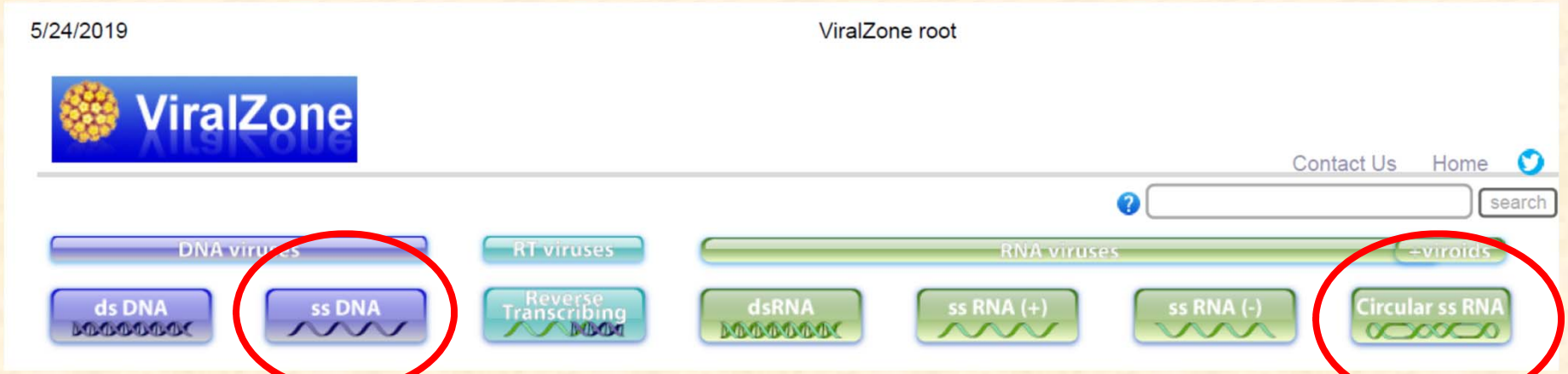
Replication dependent on a helper virus

A. Satellite Viruses
B. Virus-Dependent Nucleic Acids
C. Defective Interfering RNAs/DNAs

Replication is independent of a helper virus

D. Viroids

Only some of the subviral agents are included in the Baltimore Classification System



ssDNA satellites

Viroids

A. Satellite Viruses

- Viruses that are dependent upon a helper virus for replication

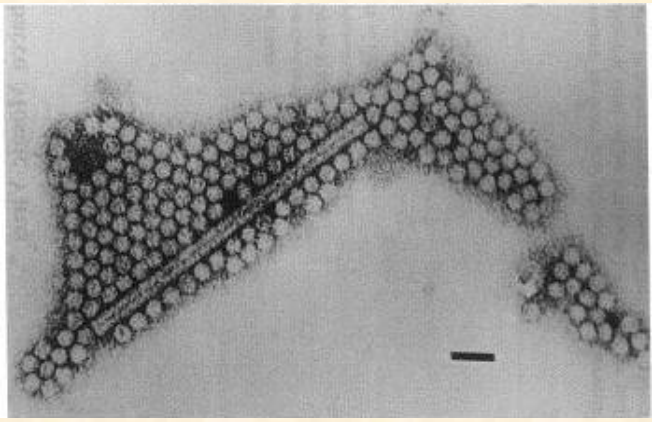
- Are encapsidated by their own coat protein

(ie satellite virus genome codes for its own coat protein)

- Have genomes that are distinct from their helper virus

- Often have a virion structure different from their helper virus

- Found as distinct components in preparations of particles of helper viruses



Electron micrograph of a negatively stained purified preparation of TMGMV and STMV from doubly infected tobacco showing a full-length rod-shaped virion of TMGMV (300 nm) surrounded by spherical virions of STMV (16-17 nm). Bar represents 50 nm. (Valverde and Dodds 1987)

A. Satellites Viruses – Classification and Taxonomy

1: Chronic bee-paralysis virus associated satellite
Contains no known plant virus satellite viruses

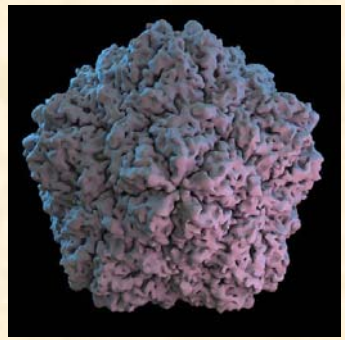
2: Satellites that resemble *Tobacco necrosis satellite virus*
Satellite viruses of plant viruses are in this subgroup

3: Nodavirus associated satellite viruses
Contains no known plant virus satellite viruses

4: Adenovirus-associated satellite viruses
Contains no known plant virus satellite viruses

5: Mimivirus-associated satellite viruses
Contains no known plant virus satellite viruses

A. Satellite Viruses (Subgroup 2):



Not a homogeneous taxon
4 approved species (not italized):

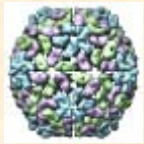
Helper Virus Family	Satellite Virus	Particle Size (nm)	Coat Protein MW (d)	Satellite Genome (nt)
Tombusviridae	Tobacco necrosis satellite virus	17	21,600	1,239
	Panicum mosaic satellite virus	17	17,000	826
	Maize white line mosaic satellite virus	17	23,961	1,168
Virgaviridae	Tobacco mosaic satellite virus	17	17,500	1,059

Satellite Viruses (Subgroup 2)

Example 1:

Satellite virus:
Tobacco necrosis satellite virus (STNV)

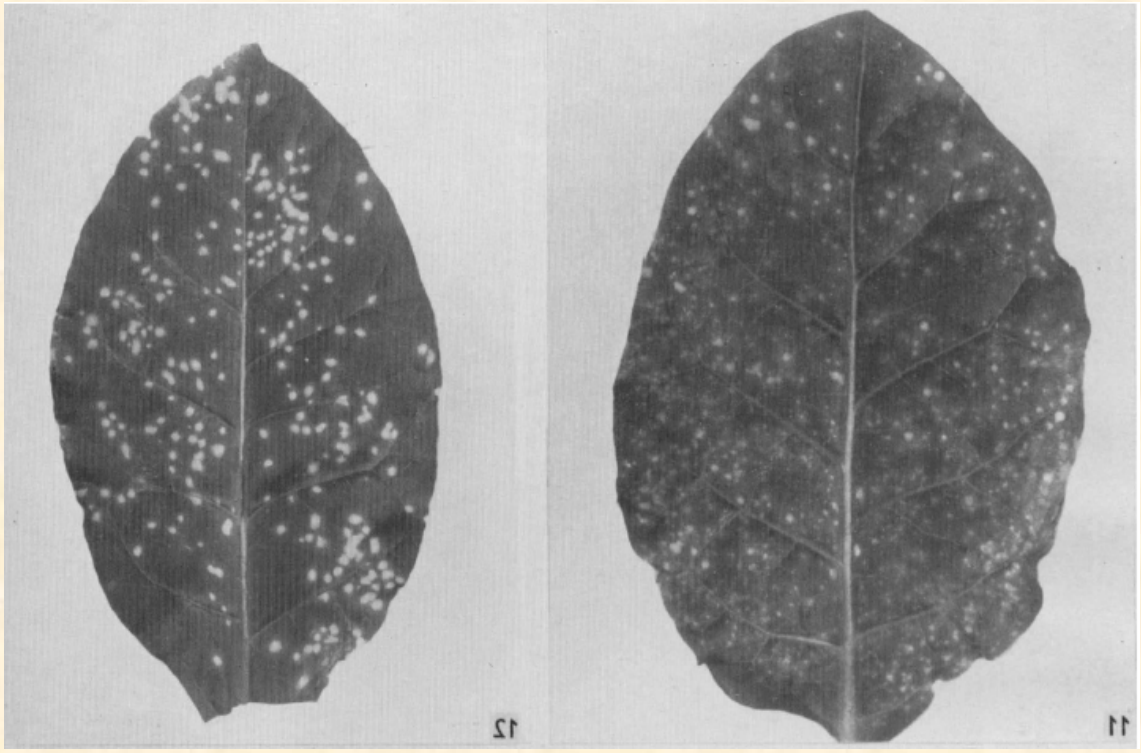
Helper virus: ***Tobacco necrosis virus*** (TNV)
(Family: *Tombusviridae* Genus: *Necrovirus*)



