



# Viroids and Virusoids

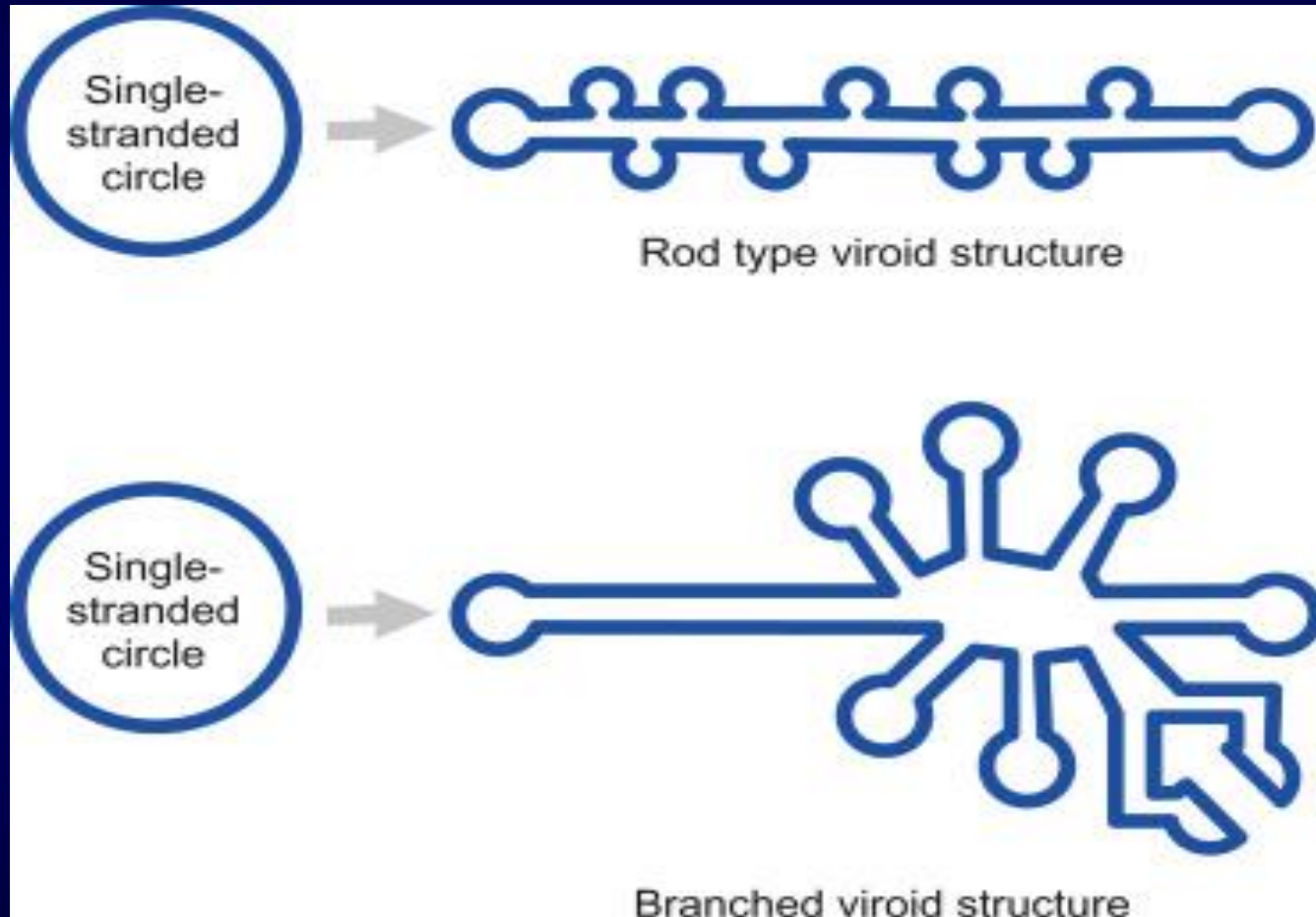
## MSc Microbiology

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# Viroids

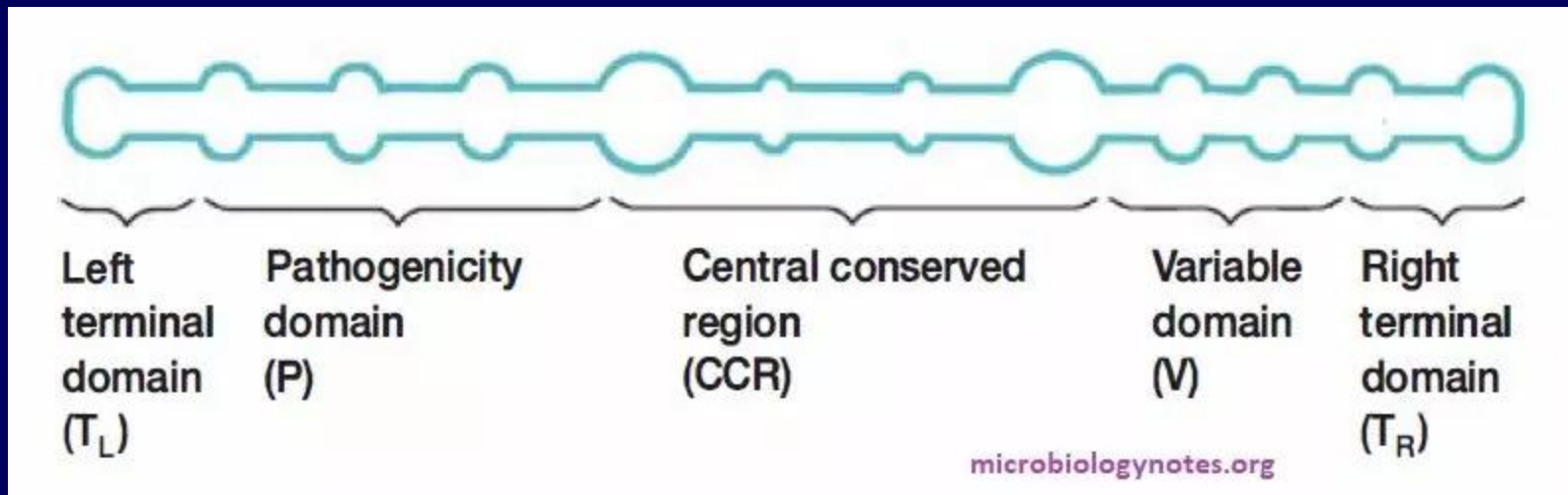
- 1971. Ted Deiner- Potato Spindle Tuber Viroid (PSTVd)
- Very small, covalently closed, circular RNA molecules capable of **autonomous replication** and induction of disease
- Sizes range from 250-450 nucleotides
- High GC content, low MW
- Circularized with secondary structures
- **No coding capacity** - do not program their own polymerase
- Use **host-encoded** polymerase for replication
- Mechanically transmitted; often seed transmitted
- More than 40 viroid species and many variants have been characterized
- “Classical” viroids have been found only in plants
- Some code for ribozymes

