



Review Paper

Viruses and Viroids: Insights of Pathogenicity

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Abstract

Viruses are simple, a cellular obligate host parasite contains one or more either double or single stranded DNA or RNA molecules enclosed in a protein coat. More variety is found in the genomes of viruses than in those of prokaryotes and eukaryotes. They are classified on the basis of their nucleic acid characteristics, capsid symmetry, the presence or absence of envelope and their host. They are capable of causing disease ranging from prokaryote like bacteria to eukaryotes including humans, animals and even plants. Different viruses are able to cause disease as they contain one or more pathogenic genes encoding various antigenic proteins found present in capsid or envelope. Viroids are sub viral particles. Viroids are single stranded RNA stretches without capsid, having a few hundred nucleotide length. Their genome is quite smaller than smallest known virus genome. In spite of absence of any protein encoding genes, they are important plant pathogens as they contain RNA structural elements which interact with various host factors. The viroid plant interactions enable them to use the host machinery for replication and transport. Viroids RNAs transcription occurs by rolling circle mechanisms in the plant host's nuclei or chloroplasts. Recent evidence indicates that viroid-derived small RNAs acting through host RNA silencing pathways play a key role in viroid pathogenicity. Viroid RNAs have sizes similar to endogenous small interfering RNA and microRNA and so capable of alteration of the normal gene expression in the host plant. Viroids have the ability to induce both RNA-mediated transcriptional gene silencing and posttranscriptional gene silencing in infected plants. To discover and understanding molecular biology of these fascinating RNA molecules can be just regarded as beginning.

Keywords: Virus, viroid, pathogenesis, RNA Silencing.

Introduction

Viruses can be seen only with a powerful electron microscope as they are too small acellular entities. They contain either double/single stranded DNA or RNA, enclosed in a protein coat and sometimes even with an envelope. As they are acellular, they are not able to reproduce on their own, lack reproduction and protein synthesis machinery and are obligate host parasite. But unlike still simpler infectious agents; they contain genes required for mutation and evolution. Over 5000 species have been discovered¹. How viruses have been evolved is still unclear. They might be evolved from plasmid or from bacteria. The discovery of virus was not possible before 1884 until Chamberland's discovery of bacteriological filter. In 1886, Adolf Mayer first described the tobacco mosaic disease that could be transferred between plants, similar to bacterial infections^{2,3}. In 1892, Dmitri Iwanowsky gave the first concrete evidence for the existence of a non-bacterial infectious agent, showing that infected sap remained infectious even after filtering through finest Chamberland filtercandles^{3,4}. In 1898, Martinus Beijerinck independently replicated Iwanowsky's filtration experiments and then showed that the infectious agent was able to reproduce and multiply in the host cells of the tobacco plant. Beijerinck coined the term of "virus" to indicate

that the causal agent of tobacco mosaic disease was of non-bacterial nature. Tobacco mosaic virus was the first virus to be crystallized^{3,5}. Invention of electron microscope by Ernst Ruska and Max Knoll in 1931 gave the first image of virus.

Viroids are considered as sub viral particles. They are small, circular, single stranded RNA. Before the non-coding nature of viroid genomes was established, how small RNA molecule can produce disease was a mystery for science. The spindle tuber disease of potato was first time described by Martin in 1922. The disease was transmitted through contact of healthy plants with diseased plants or by contaminated equipment and by means of seed and pollen⁶. Initially for longer time scientist thought that the disease of the potato plant is caused by virus but due to properties found present in the pathogen revealed that pathogen is a short, free RNA molecule and was termed as viroid. Viroids are a group of non-coding RNAs that are able to regulate the host gene expression by means other than encoding proteins for specific functions⁷. Viroids are important and smallest plant pathogens. They are able to cause disease to many economically important plants like herbal woody or ornamental plants⁸. Though viroids do not possess coding genes but they are functional entities. Viroid genome possesses all necessary genetic information required for autonomous

replication. Moreover genome has also found to possess information for host specificity and cell to cell movement and is also able to stimulate pathogenicity in host plant. Viroids are tiniest RNA molecules that do not need to encode even own pathogen specific proteins and are merely 246 to 401 nucleotides (nt) in length⁹. After discovery of PSTVd, CEVd and CSVd were also discovered. Over 30 different viroid species have been detected and found to cause disease in plants like potato, tomato, hop, apple, avocado, coconut, peach, grapevine, citrus, pear, coleus and chrysanthemum. Their genome can be retrieved from Subviral RNA Database¹⁰, which contains sequences of more than 900 viroid variants¹¹.