- STNV genome has no significant sequence similarity with TNV genome
- The replication of TNV is not dependent on the presence of STNV
- Replication of STNV is dependent on the presence of TNV
- Replication of STNV significantly suppresses TNV replication

TNV

TNV plus STNV



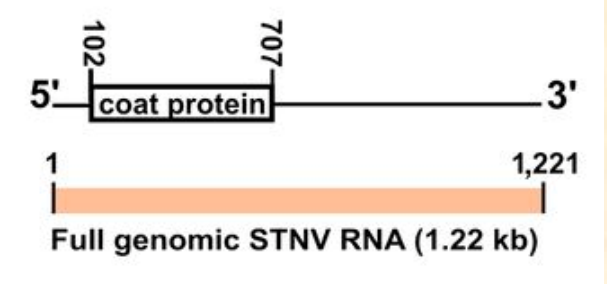
Large local lesions

Large and small local lesions

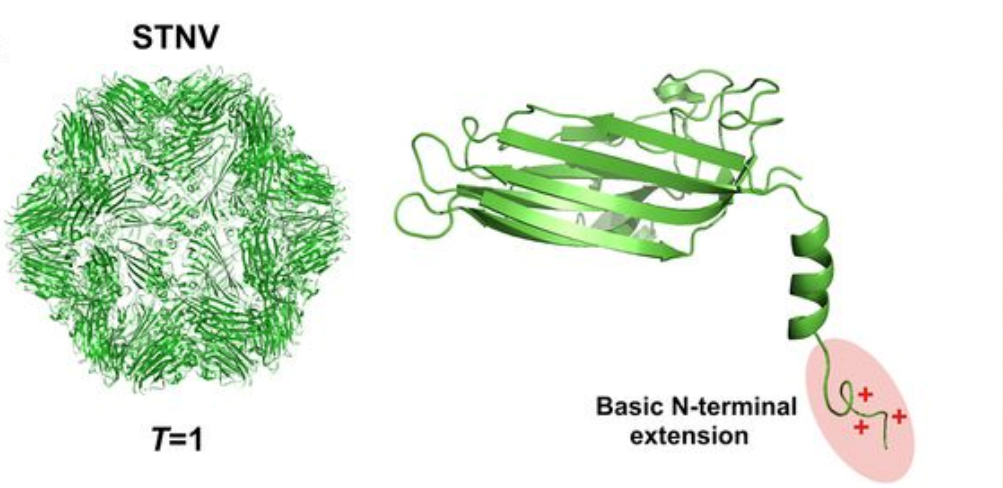
Small local lesions = TNV + STNV)

Kassanis and Nixon 1961

Genome organization of STNV:



Particle structure and coat protein molecule of STNV:

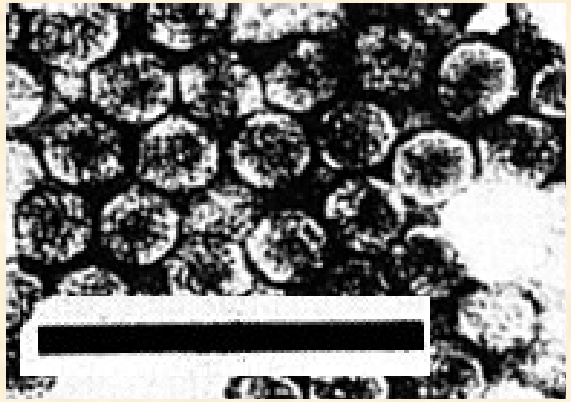


Example 2. **Panicum mosaic satellite virus (SPMV)**

Panicum mosaic virus (PMV)
(formerly known as St. Augustine decline virus*)*
Family *Tombusviridae*, genus *Panicovirus*

17 nm

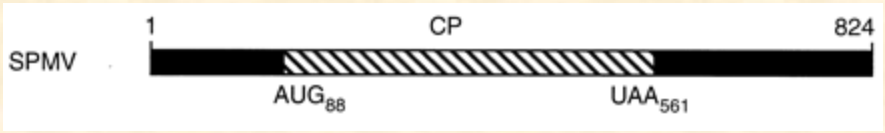
Symptoms of PMV without satellite



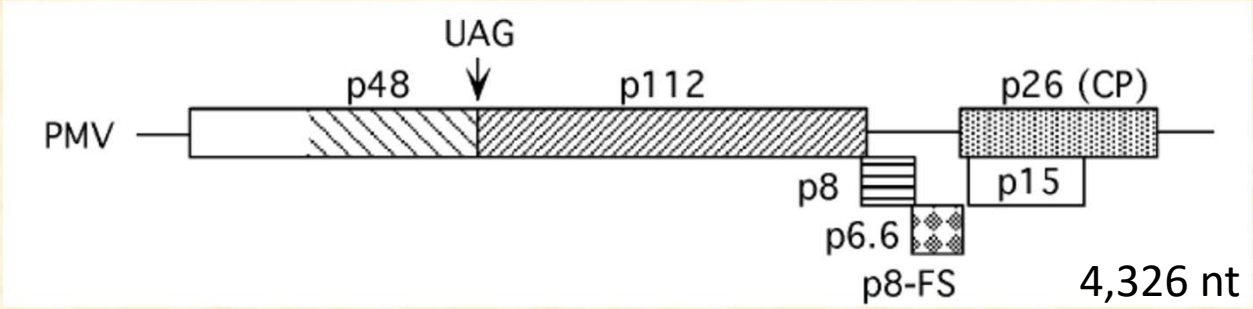
Panicum mosaic virus
25-30 nm

Bar represents 110 nm

Genome organization of Panicum mosaic satellite virus



One ORF – codes for coat protein (CP)



Genome organization of *Panicum mosaic virus*

PMV and SPMV: Example of a synergistic interaction between a satellite virus and its helper virus

Healthy PMV PMV + SPMV

Healthy PMV PMV + SPMV

Plants and panicles of pearl millet

From: Scholthof 1999 MPMI 12:163-166

Subviral Agents

There are 4 types of subviral agents in plants:

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B. Virus-Dependent Nucleic Acids

C. Defective Interfering RNAs/DNAs

Replication is independent of a helper virus

D. Viroids

B. VIRUS-DEPENDENT NUCLEIC ACIDS
(SATELLITE RNAs/ SATELLITE DNAs)

Again, these are a diverse range of DNA and RNA molecules that do not code for a capsid protein, but are packaged in capsids encoded by the helper virus

•Dependent on helper virus for replication

- May encode non-structural proteins or may not encode proteins but do not code for their own coat protein
- Are encapsidated by the coat protein of the helper virus
- Have nucleotide sequences substantially distinct from those of the genomes of their helper virus or hosts
- Some satellites have short sequences at the termini that are the same as those of the helper virus.