# Structure



- <https://www.sciencedirect.com/topics/neuroscience/viroids>

# Viroid



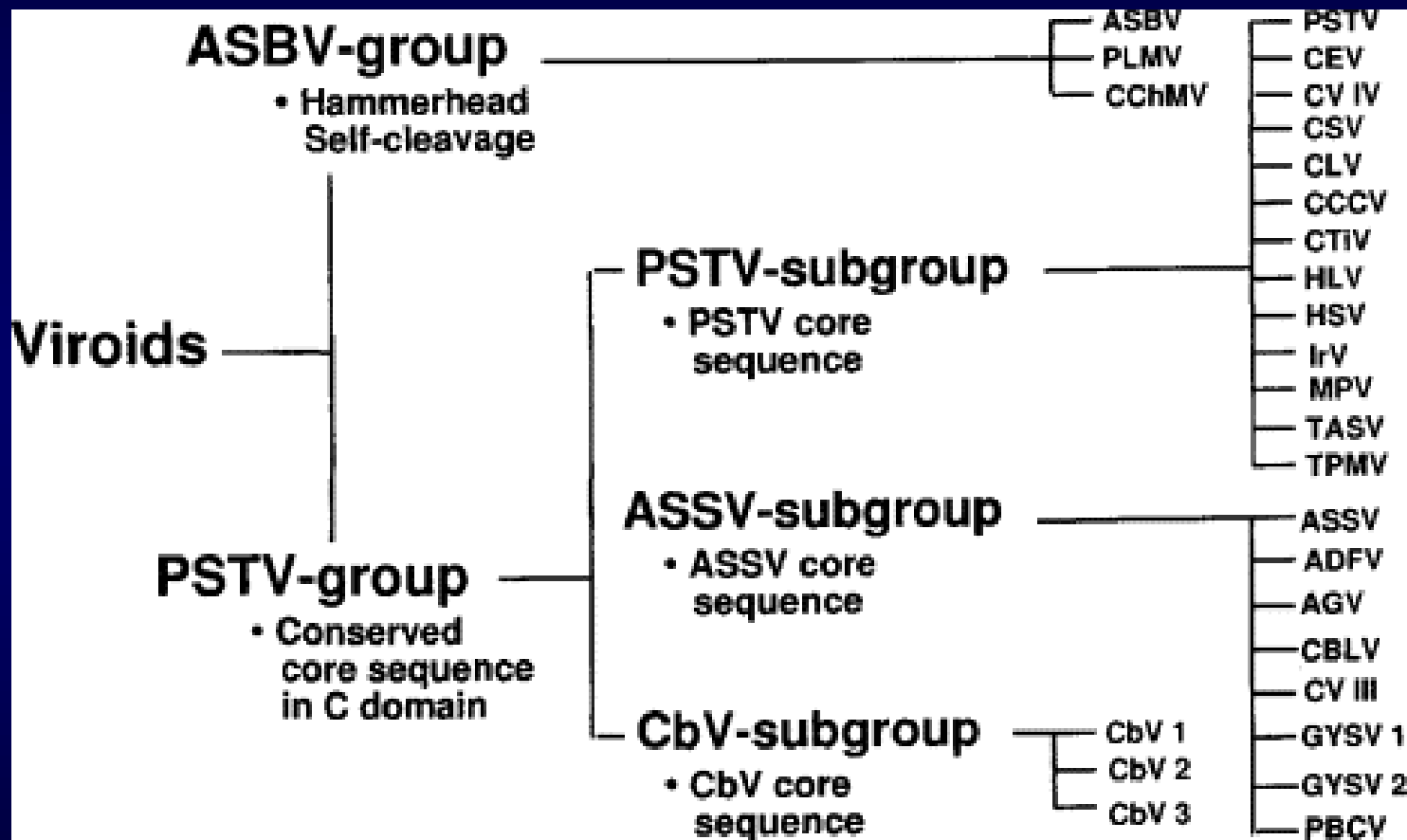
- <https://www.sciencedirect.com/topics/neuroscience/viroids>

# Classification

## 2 Groups

- Pospiviroidae (type species PSTVd)
  - Potato Spindle Tuber Viroid
  - Replicate in nucleus-host RNA pol II
  - Rod like structures
  - Asymmetric rolling circle model
- Avsunviroidae (type species ASBVd)
  - Avocado Sun Blotch Viroid
  - Replicate in chloroplast
  - Diverse
  - Branched structures
  - Symmetric Rolling Circle Model
  - Hammerhead self cleavage ribozyme

