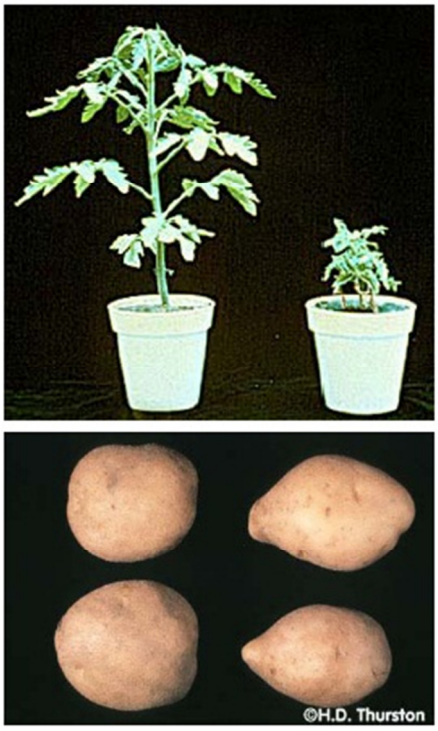
VIROIDS & VIRUSOIDS

Viroids

Viroids are small, circular RNA molecules that do not encode any protein. They do not code for proteins and thus depend on plant host enzymes for their replication and other functions. They can infect several crop plants and can cause diseases in plants of economic importance such as potato, tomato, hop, coconut, grapevine, citrus, avocado, peach, apple, pear, etc. It was first described in 1922 by Martin by working on spindle tuber disease of potato. He saw that the affected plants are smaller, their leaves are much narrower and pointed than typical leaves, and their tubers are long, narrow, smooth, skinned and have more eyes. For over 40 years, it was thought that the disease is caused by a plant virus. Later, Theodor O. Diener in 1971 in USA discovered that the infectious agent is 80 times smaller than a virus and is sensitive to treatment with ribonuclease but insensitive to treatment with deoxyribonuclease, phenol, chloroform, n-butanol, and ethanol. Because of these properties, it was concluded that the infectious agent is a short, free RNA molecules and designated as potato spindle tuber viroid disease (PSTVd). It was Diener, who coined the term "Viroid" after clarifying all physio-chemical parameters of this pathogen in order to differentiate from conventional viruses. Thereafter, several other viroids have been discovered. To date, over 30 different viroid species have been identified. Some of which are as follows; Potato Spindle Tuber Viroid (PSTVd), Citrus Exocortis Viroid (CEVd), Chrysanthemum Chlorotic Mottle Viroid (CChMVd), Hop Stunt Viroid (HSVd), Peach Latent Mosaic Viroid (PLMVd), etc.



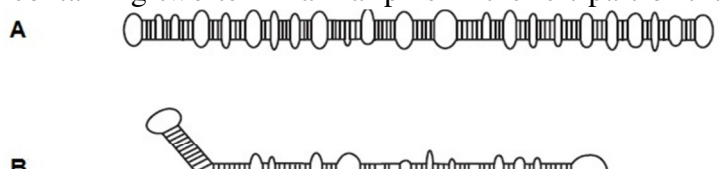
Classification of Viroids

The viroids are divided in two families named as *Avsunviroidae* and the *Pospiviroidae*. Members of the *Avsunviroidae* are able to catalyze self-cleavage of multimers produced during replication and do not possess a central conserved region (CCR), while members of the *Pospiviroidae* family don't have self-cleaving properties but possess a central conserved region (CCR). The species are primarily defined on the basis of sequence data. An arbitrary level of 90% sequence identity is accepted as separating species from variants, whereas the presence and type of CCR serve to define the genus.

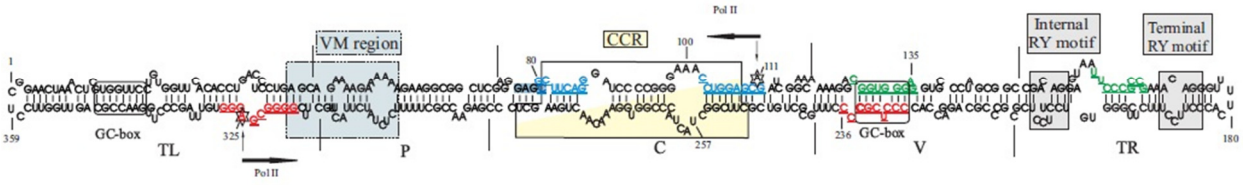
Structure of Viroids

The first viroid sequence determined was the sequence of the Potato Spindle Tuber Viroid (PSTVd) intermediate strain (PSTVd-DI). It is single stranded covalently-closed circular RNA molecule containing 359 nucleotides. By convention this RNA is referred to as (+) sense RNA. In general, viroids RNA varies from 250 to 400 nucleotides in number. Recently, it a model of a unique rod-like structure with a serial arrangement of double-helical section and small internal loops was proposed. This secondary structure was later proposed for most other *Pospiviroidae*. However, viroids of the *Avsunviroidae* family differ in this regards. Member of *Avsunviroidae*