B. VIRUS-DEPENDENT NUCLEIC ACIDS

- Effects of virus-dependent nucleic acids vary with helper virus, host plant, and the satellite
- Virus-dependent RNAs/DNAs often interfere with the replication of their helper virus
- Some attenuate disease (of interest for viral disease control and the engineering of new sources of viral resistance).
- Some satellites exacerbate symptoms produced by the helper virus alone or may produce new symptoms not associated with the helper virus alone.

B. Three Groups of Virus-Dependent Nucleic Acids:

Distinctions based on size and type of nucleic acid (not sequence)

1) Single stranded satellite DNAs –

a) Alpha satellites – (a.k.a. DNA 1 satellites) derived from *Nanovirus* components, ~ 1000 nt in size, more than 39 genomes

b) Beta satellites - ~ 1300 nt in size, code for one protein, contain a silencing suppressor, often modify symptoms of the helper virus, More than 60 genomes described, origin unknown

2) Double stranded satellite RNAs –

- Associated with viruses in the *Totiviridae* and *Partitiviridae*
- Approximately 20 genomes

3 Groups of Virus-Dependent Nucleic Acids:

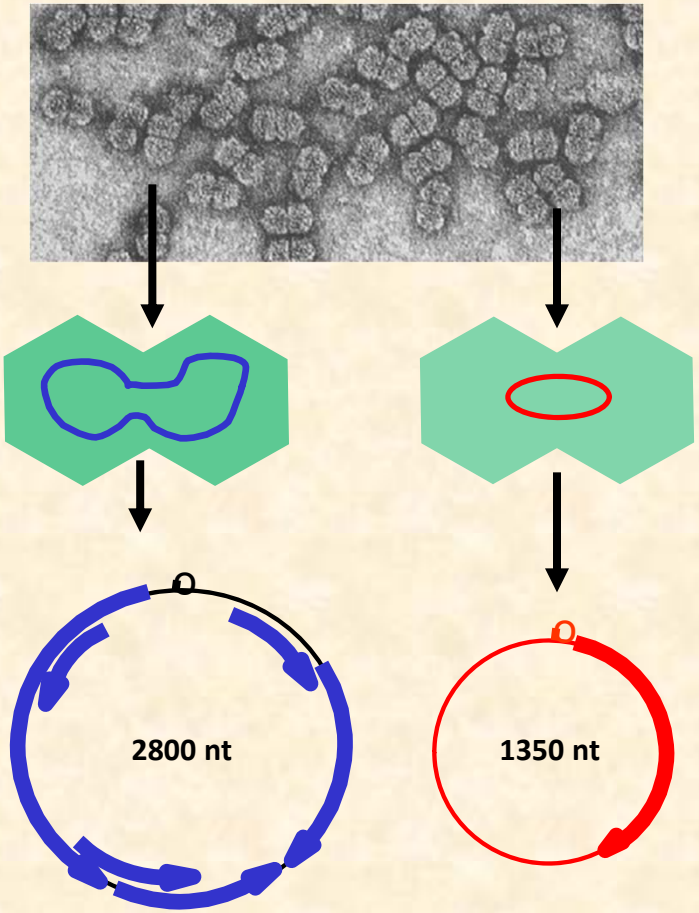
Distinctions based on size and type of nucleic acid

3) Single stranded satellite RNAs –

Distinguished by type of genome (size and strandedness)

a) large single stranded satellite RNAs, 800-1500 nt, (10 genomes)
b) small, linear RNA satellites, < 700 nt, (15 genomes)
c) circular single stranded (viroid-like) RNA satellites, 350 nt, (9 genomes)

1) Single-stranded satellite DNAs – Ex.: Begomovirus Beta Satellites



- Known as Beta or β
- ssDNA, 1350 nt in length
- Associated with monopartite begomoviruses
- No sequence homology with begomoviruses
- Require a begomovirus for replication, movement, and transmission

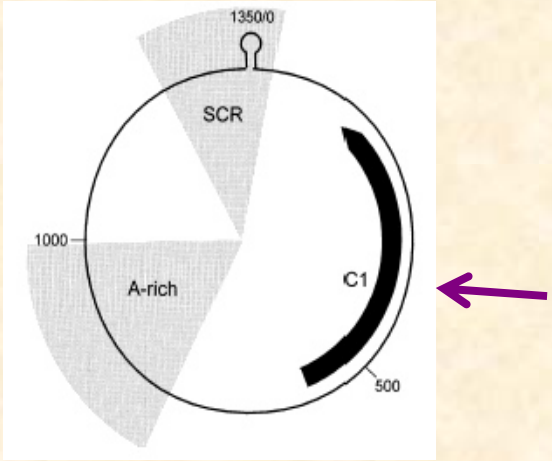
Only some of the subviral agents are included in the Baltimore Classification System

ssDNA satellites

1) Begomovirus Beta Satellites

- **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**

Consensus genome map of DNA β



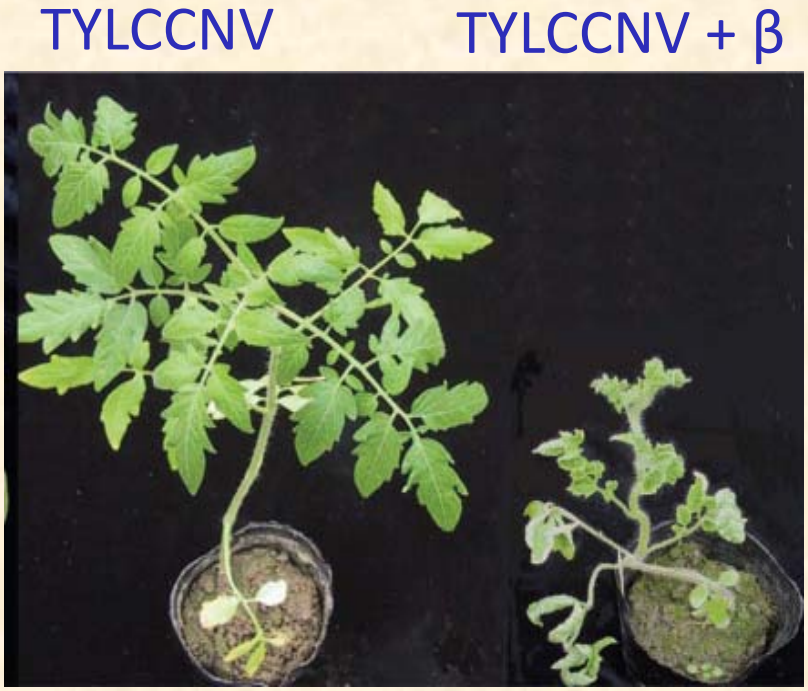
Diversity of DNA β, a satellite molecule associated with some monopartite begomoviruses, *Virology, Volume 312, Issue 1, 20 July 2003, Pages 106-121*

Virus-Dependent RNAs/DNAs:

DNA β:

1) Can alter symptoms of helper virus

TYLCCNV = tomato yellow leaf curl China virus
Host = tomato



Zhou et al. 2003. Characterization of DNAb associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. J. of Gen. Virol. 84: 237-247.