# Classification



- <https://www.sciencedirect.com/topics/neuroscience/viroids>

# Viroids are divided into two groups, based on site and details of replication

## Compilation of viroids

RNA species	Abbreviation	Number of sequences	RNA species	Abbreviation	Number of sequences
<b>Viroids</b>					
<b>ASBVd-type (group A)</b>					
<u>Avocado sunblotch viroid</u>	ASBVd	19			
<u>Peach latent mosaic viroid</u>	PLMVd	6			
<b>PSTVd-type (group B)</b>					
<b>PSTVd group (subgroup B1)</b>					
<u>Coconut cadang-cadang viroid</u>	CCCVd	7			
<u>Citrus exocortis viroid</u>	CEVd	33			
<u>Columnnea latent viroid</u>	CLVd	3			
<u>Chrysanthemum stunt viroid</u>	CSVd	4			
<u>Coconut tinangaia viroid</u>	CTiVd	2			
<u>Citrus viroid species</u>	CVdIV	1			
<u>Hop latent viroid</u>	HLVd	1			
<u>Hop stunt viroid</u>	HSVd	32			
<u>Iresine viroid</u>	IRVd	1			
<u>Mexican papita viroid</u>	MPVd	9			
<u>Potato spindle tuber viroid</u>	PSTVd	29			
<u>Tomato apical stunt viroid</u>	TASVd	3			
<u>Tomato planta macho viroid</u>	TPMVd	1			
<b>PSTVd-type (group B)</b>					
<b>ASSVd group (subgroup B2)</b>					
<u>Australian grapevine viroid</u>	AGVd	1			
<u>Apple dimple fruit viroid</u>	ADFVd	1			
<u>Apple scar skin viroid</u>	ASSVd	4			
<u>Citrus bent leaf viroid</u>	CBLVd	3			
<u>Citrus viroid species III</u>	Cvd-III	2			
<u>Coleus blumei viroid I</u>	CoVd	9			
<u>Grapevine yellow speckle viroid</u>	GYSVd	34			
<u>Grapevine 1B viroid</u>	G1BVd	1			
<u>Pear blister canker viroid</u>	PBCVd	1			

- <https://www.sciencedirect.com/topics/neuroscience/viroids>

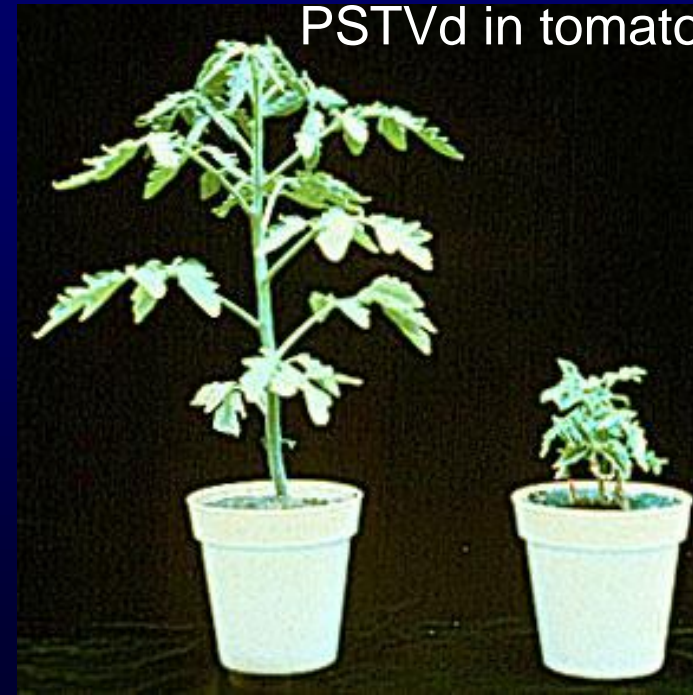
# Viroid Diseases

PSTVd in potato

- *Potato spindle tuber viroid (PSTVd)*
  - May be limiting to potato growers
  - First viroid characterized
  - Many variants described
  - Control with detection in mother stock, clean seed



PSTVd in tomato





# Viroid Diseases

- *Citrus exocortis viroid* (CEVd)
  - Causes stunting of plants, shelling of bark
  - May result in little yield loss
  - May be useful to promote dwarfing for agronomic advantage
  - Transmitted through stock, graft
  - Control by removal of infected plants, detection, clean stock