Classification of Viruses and Viroids

Currently there are 2 main schemes, by which viruses are classified: The international committee on taxonomy of viruses (ICTV) and Baltimore classification. As classification given by ICTV in 2013, there are 7 orders, 103 families, 22 sub families, 420 genera and 2,618 species of viruses have been identified. They may contain various shapes like helical (Tobacco mosaic virus-TMV), icosahedral (Adenovirus) or complex (Influenza virus and T phages) structures and size is ranging from 20-300 nt. Viral capsid is made up of many subunits called capsomeres and protects nucleic acid from attack of nucleases. Additionally a nucleocapsid may contain envelope, enzyme and other structural proteins. The genome may be made up of whole or fragmented DNA or RNA which may be single or double stranded. In case of single stranded nucleic acid sequence, if a sequence is similar to that of viral m-RNA it is called positive sense genome. If a sequence is complementary to that of viral m-RNA, it is called negative sense genome.

Viroid classification scheme is presented in table-1 suggested by Flores *et al*¹² which is also showing full names of abbreviations of viroids. According to this classification viroids are divided in two families, the *Avsunviroidae* and the *Pospiviroidae*. Members of the *Avsunviroidae* family are able to catalyze self-cleavage of multimers produced during replication and do not possess a central conserved region (CCR). Members of the *Pospiviroidae* family possess a CCR and have no self-cleaving properties. 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. The presence and type of CCR serve to define the genus¹³. PSTVd, CEVd, HSVd and CCCVd are major representatives of family *Pospiviroidae*. They have adopted a rod like secondary structure shown in figure-1A^{14,15} and are mainly found in the nucleolus and nucleoplasm of infected plants^{7,16,17}. They use circular plusstranded RNA as template and with the help of enzyme DNA dependent RNA polymerase to synthesizing many copies of linear minus strand. The minus strand will further act as a template for synthesis of linear plus RNA strand. As shown in figure-1B^{18,19} linear plus stranded RNA molecules are converted to monomeric circles by unknown enzymes of the host and follow an asymmetric rolling circle mechanism^{20,21}. Scientist has proposed that PSTVd and other related viroids have five

structural and functional domains as shown in figure-1A²². They contains a conserved central domain (C) which is able to form alternative structures for regulation and replication of cycle, pathogenicity domain (P), high sequence variability domain (V) and two terminal interchangeable domains namely left terminal (TL), right terminal (TR).

Table-1
Classification of Viroids (Flores *et al.*, 1998)

Family <i>Avsunviroidae</i>		
Species name	Abbreviation	Genus
Avocado sunblotch viroid	ASBVd	<i>Avsunviroid</i>
Chrysanthemunchlorotic mottle viroid	CChMVd	
Peach latent mosaic viroid	PLMVd	<i>Pelamoviroid</i>
Eggplant latent viroid	ELVd	
Family <i>Pospiviroidae</i>		
Species name	Abbreviation	Genus
Coleus blumei viroid	CbVd	<i>Coleoviroid</i>
Coleus blumei viroid-1	CbVd-1	
Coleus blumei viroid-2	CbVd-2	
Coleus blumei viroid-3	CbVd-3	
Citrus viroid IV	CVd IV	<i>Cocadviroid</i>
Coconut cadang-cadang viroid	CCCVd	
Coconut tinangaja viroid	CTiVd	
Hop latent viroid	HLVd	
Hop stunt viroid	HSVd	<i>Hostuviroid</i>
Apple dimple fruit viroid	ADFVd ASSVd ACJVd AGVd CBLVd CVd-II CVd-LSS CVd-III CVd-OS GYSVd-1 GYSVd-2 JCVd PBCVd	<i>Apscaviroid</i>
Apple scar skin viroid		
Apple/citrus junos fruit viroid		
Australian grapevine viroid		
Citrus bent leaf viroid		
Citrus viroid II		
Citrus viroid I LSSI		
Citrus viroid III		
Citrus viroid OS		
Grapevine yellow speckle viroid-1		
Grapevine yellow speckle viroid-2		
Japanease citrus viroid I		
Pear blister canker viroid		
Chrysanthemum stunt viroid		
Citrus exocortis viroid		
Columnea latent viroid		
Iresine viroid		
Mexican papita viroid		
Potato spindle tuber viroid		
Tomato apical stunt viroid		
Tomato chlorotic dwarf viroid		
Tomato planta macho viroid		

Another small family of viroids is *Avsunviroidae*, which is found to localize in chloroplasts²³. This family contains only four members like ASBVd, PLMVd, ELVd and CChMVd. They contain flexible linear or branched structure as shown in figure-2A and B²⁴. Members of this family synthesize more RNA molecules with the help of chloroplastic RNA polymerase and replication occurs by a symmetric rolling circle mechanism as shown in figure-2C^{25,26}. Here hammerhead ribozyme motifs process multimeric replication intermediates into monomeric units^{27,28}.