Virus-Dependent RNAs/DNAs:

DNA β:

2. Can increase the host range of a begomovirus

   In Pakistan, Begomoviruses of cotton cannot infect tomato ….. unless a β satellite is present

   Result: several new diseases of tomato are caused by cotton begomoviruses + β satellite

Virus-Dependent RNAs/DNAs:

DNA β:

3. Can break resistance to the helper virus

Many tomato cultivars possess the *TY-1* gene for resistance to TYLCV:

- Inoculation with TYLCV = resistance (no or very mild symptoms)
- Inoculation with TYLCV plus satDNA β = no resistance = severe symptoms

a) Partial list of Virus-Dependent large RNAs

Virus Genus	Helper Virus	Satellite	
		Size (nt)	Symptoms of the helper virus
Nepovirus	Arabis mosaic virus – L1	1104	+
	Strawberry latent ringspot virus	1200	0
	Tomato black ring virus	1375	0
	Grapevine fan leaf virus	1114	-
	Myrobalan latent virus	1400	-

+ = enhances
0 = no change
- = attenuates

2) Small linear satellite RNAs: Example

Effect of satellite RNA on symptoms of CMV in tomato:

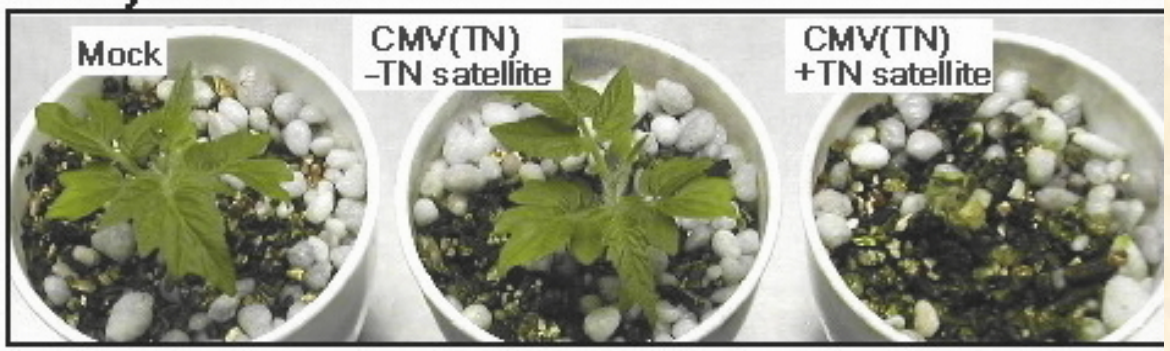


Table 1. Frequency of systemic infection and symptom expression on virus-inoculated "Micro-Tom" plants

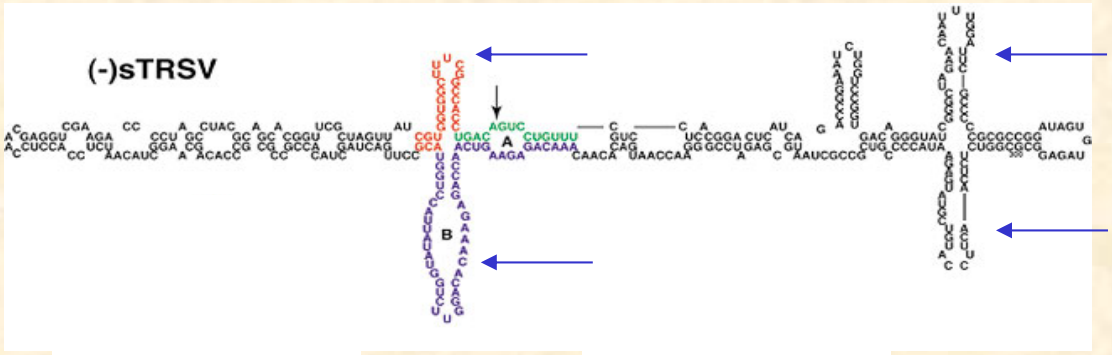
Virus	Number of virus-inoculated plants	Number of plants systemically infected with virus*	Number of plants showing symptoms** (type of symptom***)
CMV-TN(-)sat	15	15	0
CMV-TN(+)sat	15	15	15 (Lethal systemic necrosis)

* Systemic infection of virus was confirmed immunologically.
** Number of plants showing symptoms was counted at 16 days after inoculation.
*** Symptoms were shown in Fig. 1.

(by Ayano Shimizu & H. Takahashi)

www.agri.tohoku.ac.jp/ppathol/tomato/sub5CMV.htm

3) Circular single stranded satellite RNAs: Example



Genome of Virus-Dependent RNA satellite of *Tobacco ringspot virus* showing:

1) Small genome size
2) nucleotide sequence
3) secondary structure (blue arrows)

Amount and location of secondary structure varies among satellite RNAs

Subviral Agents

4 types of subviral agents in plants:

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C. Defective Interfering RNAs/DNAs

Replication is independent of a helper virus

D. Viroids

C. Defective interfering (DI) RNAs

Defective interfering RNA/DNAs are distinct from Virus-Dependent Nucleic Acids …..they are deletion and/or rearrangement derivatives of their helper virus.

- Defective interfering (DI) RNAs/DI DNAs arise as a result of mistakes made during viral RNA/DNA synthesis.
- DI RNAs/DNAs may interfere with the replication of the helper virus
- DI RNAs/DNAs require the helper virus for replication
- Range in size from 10 - 25% of the parental viral genome
- Encapsidated by helper virus coat protein

C. Defective interfering (DI) RNAs/DNAs

- Effect on helper virus symptoms:
  - no effect
  - reduction of symptom severity
  - mild - marked increase in disease severity

- Once formed DI RNA molecules replicate and accumulate in infected tissues together and in competition with the helper virus.

Associated with several plant virus genera: *Begomovirus, Bromovirus, Tombusvirus, Potexvirus, Carmovirus, Furovirus, Closterovirus, Tospovirus,* and others