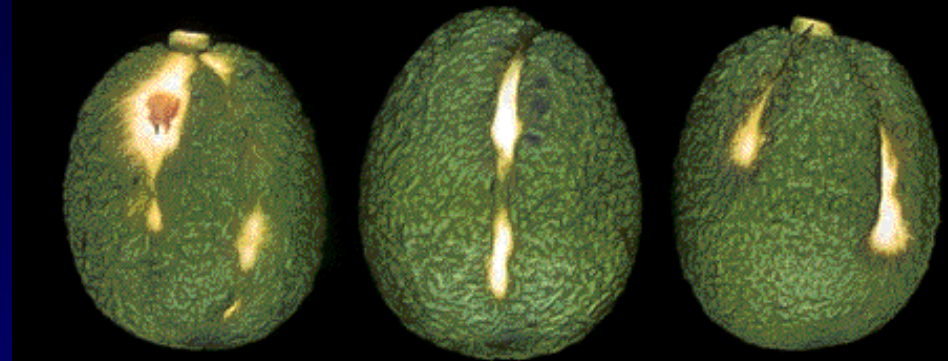
Citrus exocortis viroid



Healthy

Infected

Apple crinkle fruit viroid



Avocado sun blotch viroid

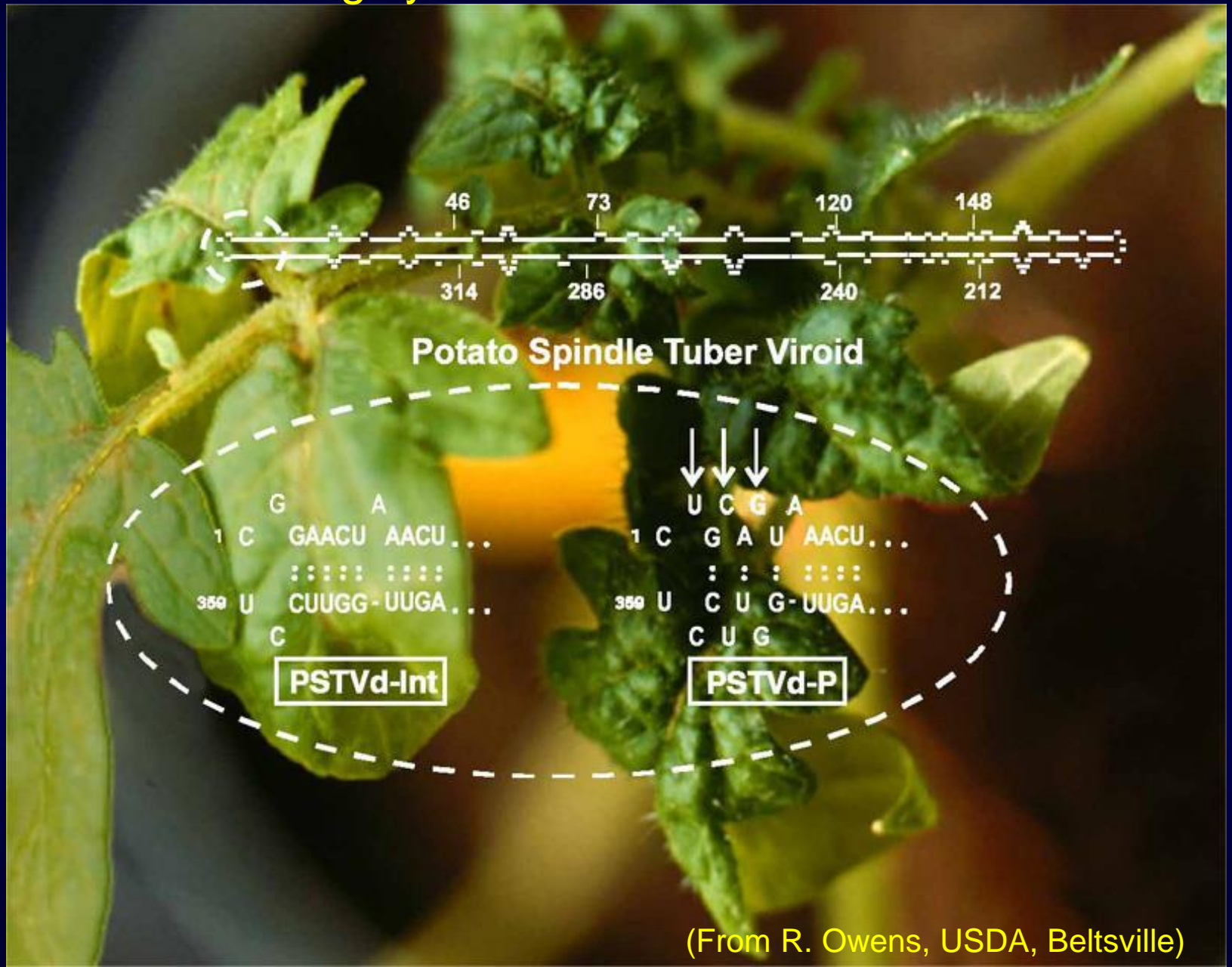


UC Statewide IPM Project  
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Citrus exocortis viroid

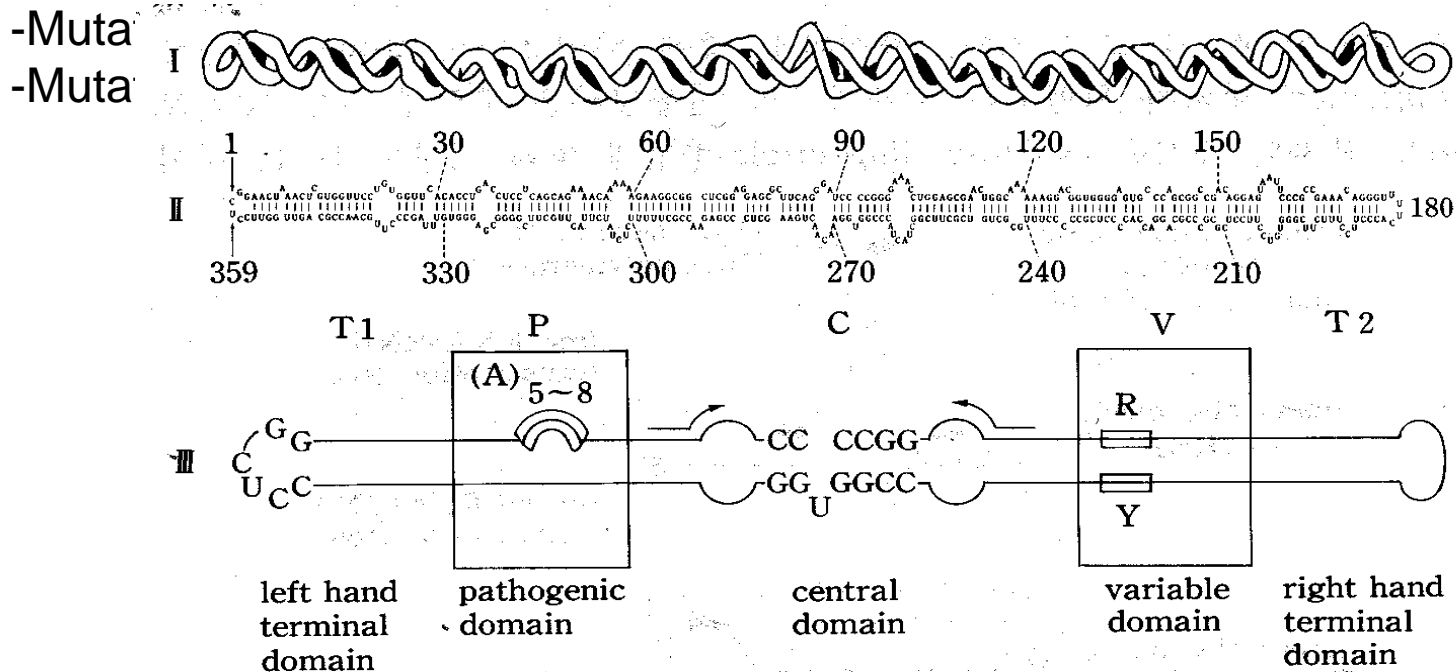
# Potato spindle tuber viroid (PSTVd) is the most thoroughly characterized viroid disease