family assumes quasi-rod-like structure, containing two terminal hairpins in the left part of the molecule. The Pospiviroidae viroids contain five structural domains such as TL (terminal left), P (pathogenicity), C (central), V (variable), and TR (terminal right) domains. The CCR within the C domain consists of conserved nucleotides (core sequences) located in the upper and lower strands which are crucial for the viroids replication and processing. Domain P is associated with symptom expression and is characterized by an oligo (A5-6) sequence present in all viroids of the PSTVd family. Domain V shows the highest sequence variability between closely related viroids. The only significant sequence relationship between viroids in the V domain appears to be the presence of an oligopurine**:**oligopyrimidine helix, usually with a minimum of three G:C pairs. Domains TR and TL are interchangeable between viroids; thus their role in RNA rearrangements during viroid evolution has been suggested. These domains may play a role in viroid movement in plants. Viroids of the *Avsunviroidae* family lack CCRs but contain sequences which can assemble into self-cleaving hammerhead structures. During thermal denaturation, several structural transitions from a rod-like conformation to a single stranded circle takes place. During the major thermal transition phase, all base pairs of the native structure are disrupted and a metastable structure with three hairpins (HP I, HP II and HP III) is observed. HP I is formed in the CCR of the PSTVd-type molecule. HP II is formed by base pairing of sequences of the pathogenicity (P) and variable (V) domains. HP III is only found in PSTVd. The position, length and GC content of the regions forming HP I and HP II are conserved. Therefore, it was argued that the ability to form these hairpins is not coincidental and results from biological.



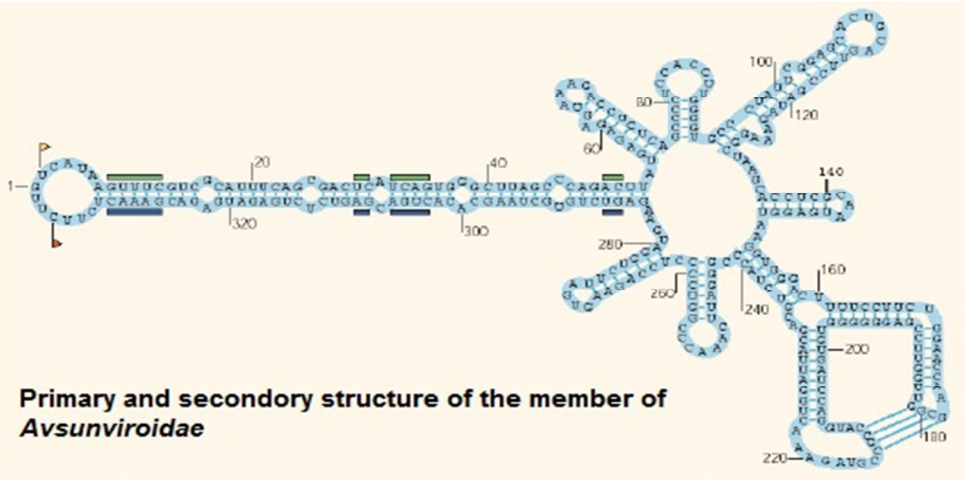
(A) Rod-like secondary structure proposed for PSTVd, the type member of family *Pospiviroidae*.
(B) Quasi rod-like secondary structure proposed for ASBVd, the type member of family *Avsunviroidae*.
(C) Complex branched conformation proposed for PLMVd.



Primary and secondary structure of the type member of *Pospiviroidae* family.

