

# Discovery of Viroids

Potato spindle tuber and at least 15 other crop diseases are caused by viroids, an entity that nobody had ever heard of before 1971, its official date of discovery. Theodor O. Diener, the Agricultural Research Service plant pathologist who discovered the pathogen, named it the “viroid,” because it is “like a virus.”

Like a virus, the viroid invades a cell and hijacks its reproductive mechanisms. It forces the cell to duplicate the viroid's RNA instead of its own. The viroid has no DNA. RNA and DNA are nucleic acids, the molecules of heredity; with the exception of viroids and some viruses, all genes are made of DNA.

The difference between viroids and RNA viruses is that viroids have no protective protein coat. The scientific dogma in 1971 was that an organism with no protein wasn't supposed to be able to replicate itself, even with a host cell's help. And an entity as small as the PSTV (potato spindle tuber viroid)—130,000 daltons—wasn't supposed to be able to infect anything, even a potato.

# The International Committee on Taxonomy of Viruses



## Virology Division

International Union of Microbiological Societies

### *Viroids Classification*

#### **Taxonomic Structure of the Family**

Family	80.001. <i>Pospiviroidae</i>
Genus	80.001.0.01. <i>Pospiviroid</i>
Genus	80.001.0.02. <i>Hostuviroid</i>
Genus	80.001.0.03. <i>Cocadviroid</i>
Genus	80.001.0.04. <i>Apscaviroid</i>
Genus	80.001.0.05. <i>Coleviroid</i>
Family	80.002. <i>Avsunviroidae</i>
Genus	80.002.0.01. <i>Avsunviroid</i>
Genus	80.002.0.02. <i>Pelamoviroid</i>

[http://www.ictvdb.iacr.ac.uk/Ictv/fs\\_viroi.htm](http://www.ictvdb.iacr.ac.uk/Ictv/fs_viroi.htm)

*Viroids* are unencapsidated, small, circular, single-stranded RNAs which replicate autonomously when inoculated into host plants. Some are pathogenic, others replicate without eliciting symptoms.