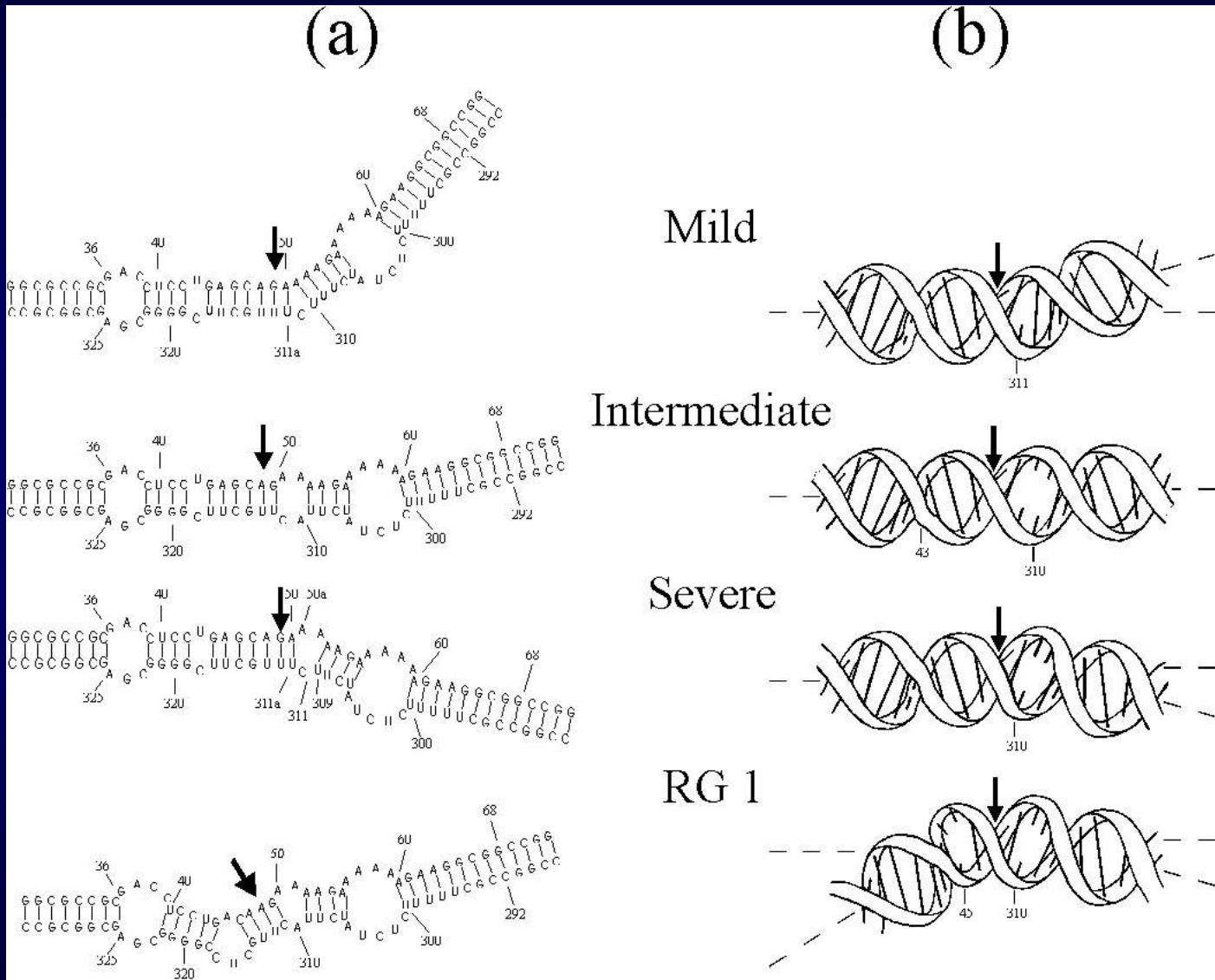
(From R. Owens, USDA, Beltsville)

# Viroid structures

- All are covalently closed circular RNAs fold to tightly base-paired structures
- Two main groups of viroids: self-cleaving and non-self-cleaving
- Non-self cleaving viroids replicate in nucleus and fold into “dog bone” or rod-like structure
- Five domains identifiable in non-self-cleaving
  - Left hand (LH) and right hand (RH) domains are non-base-paired loops
  - Single mutations to pathogenic domain often alter virulence

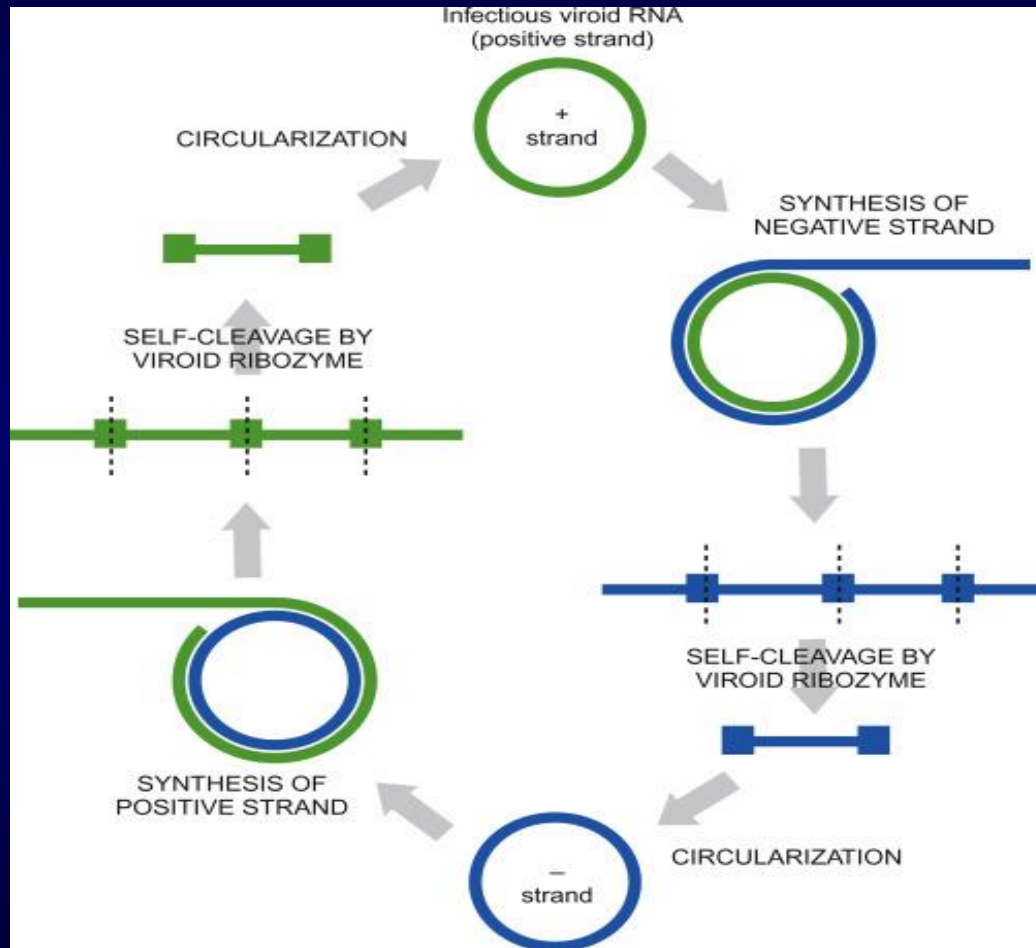


Minor variations in viroid sequence, and presumably attendant RNA structure changes, are associated with virulence differences