The base sequences of viroids have repeats, both direct and inverted, which suggest relatedness to transposing elements. Moreover, they possess a sequence similar to that used by retroviruses. However, viroids are not transcribed into DNA, and no sequences homologous to viroids are found in the DNA of infected cells. cDNA of the viroid is also infectious and can be transcribed into regular infectious viroid particles. A striking feature of viroid RNA is the presence of sequences highly homologous to some of the small nuclear RNAs U_1 and U_3, which are involved in the splicing of introns in animal cells. This suggests that viroids may have originated from introns and their pathogenecity might be due to interference with the normal splicing of introns

in cells. *A candidate for a viroid-like agent in humans is the delta agent which is much larger (1678bp) and is surrounded by a coat.*



Primary and secondory structure of the member of *Avsunviroidae*

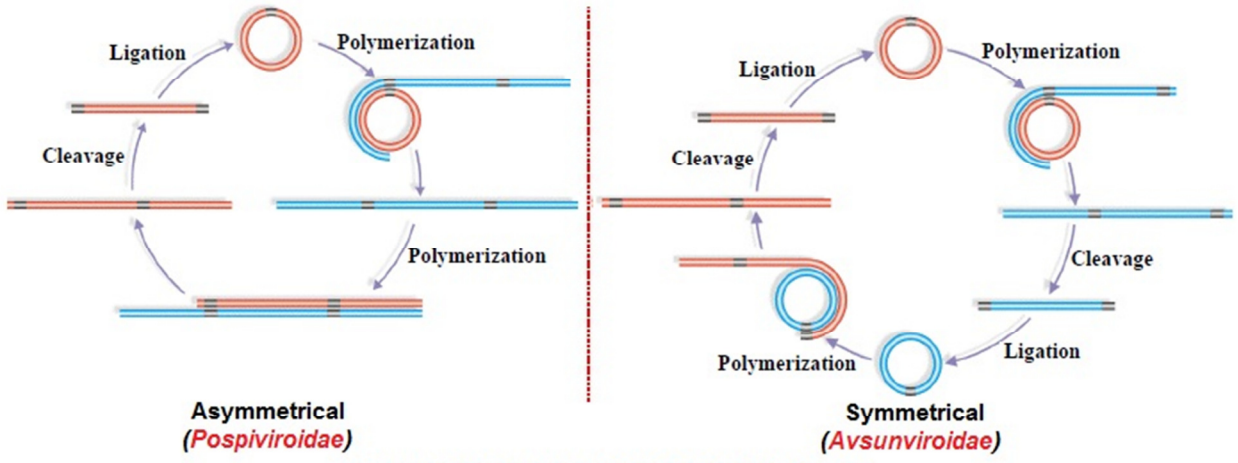
Replication of Viroids

There is no evidence that the RNA is translated. They are replicated in the nucleus of infected cells by host enzymes through double stranded intermediates. Replication is blocked by alpha-amantine, which inhibits RNA polymerase II (the RNA polymerase responsible for generating the transcripts for mRNAs). In the cell, this enzyme is responsible for mRNA synthesis and is partly associated with chromatin. Interestingly, viroids RNA are found subnuclear localization in nucleolar compartment suggesting that a replication intermediates shuttle between the nucleolar and nuclear compartment. A recent report suggests intranuclear trafficking of the (+) strand RNA but not of the (-) strand RNA. Synthesis of (-) and (+) strand RNA occurs in the nucleoplasm. The (–) strands are anchored in the nucleoplasm but the (+) strands are transported selectively into the nucleolus. Two alternative pathways for the processing and trafficking of the (+) strands are possible. Multimeric (+) PSTVd migrates into the nucleolus where its cleavage to monomers and circularization occurs. The second possibility is that multimeric (+) RNA is cleaved and circularised in the nucleoplasm, after which some circular molecules migrate into the nucleolus. In either pathway, circular monomers migrate into the cytoplasm and then to neighbouring cells through the plasmodesmata.

For replication, circular (+) strand acts as template to synthesize (-) strand in the nucleoplasm and initiation starts at two sites i.e. nucleotides A111 (C domain) and A325 (TL domain). The replication of viroids follows a rolling circle mechanism with two variants i.e. first (symmetric) variant and the second (symmetric) variant.

In the first variant the infectious circular (+) RNA is copied continuously by RNA polymerase into a concatameric (–) strand. The concatameric (–) RNA then serves as a template for the production of concatameric (+) RNA strands that are cleaved to monomers, and finally produce circular progeny.

In the second variant, concatameric (–) RNA strands created as in the first mechanism are cleaved producing monomers that are circularized and then copied to yield concatameric viroid (+) RNA molecules. Specific cleavage of these strands produces (+) monomers that are circularized to yield the progeny RNA.