# Completed Genome of Viroids

No.	Description	Seqlength (nt)	Genome	Proteins
1	Apple dimple fruit viroid	306	<a href="#">X99487</a>	<a href="#">SP-astA</a>
2	Apple scar skin viroid	329	<a href="#">X17696</a>	<a href="#">SP-astA</a>
3	Avocado sunblotch viroid	247	<a href="#">X13000</a>	<a href="#">SP-astA</a>
4	Chrysanthemum chlorotic mottle viroid	399	<a href="#">AJ247112</a>	<a href="#">SP-astA</a>
5	Chrysanthemum stunt viroid	354	<a href="#">AJ000046</a>	<a href="#">SP-astA</a>
6	Citrus bent leaf viroid	315	<a href="#">U21125</a>	<a href="#">SP-astA</a>
7	Citrus exocortis viroid	370	<a href="#">AJ490825</a>	<a href="#">SP-astA</a>
8	Citrus exocortis viroid (CEV-g)	369	<a href="#">Y00328</a>	<a href="#">SP-astA</a>
9	Citrus viroid IIIb	296	<a href="#">AF184148</a>	<a href="#">SP-astA</a>
10	Citrus viroid IIIc	291	<a href="#">AF184149</a>	<a href="#">SP-astA</a>
11	Citrus viroid IIa	299	<a href="#">X69518</a>	<a href="#">SP-astA</a>
12	Citrus viroid IIb	302	<a href="#">X69519</a>	<a href="#">SP-astA</a>
13	Citrus viroid IV	284	<a href="#">X14638</a>	<a href="#">SP-astA</a>
14	Citrus viroid Ia	326	<a href="#">AF040721</a>	<a href="#">SP-astA</a>
15	Coconut cadang-cadang viroid RNA 1 fast	246	<a href="#">J02050</a>	<a href="#">SP-astA</a>
16	Coconut cadang-cadang viroid RNA 1 slow	287	<a href="#">J02051</a>	<a href="#">SP-astA</a>
17	Coconut tinangaja viroid	254	<a href="#">M20731</a>	<a href="#">SP-astA</a>
18	Coleus blumei viroid	248	<a href="#">X52960</a>	<a href="#">SP-astA</a>
19	Coleus yellow viroid	248	<a href="#">X69293</a>	<a href="#">SP-astA</a>
20	Columnnea latent viroid	370	<a href="#">X15663</a>	<a href="#">SP-astA</a>
21	Cucumber pale fruit viroid	301	<a href="#">X07405</a>	<a href="#">SP-astA</a>
22	Dapple apple viroid	331	<a href="#">X71599</a>	<a href="#">SP-astA</a>
23	Dapple peach fruit disease viroid	297	<a href="#">D13765</a>	<a href="#">SP-astA</a>
24	Dapple plum and peach fruit disease viroid	297	<a href="#">D13764</a>	<a href="#">SP-astA</a>
25	Grapevine viroid grapevine isolate	296	<a href="#">M35717</a>	<a href="#">SP-astA</a>
26	Grapevine yellow speckle viroid 1	368	<a href="#">Z17225</a>	<a href="#">SP-astA</a>
27	Hop stunt viroid	297	<a href="#">Y09345</a>	<a href="#">SP-astA</a>
28	Hop stunt viroid citrus isolate (HSV-cit)	302	<a href="#">X06719</a>	<a href="#">SP-astA</a>
29	Hop stunt viroid grapevine variant	298	<a href="#">X87928</a>	<a href="#">SP-astA</a>
30	Hop stunt viroid strain yellow corky vein	295	<a href="#">AJ490824</a>	<a href="#">SP-astA</a>
31	Iresine viroid 1	370	<a href="#">X95734</a>	<a href="#">SP-astA</a>
32	Peach latent mosaic viroid	337	<a href="#">AF170519</a>	<a href="#">SP-astA</a>
33	Pear blister canker viroid PBCVd	315	<a href="#">Y18035</a>	<a href="#">SP-astA</a>
34	Potato spindle tuber viroid strain I-818	359	<a href="#">X76847</a>	<a href="#">SP-astA</a>
35	Potato spindle tuber viroid strain PSTVd-NT	359	<a href="#">X97387</a>	<a href="#">SP-astA</a>
36	Potato spindle tuber viroid strain S27-I-8	359	<a href="#">Y09381</a>	<a href="#">SP-astA</a>
37	Rice yellow mottle virus satellite	220	<a href="#">AF039909</a>	<a href="#">SP-astA</a>
38	Tomato apical stunt viroid	363	<a href="#">X06390</a>	<a href="#">SP-astA</a>
39	Tomato chlorotic dwarf viroid	360	<a href="#">AF162131</a>	<a href="#">SP-astA</a>
40	Tomato planta macho viroid	360	<a href="#">K00817</a>	<a href="#">SP-astA</a>

Viroids are catalytic RNAs that cleave RNA to produce fragments containing a 5' hydroxyl and 2'3' cyclic phosphates.

Differences between viroids and viruses.

1. Viroids exist in vivo as non-encapsidated, low molecular weight RNAs.
2. Infected tissues do not contain virus-like particles
3. Only a single species of low molecular weight RNA is required for infectivity.
4. Viroids do not code for any protein
5. Despite their small size viroids are replicated autonomously in susceptible cells.

Thus these are not viruses.

**Group A:** e.g. avocado sunblotch viroid, peach latent mosaic viroid

**Group B:** Subgroup B1: potato spindle tuber viroid, coconut cadang cadang viroid, tomato plant macho viroid

Subgroup B2: citrus bent leaf viroid, pear blister canker viroid

The RNA genomes of viroids are 246-375 nucleotides in length and share many similarities:

- They are all single stranded covalent circles
- There is extensive intramolecular base pairing
- A DNA-directed RNA polymerase makes both plus and minus strands
- Replication does not depend on the presence of a helper virus
- No proteins are encoded

Group A viroids probably replicate via a symmetric rolling circle mechanism, whereas Group B viroids probably use an asymmetric mechanism.