(From R. Owens, USDA, Beltsville)

# Viroid Replication-rolling circle model



Venkataraman, Srividhya & Badar, Uzma & Shoeb, Erum & Hashim, Ghya & Abouhaidar, Mounir & Hefferon, Kathleen. (2021). An Inside Look into Biological Miniatures: Molecular Mechanisms of Viroids. *International Journal of Molecular Sciences*. 22. 2795. 10.3390/ijms22062795.

# Rolling Circle Model

The viroids own ribozyme activity is used for self-cleavage of the multimeric RNA generated during replication. Host enzymes provide all other functions.

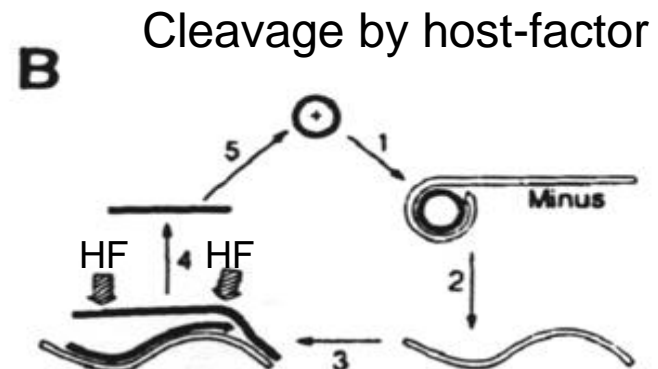
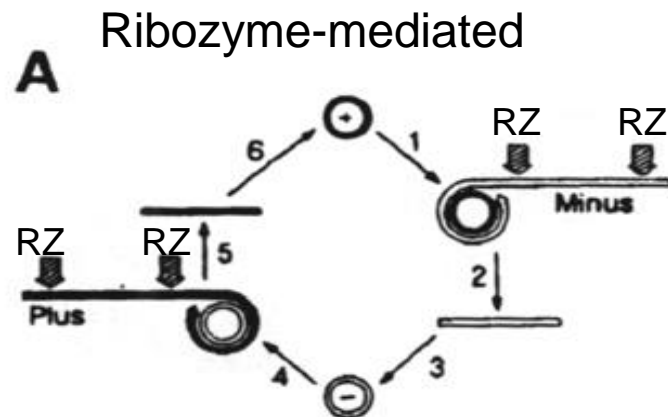
First, host RNA polymerase copies the circular plus strand to form a multimeric minus strand. Site-specific cleavage of this strand by the viroid ribozyme gives monomers that are circularized by a host RNA ligase.

The minus-stranded circles are the templates for a second round of rolling circle replication by RNA polymerase. The resulting multimeric plus strand undergoes ribozyme cleavage to create monomers.

These are circularized to produce the progeny viroids (circular, positive single-stranded RNA).

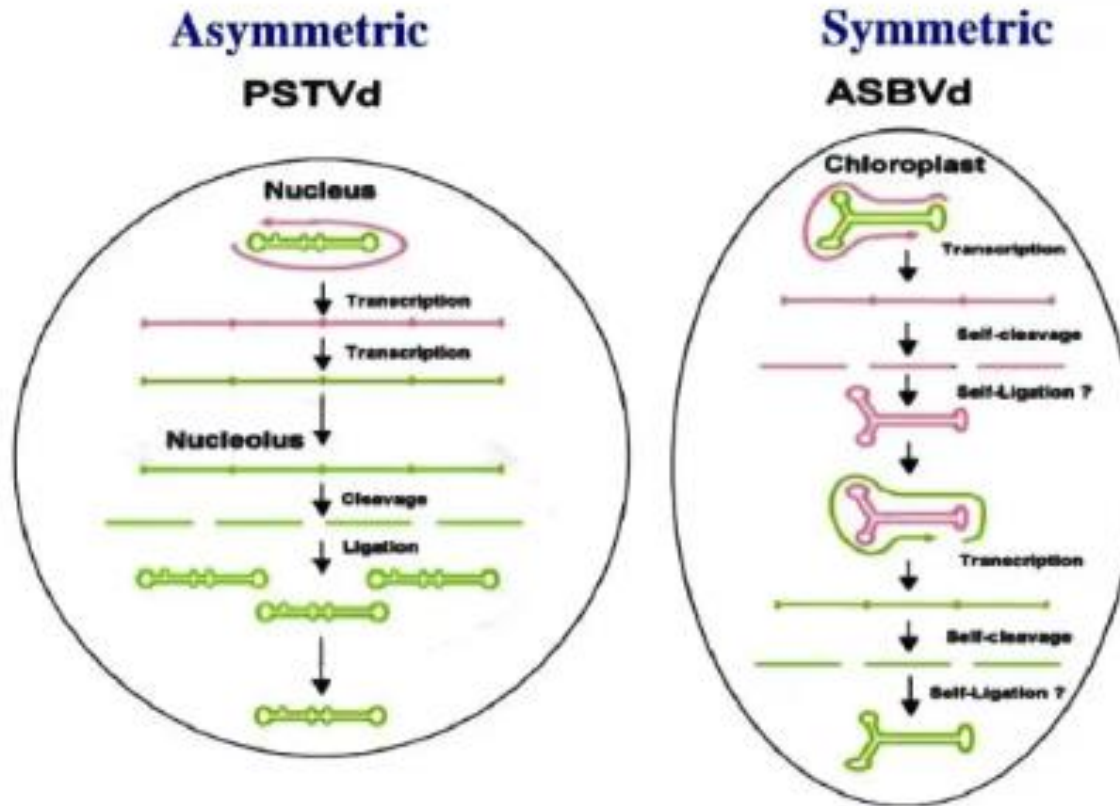
# Viroid replication

- In nucleus or chloroplasts, depending on class of viroid
- Chloroplast-associated viroids process into monomers by ribozyme-mediated cleavage
- Nucleus-associated viroids process into monomers by using host-derived enzyme
- In both classes, host DNA-dependent RNA polymerase is the performs RNA polymerization on + and – strand RNA templates