C. Defective interfering (DI) RNAs

Example 1
DI RNAs of TSWV L-RNA

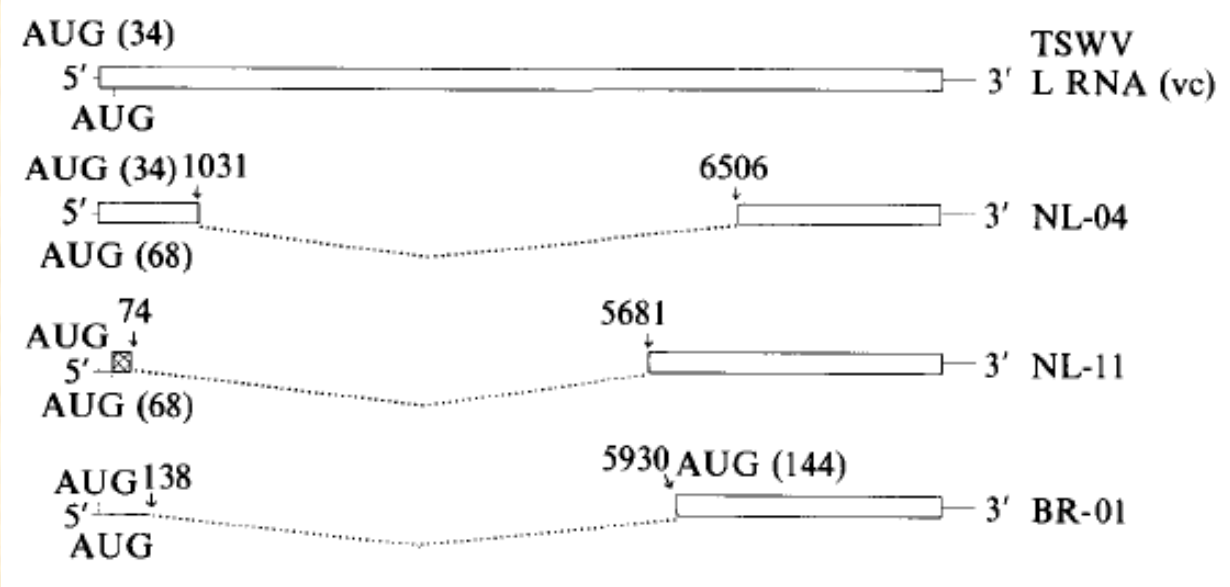
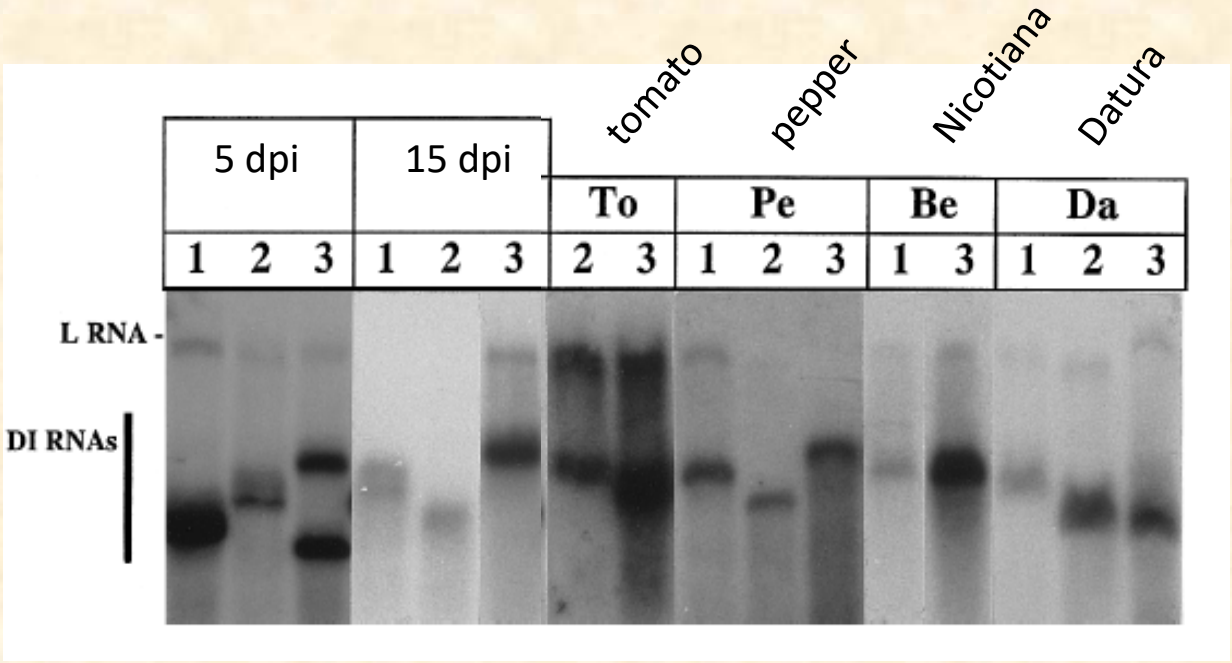


Fig. 5. Schematic representation of the ORFs present in the TSWV DI L RNAs. The wild-type ORF is presented at the top. The open boxes in the three DI RNAs indicate encoded amino acid sequences identical to those in the L protein and the shaded box the novel sequences encoded by the ORF generated by the NL-11 DI RNA. The numbers with an arrow indicate the position of the nucleotides at the junction sites; the position of the AUG start codon of each ORF is given in parentheses.

Resende et al 1992. J. of General Virology 73: 2509-2516

Northern blot showing different sizes of DI RNAs in different plants

- Size and number of DI RNAs can vary from plant to plant
- Size and number of DI RNAs can change over time in the same plant



Inoue-Nagata et al 1998. Virology 248, 342–356

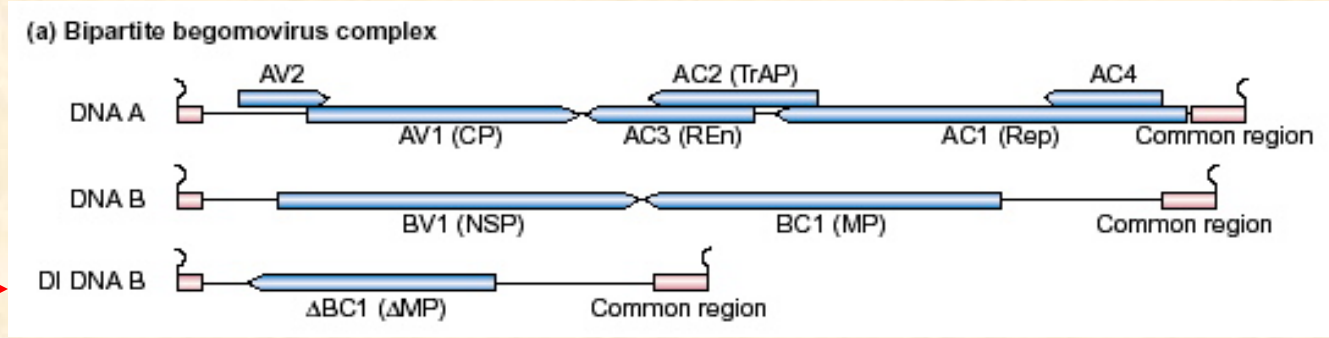
C. Defective Interfering (DI) DNAs

Example 3:

Defective Interfering DNAs of Begomoviruses (multipartite genome)

Some Begomoviruses may spontaneously produce 1/2 sized defective DNA A or B components

DI DNA of DNA-B



DI DNAs often reduce the severity of the disease symptoms caused by bipartite begomoviruses

Subviral Agents

4 types of subviral agents in plants:

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3. Defective Interfering RNAs/DNAs

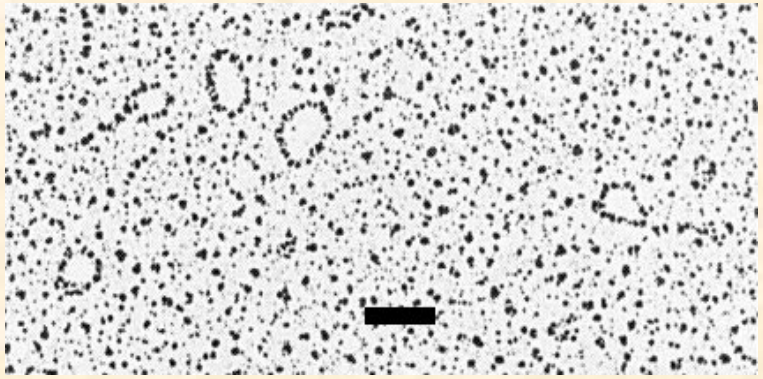
Replication is independent of a helper virus