In this case the +ve infecting circular RNA strand of a viroid serves as a template to make a large linear multimeric -ve strand. RNA pol II is probably the enzyme which does this.

Group B viroids with an asymmetric replication pathway then make +ve RNA from this long linear molecule. A host RNase activity cleaves the +ve strand into unit viroid lengths. This molecule is then ligated to form a circular viroid.

In Group A viroid replication the long -ve RNA is self cleaved by the associated ribozyme activity. The RNA circularizes to form a -ve circle. A second rolling circle event makes a long linear +ve strand which is again cleaved by the ribozyme activity. The short viroid RNA is then ligated to the circular form.

Group A viroids lack a central conserved region (CCR) and possess a ribozyme activity (a ribozyme is a catalytic RNA molecule, in this case RNA cleavage is the ribozyme activity).

Additionally it is speculated that Group A viroids may replicate in chloroplasts whereas

Group B viroids replicate in the nucleus and nucleolus. 3 enzymatic activities are required for viroid replication, an RNA polymerase, an RNase and an RNA ligase.

## Pathogenesis of Viroids

Probably there is more than one mechanism responsible for viroid pathogenesis. Recent evidence suggests that one pathway is due to viroid RNA activating a plant RNA activated protein kinase, or **PKR** (analogous to the PKR enzyme activated by viral RNAs in mammalian cells). Protein synthesis is reduced and this causes pathogenic effects. In the case of potato spindle tuber viroid, there is a good correlation between a strains pathogenicity and its ability to activate PKR in vitro.

## Virusoids and Satellites

Virusoids or satellite RNAs are also several hundred nucleotides long circular and single stranded. They depend on a helper virus for replication. This helper virus also encapsidates them, e.g:

- Barley yellow dwarf virus satellite RNA: Helper - *Luteovirus*
- Tobacco ringspot virus satellite RNA: Helper - *Nepovirus*
- Subterranean clover mottle virus satellite RNA: Helper - *Sobemovirus*

Virusoids replicate in the cytoplasm using an RNA-dependent RNA polymerase. This enzymatic activity is common in plants but not found in animal cells.

## Hepatitis delta virus (HDV)

There are similar infectious agents which infect animals, e.g. newt satellite 2 transcript. One such agent infecting humans is the **HDV**.

HDV was first identified in the 1970s in Australia as a nuclear antigen, the delta antigen. Subsequently, it was found to be the cause of a particularly virulent form of hepatitis known as type D hepatitis.

Common in indigenous natives of S.America, method of transmission not understood. Can be transmitted perinatally. In the West, transmission is associated with drug abuse and transfusion of blood products. It seems prudent to assume that it can also be transmitted sexually. No specific treatment.

The delta antigen is associated with a defective pathogen which is obligatorily associated with Hepatitis B helper virus:

This virus has a circular single stranded RNA genome of about 1700 nucleotides. It has a ribozyme (RNA cleavage) activity. This is the smallest known genome for an animal virus.

RNA and delta antigen (195 AA) are packaged in a Hepatitis B particle. No DNA intermediate has been detected during the replication phase and it is thought that replication occurs by RNA directed RNA synthesis using a DNA dependent RNA polymerase. Certain parts of the genome and the pattern of replication is of course similar to a viroid. One difference is that mRNA and a protein - the delta antigen - are made.

The encapsidated RNA is (-)ve sense strand so (+)strand RNA synthesis to make the mRNA is required. These RNAs are nuclear associated and in any case circular RNAs are not good templates for protein synthesis. About 10% of the antigenomic strand is cleaved and polyadenylated and serves as a 800nt mRNA. RNase activities tend to be exonucleolytic rather than endonucleolytic. The fact that the genomic strands are circular probably contributes to the agents stability.