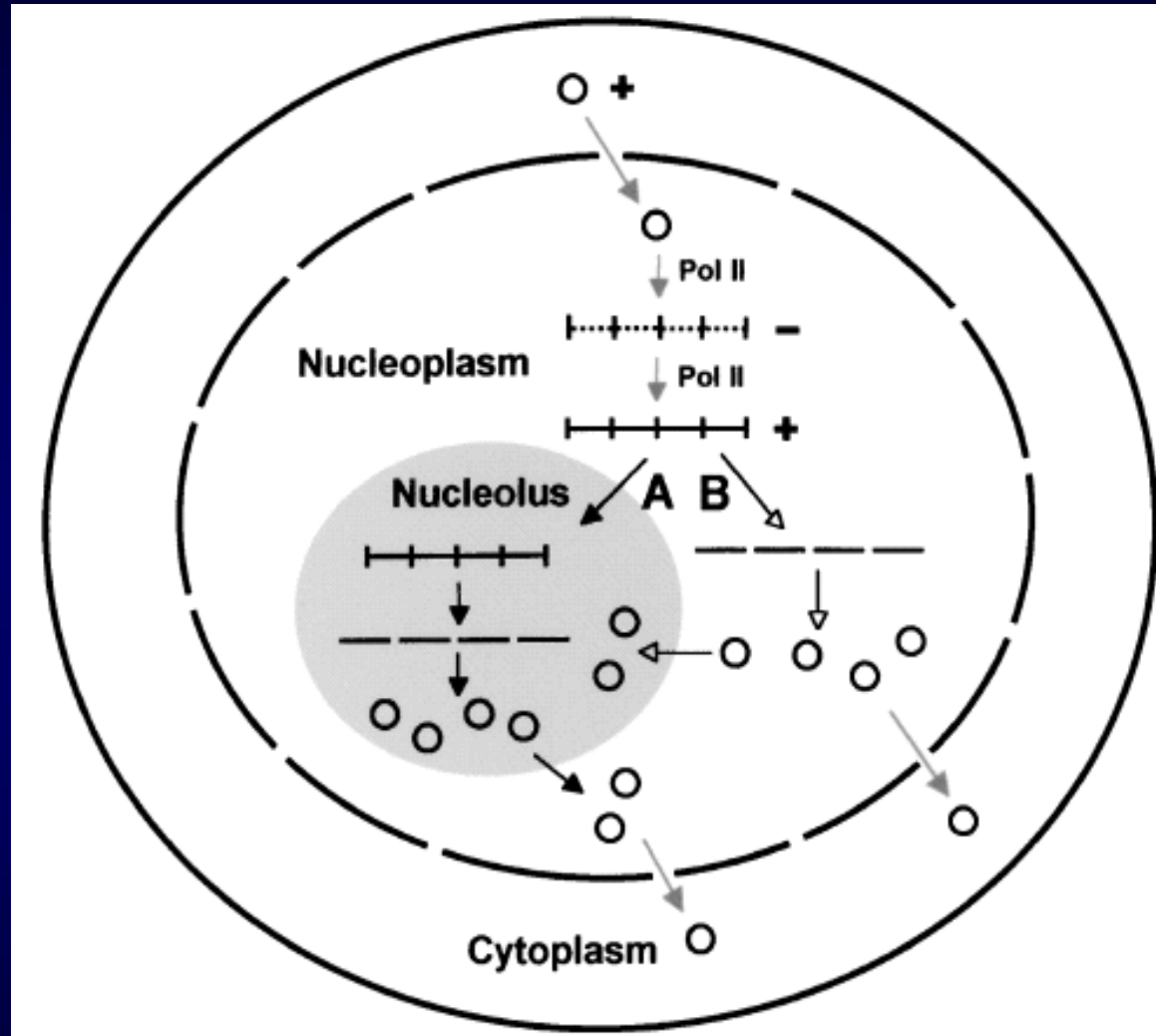
# Viroid Replication



Replication of Viroids

# Viroid movement

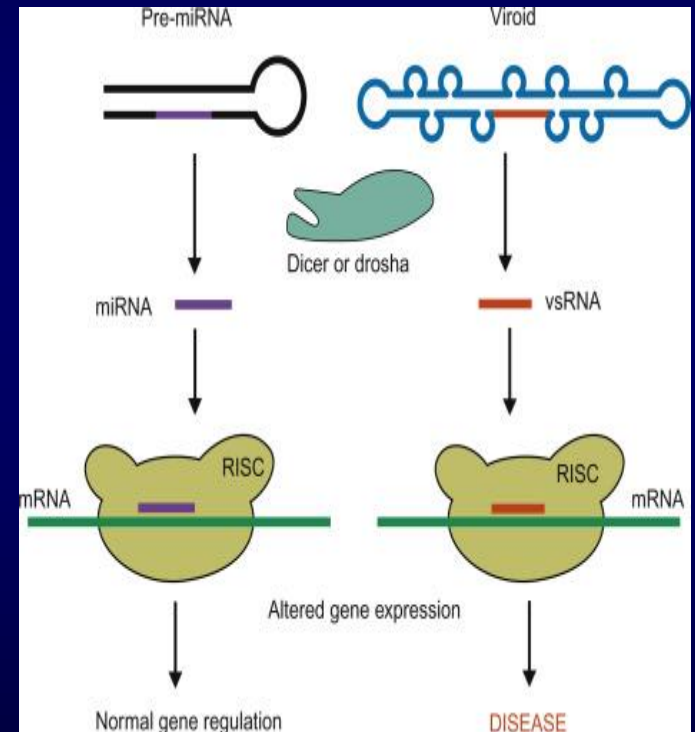
- Traffic within cell through nuclear pores using VirP1, a nuclear localization protein that binds viroid RNA
- Traffic cell-to-cell through plasmodesmata
- Traffic long distance through phloem
- All of these processes are associated with host proteins



**Figure 5.** Model of PSTVd Replication Featuring Intranuclear Trafficking of the (+)-Strand PSTVd.

# Cause for Disease- trigger RNAi

Viroid and/or its replicative intermediates trigger RNA interference. This results in the production of short single-stranded RNA molecules (known as vsRNA—viroid short RNA) of the same size as the microRNAs widely used by plant cells to regulate genes. This decreases expression of multiple plant genes, hence causing disease.