4. **Viroids**

D. Viroids

Viroids - low-molecular-weight, covalently closed, single-stranded, circular RNAs

- 246 to 375 nucleotides
- exist in their native state as **highly base-paired, rod-like structures** -
- 50 nm long
- Viroids are unencapsidated, replicate autonomously in plants, and may or may not elicit symptoms



- There are 31 ICTV approved viroid species and probably many more still to be found
- Viroids show a range of symptoms from no effect to plant death
- The same viroid can infect different kinds of plants
- Viroids are usually a concern in vegetatively propagated crops

Subviral Agents: Viroids

- Typical symptoms produced by viroids - stunting, mottling, leaf distortion, and necrosis
- Symptoms can be indistinguishable from those caused by viruses

3 wks post inoculation **6 wks post inoculation**

PSTVd –
causes elongation
of potato tubers.

Potato spindle tuber viroid (PSTVd)

Chrysanthemum stunt viroid (CSVd)

Healthy

Infected

'Bonny Jean'

One viroid may induce different symptoms on different hosts

Sweet orange / Citrumelo

'Tahiti' lime

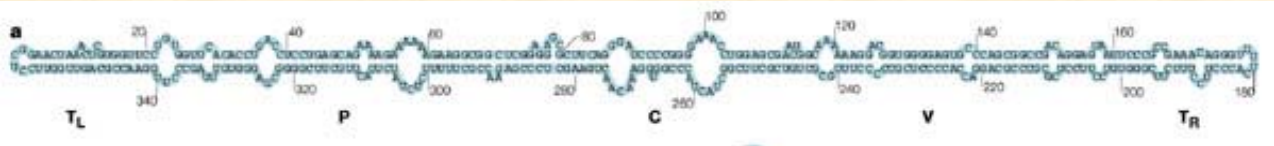
Citron

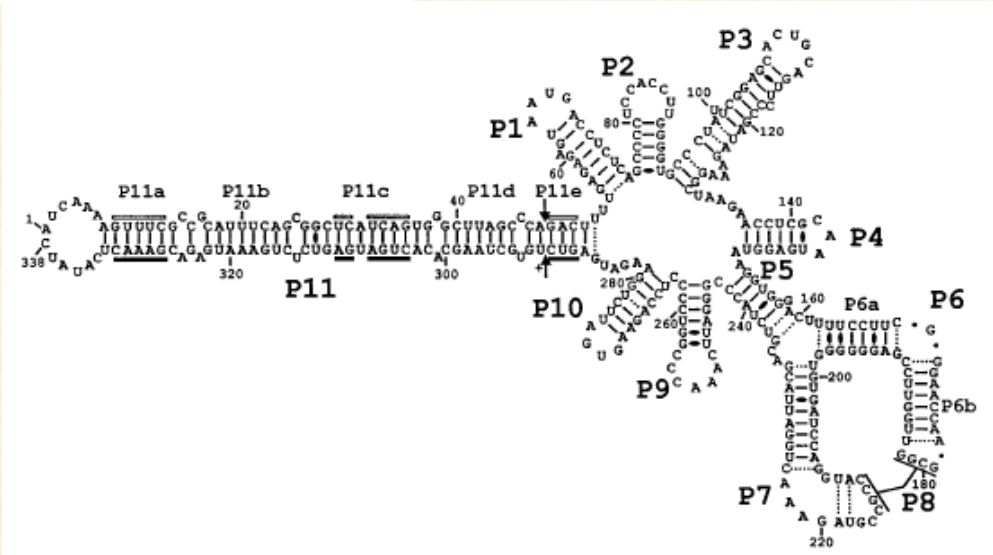
Sweet orange / 'Carrizo'

***Citrus exocortis viroid* (CEVd)**

D. Viroids

- The circularity of the ssRNA and the high degree of base-pairing make the RNA stabile (despite lack of a protective coat protein).

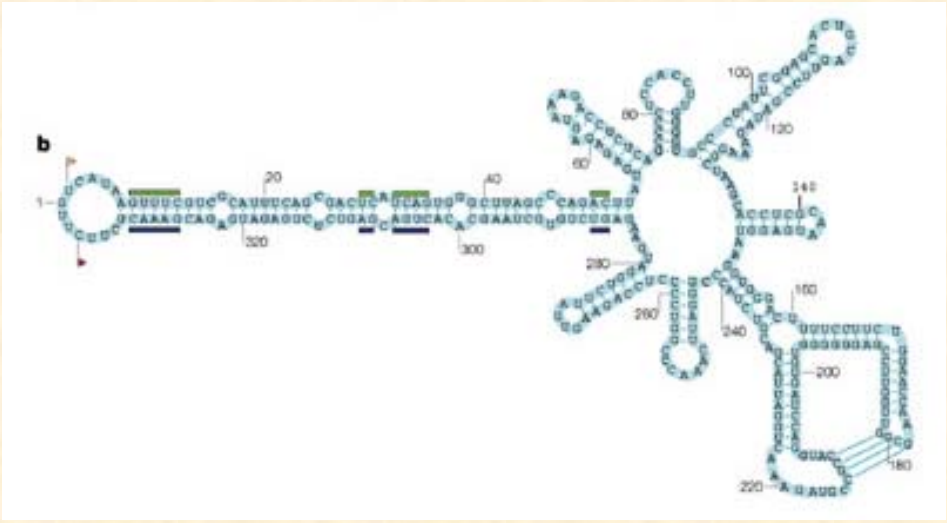




Viroids

Viroids lack mRNA activity

- no proteins are encoded
- viroids use pre-existing host nucleic acid synthesizing enzymes
- very small changes in nucleotide sequence may give rise to dramatic changes in the effects induced by a viroid



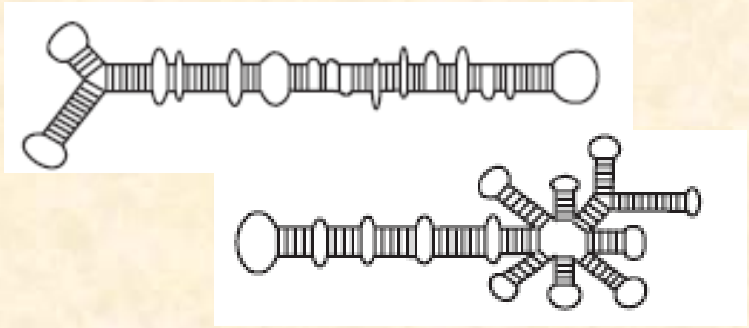
D. Viroids

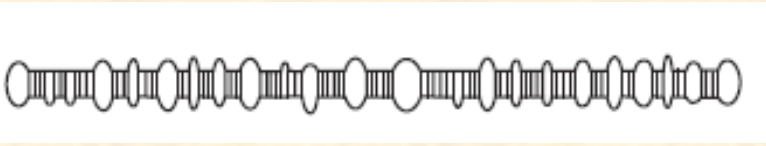
- **Viroids are unique to plants**
- Viroids move rapidly throughout the plant upon infection
- Viroids are readily transmitted by mechanical means, vegetative propagation, and through pollen and seed.
- Aphid transmission of viroids is possible (but rare) in mixed infections with certain aphid-transmitted plant viruses.