The delta antigen is a 22kd nuclear phosphoprotein essential for replication and particle formation. It is basic and associates specifically with the RNA genome thereby stabilizing it. Two forms are made differing by 19 amino acids at the C-terminus. The large form is a dominant inhibitor of genome regulation and directs genome packaging into Hep B viral particles. This packaging is due to farnesylation of a Cys residue 4 AA from the C-terminus. Recently a host protein interacting with the delta antigen has been identified. In fact sequence similarities suggest it is a cellular homologue of the delta antigen. This suggests that it may be able to modulate viral replication. It may also suggest that HDV originated from a viroid like element which then "captured" a cellular transcript. Probably the genomic strand transferred onto the mRNA for the cell homologue of the delta antigen. This was copied into the antigenomic strand and stabilize as part of the genome.

How do they replicate? Which enzyme system do they use?

DNA probe has been made, and search is now on for sequences in uninfected plant, infected plant etc.

The site of synthesis in the cell is also unknown. In the case of PSTV it has been shown that the nucleolus is the site of accumulation but the actual site of synthesis unknown.

Sequence analysis has revealed sequence similarities to introns in certain classes of plant cell genes, namely introns of mitochondrial and ribosomal RNAs. These similarities consist of short conserved sequences which place constraints on secondary and tertiary structure of all these RNAs.

Some of these introns are known to be self-splicing, that is the parental RNA acts as a catalyst to promote excision of a circular offspring intron RNA and a shortened linear RNA. Diener has proposed that viroids and virusoids may represent plant gene introns which have escaped from their genes during evolution. However more likely to be similarity in structure that gives rise to similar mechanisms of processing.

The viroids have been cloned into m13 and their DNA is equally infective. Single changes in nucleotide sequences can either enhance or lessen their pathogenicity. Most of the observed changes are located in one or two specific areas of the viroids structure, termed the virulence or pathogenicity-modulating domains.

Further analysis shows remarkable similarity between end of transposable element and retroviruses and viroids.

All contain uninterrupted stretch of 11-18 purines, and centrally conserved region. Center bordered by almost perfect repeat. Similar to copia, etc.

Could viroids be derived from retroviruses that have lost a large component part or could they be moveable genetic elements-transposons?

# SATELLITE RNAS AND VIRUSOIDS

DI particles are encapsidated in viral coats, and are characterized as being part of the viral genome. Require infection with the homologous virus to become infected. Also find similar situation with plant viruses, in which the DI may code for coat protein RNA.

**Satellite viruses.** Small virus is dependant for its replication on co-infection with an unrelated ,yet specific,helper virus that provides the replicase. There is no sequence similarity between the helper virus and the satellite virus. Example is tobacco necrosis satellite virus. Two viruses, tobacco necrosis virus and its satellite, STNV These are two unrelated viruses in terms of coat protein and nucleic acid structure..TNV is a typical infectious virus that carries all its needed information in its single RNA molecule. STNV is non-infectious on its own, however it is a small icosahedral particle, made up of 60 protein molecules. It contains an RNA of  $.4 \times 10^6$  daltons that appears to be monocistronic RNA coding only for its coat protein. All of the satellite viruses carry information for the coat protein.

STNV is an obligate parasite of a TNV. This dependence is specific, since no other virus can be substituted. TNV is a typical icosahedron plant virus. TNV normally infects plant roots. The STNV contains only 1239nt and acts as a monocistronic mRNA for the synthesis of its 22kD coat protein.

Another example of a satellite virus is satellite tobacco mosaic virus, which is somewhat smaller and contains two ORFs. One of these is the capsid protein, the other unknown function.