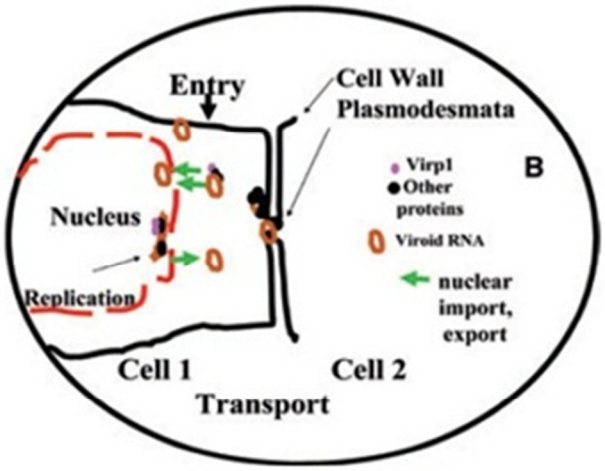


Rolling circle model of viroid replication

After replication, the progeny RNA moves through cell-to-cell movement through plasmodesmata and appears to be an active process mediated by specific sequence or by structural motifs. Long-distance trafficking takes place in the phloem which is likely sustained by viroid replication in the phloem and is governed by plant developmental and cell factors. In phloem, viroid binding protein VirP1 is involved in the transportation which contains a nuclear localization signal and a bromodomain that interacts with (+) strand RNA.

Pathogenesis of Viroids

Viroids are transmitted through either by mechanical breaks e.g. tools, breaks, and by insects, and through biologicals such as germs and co-infection. They may be latent for many years. Transmission Despite their small size and lack of mRNA activity, viroids can induce disease symptoms similar to those induced by plant viruses, and their interference with cell functions must stem from interaction between the viroid and certain host constituents. Targets for such interactions may be assigned to either host proteins or nucleic acids. Based on the sequence similarity between (+) and (–) viroid strands and various cellular RNAs such as U1 snRNA, U3B snRNA and U5 snRNA, it was proposed that viroids may interfere with mRNA splicing or pre-rRNA processing and results in the aberrated expression of respective effector molecules which causes disease appearance in infected plants.



Trafficking of viroid

Treatment of Viroids

To increase the reduced yield of crops, several attempts have been made to generate viroid-free plants. Recently, a long-term heat treatment at 35 °C for 14–37 weeks has been advocated as heat treatment for viroid infestation. Cold therapy or cold treatment has also been found effective in eliminating viroid from infected plants.

Virusoids

A second type of pathogenic RNA that can infect commercially important agricultural crops are the **virusoids** formerly called as **satellite RNAs**, which are sub-viral particles best described as non–self-replicating covalently closed circular ssRNAs, first discovered by J. W. Randles and coworkers in 1981. They are found in bacteria, plants, fungi, invertebrates and vertebrates. They are satellite, viroid-like molecules but larger than viroids (e.g. about 1000 nucleotides). Satellite viruses encode capsid proteins but depend on a helper virus for replication; therefore, they are called as 'satellite'. In humans, the hepatitis delta virus (HDV) is a virusoid which is much larger than a plant virusoid, HDV has a circular, ssRNA genome of 1,700 nucleotides and can direct the biosynthesis of HDV-associated proteins. Co-infection with hepatitis B virus and HDV results in more severe pathological changes in the liver during infection, which is how HDV was first discovered.

Virusoids are essentially viroids that have been encapsulated by a helper virus coat protein. They are thus similar to viroids in their means of replication and adopts rolling circle mode of replication, but they differ in that viroids do not possess a protein coat and do not interfere with the replication of viruses. They replicate in the cytoplasm using an RNA-dependent RNA polymerase. This enzymatic activity is common in plants but not found in animal cells. This helper virus also encapsulates them e.g. subterranean clover mottle virus satellite has a helper Sobemovirus. Once the helper virus enters the host cell, the virusoids are released and can be found free in plant cell cytoplasm, where they possess ribozyme activity. The helper virus undergoes typical viral replication independent of the activity of the virusoid.

Some examples of virusoids are: barley yellow dwarf virus satellite RNA and a helper Luleovirm: tobacco ring spot virus satellite RNA and a helper Nepovirus. These agents modify the symptoms of infection by their helper virus They can be spread by vegetative propagation, within seeds or by direct inoculation either by insects or man.