# Virus associated Satellite RNA

	Viroid-like satRNA	Linear satRNA	Viroid
<b>Host</b>	Plants	Plants	Plants
<b>Helper Virus</b>	eg. SNMV	eg. CMV	None
<b>Genome</b>	ssRNA	ssRNA	ssRNA
<b>Replication</b>	HV replicase	HV replicase	Cellular polymerase
<b>Site of replication</b>	Cytoplasm	Nucleus/cytoplasm	Nucleus
<b>Coding capacity</b>	None	None	None
<b>Encapsidation</b>	HV CP	HV CP	None

HV, helper virus; SNMV, Solanum nodiflorum mottle virus; CMV, Cucumber mosaic virus; ssRNA, single-stranded RNA; CP, capsid protein. Rao et al, 2015 Virology 479:627

# Virusoids

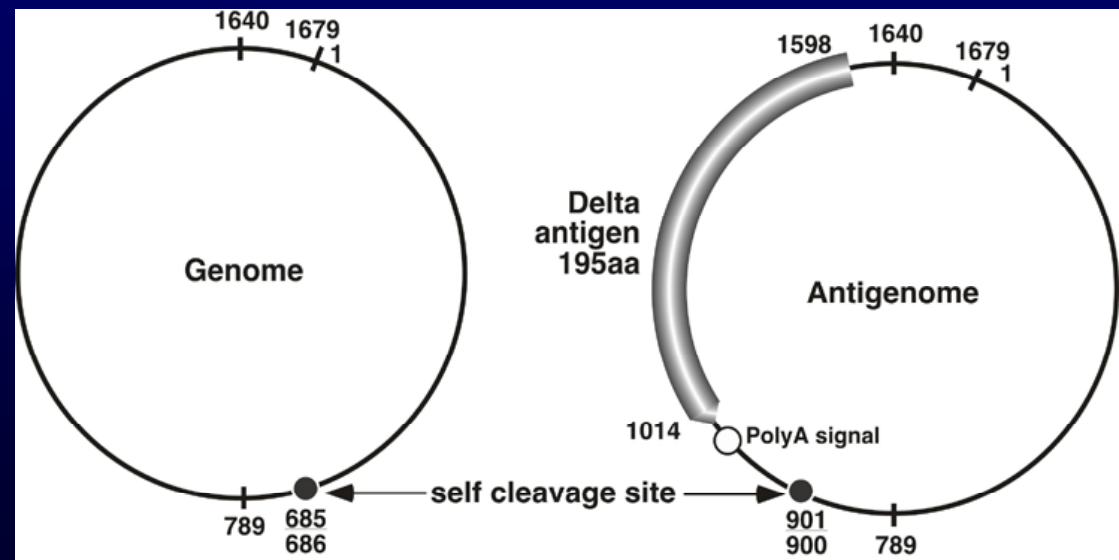
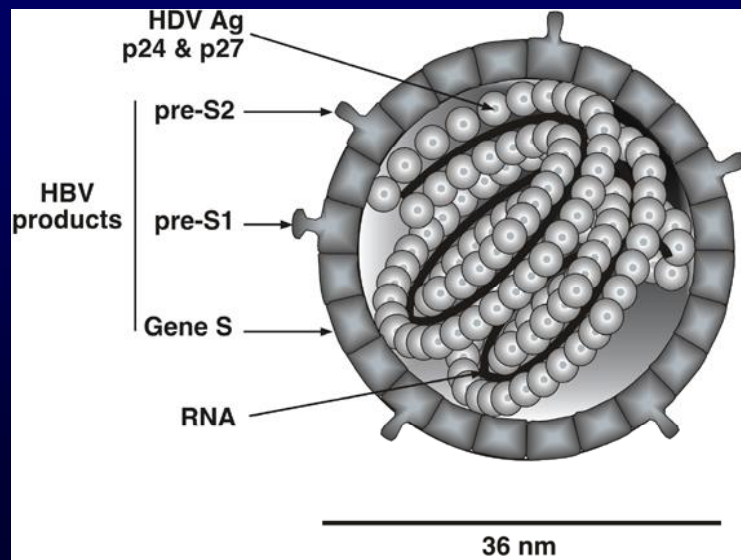
- 1981. W Randles described virusoids
- Formerly called Satellite viruses
- circular single-stranded RNA dependent on viruses for replication and encapsidation.
- 200-338 nt
- The genome of virusoids consist of several hundred nucleotides and does not code for any proteins. Some ORF have been identified

# Virusoids

- Encapsidated by protein coat of host virus
- Replication: Host cytoplasm
- Barley yellow dwarf virus satellite RNA is another virusoid, and its helper virus is Luleovirus
- Tobacco ringspot virus satellite RNA and its helper virus Nepovirus

# Hepatitis delta

- *Hepatitis delta virus* has many viroid-like properties, but the RNA is larger (1.7 kb), is encapsidated, and encodes a virion-associated protein (hepatitis delta antigen)
- Intensifies HBV infection
- HDV requires HBV as helper virus for encapsidation, so it has satellite-like properties (like a “virusoid”)
- Replicates in nucleus via cellular DNA-dependent RNA polymerase II



# Uses

- Rice yellow mottle virus small circular satellite RNA (scRYMV)- model for molecular biology studies
- Bamboo mosaic virus satellite RNA (satBaMV),<sup>[32]</sup> which possesses an open reading frame that encodes a 20-kDa P20 protein. It was observed that when this nonessential ORF region was replaced with a foreign gene, expression of the foreign gene was enhanced or overexpressed
- Delivery for RNAi/ gene expression studies/ cargo carrier