A number of other satellite viruses have been discovered. Very much like the case of adeno-associated viruses in mammals. These viruses are dependent on the replication of adenovirus or herpes virus. Likewise delta hepatitis virus appears to be a satellite virus.. It is like a satellite RNA since it requires hepatitis virus for transmission although not for replication. It is circular RNA of about 1678 nucleotides. The agent appears to replicate like a viroid. However it does contain information for a protein antigen. It is thus more similar to a defective virus, or satellite RNA.

That STNV is a specific obligative parasite is illustrated by the inability of other viruses to act as helper, and the variation in the ability of certain TNV strains to support the replication of different strains of STNV.

## Satellite RNAs

as contrasted with satellite viruses, are molecules similar in size to viroids. These RNAs are always encapsidated in the coat of their helper viruses and are usually associated only with certain strains of these virus. These are known as virusoids.

First discovered in 1972 when a lethal necrotic disease of epidemic proportions was discovered among tomato plants in France. This virus was initially identified as cucumber mosaic virus.. The ability of CMV to cause necrosis in tomatoes was found to be related to the species used to grow the virus.

Among examples are the satellite RNAs of tobacco ring-spot virus, in which the virion pool is filled with varying number of these small RNAs. They are circular single stranded RNA's of 350 nucleotides and co-exist with virion RNA of 4500 nucleotides. Little if any sequence homology between satellite RNAs and RNA of helper virus. The satellite RNA lacks mRNA activity, and has highly based paired rod like structures.

Satellite RNAs have a great effect on the pathogenicity of the virus, in some cases increasing pathogenicity, in others decreasing. A good example is in the case of satellite RNA from Cucumber mosaic virus. Satellite RNA called CARNA 5. (Cucumber mosaic virus Associated RNA). Very deadly if infects tomato crop.

Important new findings with the satellite viruses has been

1.that it occurs in dimeric and multimeric forms in the virion

2.That these are spontaneously degraded to the monomeric form in vitro at a specific -ApG-of the minus strand,yielding an -A2'3' cyclic phosphate end and an HO-5'G- and

1.that such splits are autocatalytically ligated to monomeric cyclic intermediates.

Most satellite RNAs and some viroids (i.e. Avocado Sunblotch) are self cleaving, other viroids require a factor present in the cell nucleus. Ability to assume a hammer-head structure appears to be a prerequisite for cleavage of most satellite RNAs. The mechanism of cleavage is reminiscent of splicing mechanisms. Some splicing requires a factor, others do not.

Diener argues that results of phylogenetic studies of viroids and satellite RNA are consistent with a common origin. In this view viroids arose from satellite RNAs while still free living molecules and both acquired dependence on their hosts or helper virus only after becoming intracellular parasites.

Only relatively small portion of the satellite RNA sequences are required for self splicing.

Thus, deletion of RNA sequences from the 5' and 3' end of one satellite RNA is capable of self cleavage. A synthetic 19 mer can participate in cleavage reactions with a highly specific nucleotide fragment. Thus these RNAs act like ribozymes.

Diener suggests that viroids are precursors of introns, and are very primitive RNA molecules. Could have become fixed in the cell.

# Summary of differences among sub-viral particles

**Satellite viruses** are by nature defective, that is they do not have a related replication competent virus. These viruses are present in cells infected by another unrelated virus, which acts as helper.

Good example of this would be AAV which requires adenovirus as a helper, but the virus codes for its own unrelated coat protein. Other examples would be satellite necrosis virus of plants.

**Delta agent** is different from the above in that it codes for a delta antigen, but is surrounded by hepatitis B surface antigen. is packages in hepB coat protein.

**Viroids** are naked RNA, containing between 250-400 nucleotides. Resistant to most nucleases since have no free ends, and have tight secondary structure.

**Virusoids**, are satellite of certain plant viruses and are encapsidated with their helper RNAs in the virions. Structurally resemble viroids. Non- infectious and only replicate with viral helper. Have sequence domains similar to viroids and introns.

Satellite RNA needs helper virus for replication and are encapsidated in coat of helper virus. May code for two or three proteins enhance pathogenicity of the helper virus.