[Ref. Syller et al., 1997. Eur. J. of Plant Path.103: 285-289.
Querci et al., 1997. J. of Gen. Virol. 78:1207-1211]

Viroid Taxonomy

Family	Genus
Avsunviroidae	*Avsunviroid*
	Pelamoviroid
	Elaviroid
Pospiviroidae	*Pospiviroid*
	Hostuviroid
	Cocadviroid
	Apscaviroid
	Coleviroid





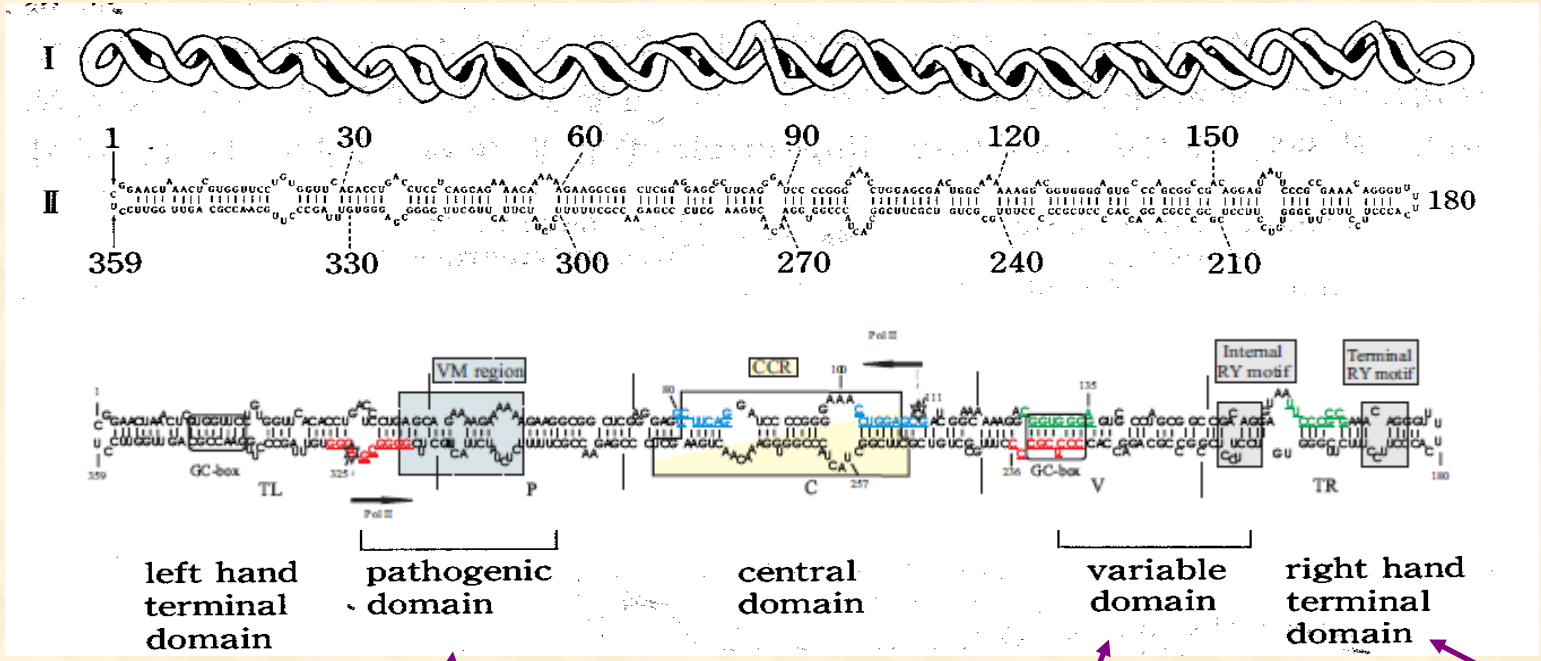
Partial List of Viroids and their Hosts

Primarily found in asexually propagated crops

TABLE 1 Viroid species with their abbreviations, accession numbers of typ variants, sizes, and genus and family to which they belong

Viroid species	Abbreviation	Accession	Size (nt)	Genus
Potato spindle tuber	PSTVd	V01465	359	Pospiviroid
Tomato chlorotic dwarf	TCDVd	AF162131	360	Pospiviroid
Mexican papita	MPVd	L78454	360	Pospiviroid
Tomato planta macho	TPMVd	K00817	360	Pospiviroid
Citrus exocortis	CEVd	M34917	371	Pospiviroid
Chrysanthemum stunt	CSVd	V01107	356	Pospiviroid
Tomato apical stunt	TASVd	K00818	360	Pospiviroid
Iresine 1	IrVd-1	X95734	370	Pospiviroid
Columnea latent	CLVd	X15663	370	Pospiviroid
Hop stunt	HSVd	X00009	297	Hostuviroid
Coconut cadang-cadang	CCCVd	J02049	246	Cocadviroid

Pospiviroidae:



replication

Implicated in pathogenicity

~95 nt, highly conserved

<50% identity among closely related viroids

replication

Pospiviroidae: Structure

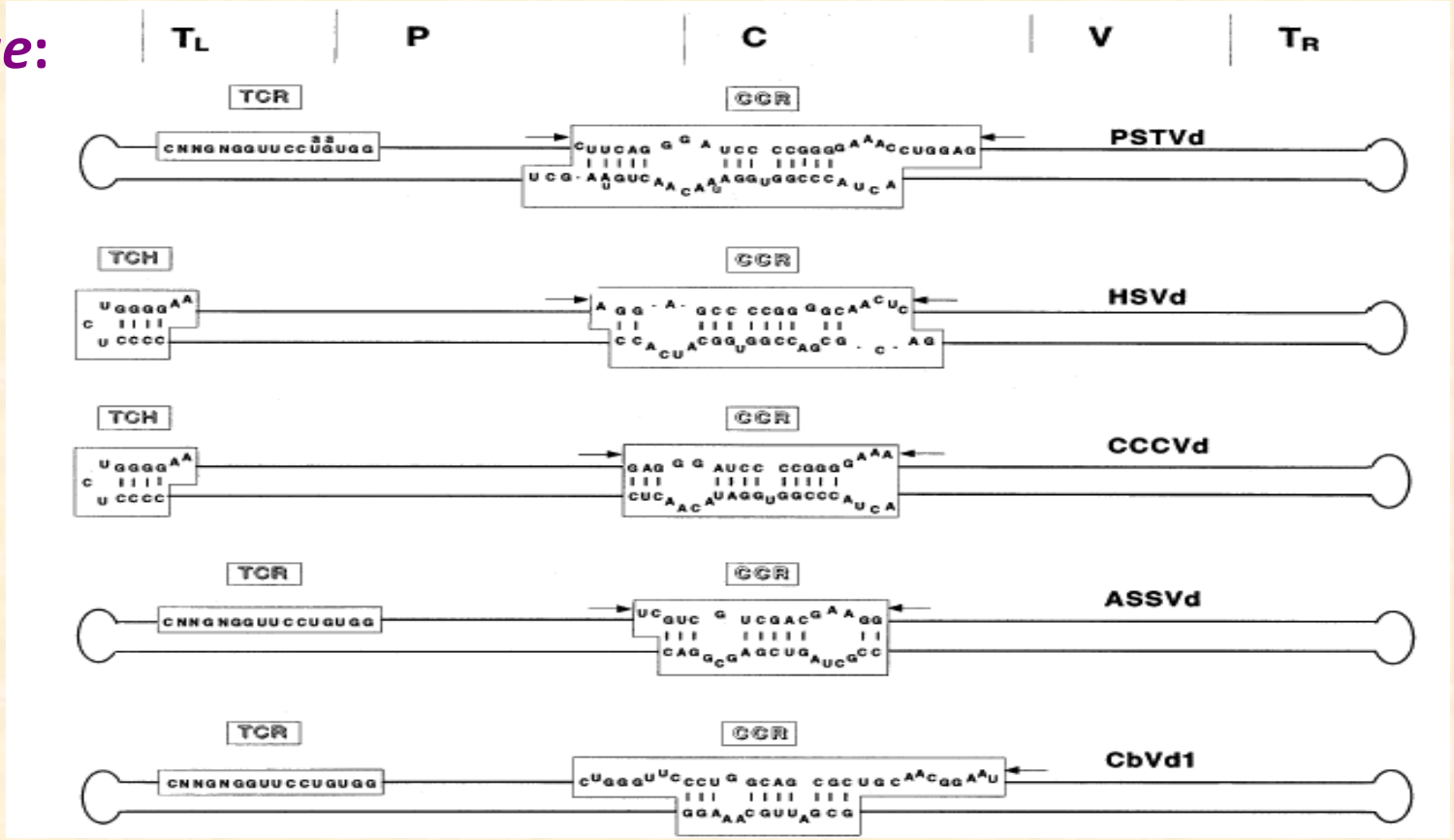
Pospiviroid

Hostuviroid

Cocadviroid

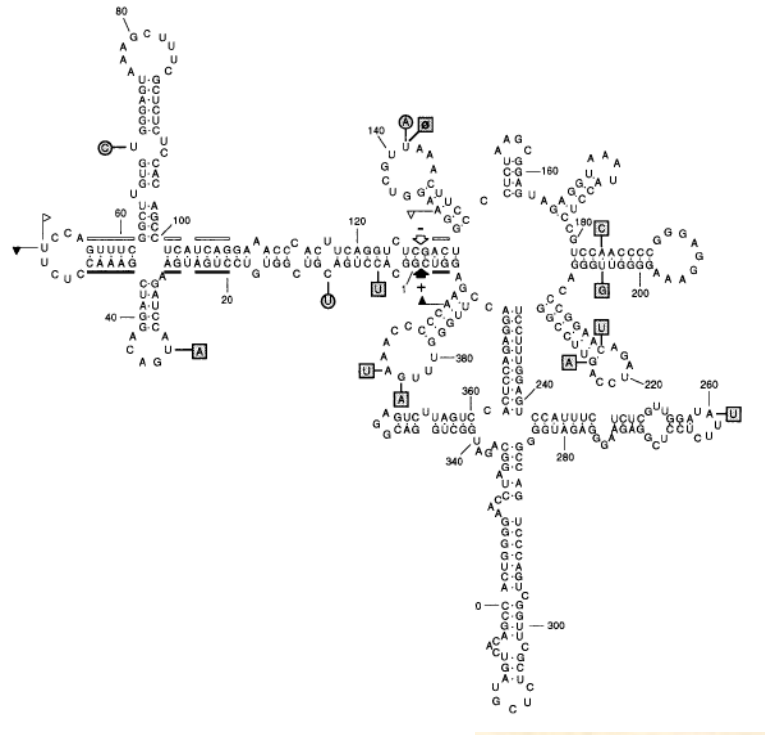
Apscaviroid

Coleviroid



- Similar secondary structures
- Some sequence homology among Pospiviroids

Avsunviroidae:



Chrysanthemum chlorotic stunt viroid

Peach latent mosaic viroid

- Different secondary structures
- Very low sequence homology

among viroids in *Avsunviroidae*

Detection of Viroids:

- Rapid and reliable detection followed by eradication of all infested material at a very early stage of infection is the only way to control the spread of viroids.

- Detection methods:
  - PAGE
  - Nucleic acid hybridization
  - RT-PCR
  - Deep sequencing of low mol. wt. RNAs and siRNAs (ecogenomics)

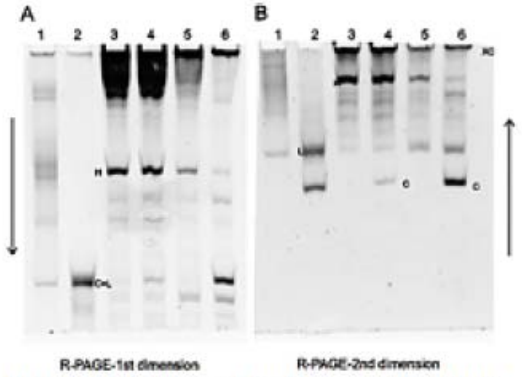


Fig. 4. Detection of *Potato spindle tuber viroid* by return polyacrylamide electrophoresis. (A) first dimension (non-denaturing) and (B) second dimension (denaturing). C, L - circular and linear forms of the viroid molecule; H - host RNA; XC-xylene cyanol marker dye.

(Helpful) Summaries

Summary of Subviral Agents

Replication dependent on a host virus

- **A. Satellite Viruses**
- **B. Virus-Dependent Nucleic Acids**

Genomes are sign. different from genome of helper virus

- **C. Defective Interfering RNAs/DNAs**

Genomes are nearly identical to helper virus genome

Replication Is independent of a host virus

- **D. Viroids**

Summary of Types of Subviral Agents

A. Satellite Viruses:

Often found as distinct nucleoprotein components in preparations of particles of helper viruses

- Have ssRNA plus sense genomes
- Have nucleotide sequences substantially distinct from those of the genomes of their helper virus or hosts
- Code for a coat protein (one structural protein that encapsidates the satellite genome)
- Coat protein is antigenically distinct from helper virus.
- May have a virion structure that is distinct from helper virus

Summary of Types of Subviral Agents

B. Virus-Dependent Nucleic Acids:

- May or may not encode non-structural proteins but do not encode a coat protein;
- May be linear or circular, may be RNA or DNA
- Are encapsidated by the helper virus's coat protein;
- Have nucleotide sequences substantially distinct from those of the genomes of their helper virus or hosts.
- Particles containing satellite nucleic acid genome are antigenically identical to that of the helper virus and may only be distinguished by differences in sedimentation rates (due to difference in genome sizes of satellite and helper)

C. Defective interfering (DI) RNAs/DNAs:

- Dependent on helper virus for replication
- Do not code for a protein;
- Sequence is derived from their helper virus;
- Are deletion and/or rearrangement derivatives of the helper virus
- Have significant sequence homology with their helper virus.

D. Viroids:

- Do not require a helper virus, do replicate independently
- Are low-molecular-weight, covalently closed, single-stranded, circular RNA
- Are not encapsidated by any coat protein
- Do not code for any proteins

Summary of Differences between the Two Families of Viroids