Pathogenesis and Genes involved

Bacteriophages and animal viruses having the same reproductive strategy, which includes phases like adsorption, penetration and uncoating, replication of viral nucleic acids, synthesis and assembly of capsids and virus release. Viruses may harm their host cells in variety of ways, ranging from direct inhibition of DNA, RNA and protein synthesis to the alteration of plasma membrane and formation of inclusion bodies. Some animal viruses establish long term chronic infections; others are dormant for a while and then become active again. Slow virus infections may take years to develop. The replication of RNA viruses occur inside cytoplasm, as it is associated with RNA-dependent RNA polymerase. Whereas DNA viruses replicate inside nucleus, utilizing host's DNA polymerase and DNA-RNA synthesizing machinery. DNA viruses especially infect cells under cell cycle. Hepadnaviruses like hepatitis B virus contain genome having full length minus sense ssDNA and shorter length plus sense ssDNA, enters inside nucleus, repaired by viral reverse transcriptase and forms circular episome. RNA viruses usually replicate without formation of dsDNA.

Exceptionally viruses of Retroviridae family contain evolutionary successful design and unique biology. They form viral complementary DNA and integrate it in to a host genome, may even pass in to a germline DNA. This latency is considered to be one of the biggest reasons that drug therapy fails to eradicate HIV from patients. Cancer can be caused by viruses like hepatitis B virus, hepatitis C virus, human papilloma virus, Epstein-Barr virus, Kaposi's sarcoma virus and human T-lymphotrophic virus type 1 and 2²⁹. Viruses may bring oncogenes into a cell, carry promoters to stimulate a cellular oncogene and transform cells in to tumor cells. In one study authors have proposed that many DNA and RNA viruses have been proved to be oncogenic in many animals that ranging from amphibian to primates³⁰. Epstein Bar Virus (EBV) was discovered from Burkitt's lymphoma cell line in the year 1963³¹. This virus was the first to be identified from a human neoplastic cell and later on Human Papilloma Virus (HPV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human T Cell Leukemia Virus 1 (HTLV 1)³². It has been estimated that 15% of human cancers worldwide are viralborne²⁹. The HPVs are small DNA tumor viruses that implicated in the development of many cancers like cervix, oral and laryngeal cancers³³. E6 and E7 are early viral gene products that enhance p53 degradation, which block apoptosis process and decrease the activity of p21 and prevents inhibition of cell proliferation³⁴. The EBV found to cause Burkitt's lymphoma, B cell lymphoma, Hodgkin's lymphoma and nasopharyngeal carcinoma. Viruses are not replicating and do not killed cells but infected B cells are getting ability to propagate indefinitely. Moreover, EBV is also found to associated with malignant neoplasms and gastric carcinoma^{35,36}.

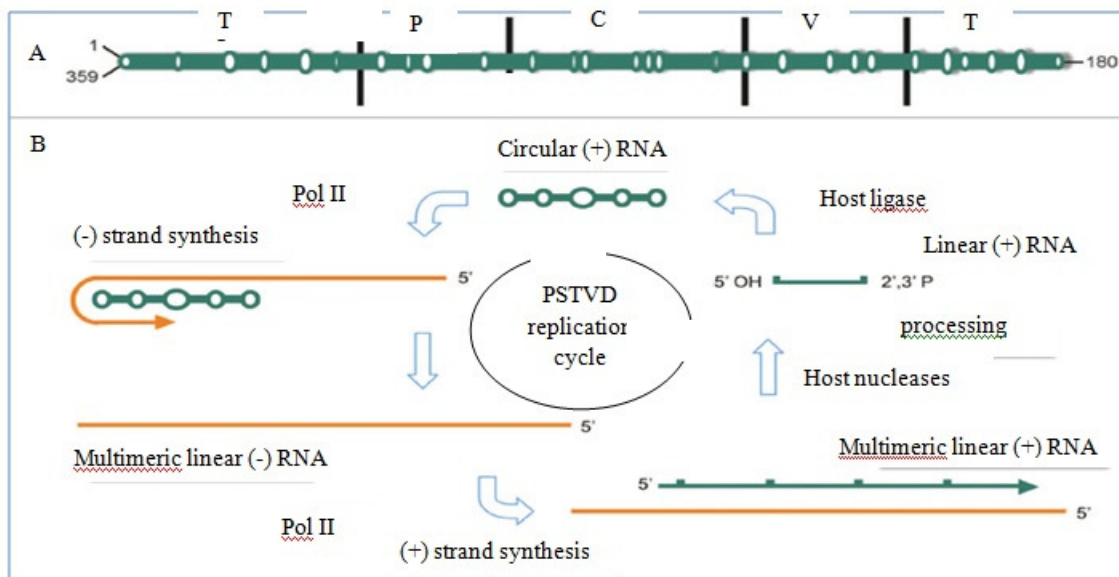


Figure-1

Structure and replication of *Pospiviroidae* (Steger and Riesner, 2003; Riesner and Gross, 1985; Warrilow and Symons, 1999; Kolonko *et al.*, 2005) (A) Schematic representation of the consensus secondary structure of the 359 nt circular plus PSTVd with the five functional domains. TL: Left terminal domain, P: pathogenicity-modulating domain, C: conserved central core, V: variable domain, TR: right terminal domain. (B) Replication follows an asymmetric rolling-circle mechanism

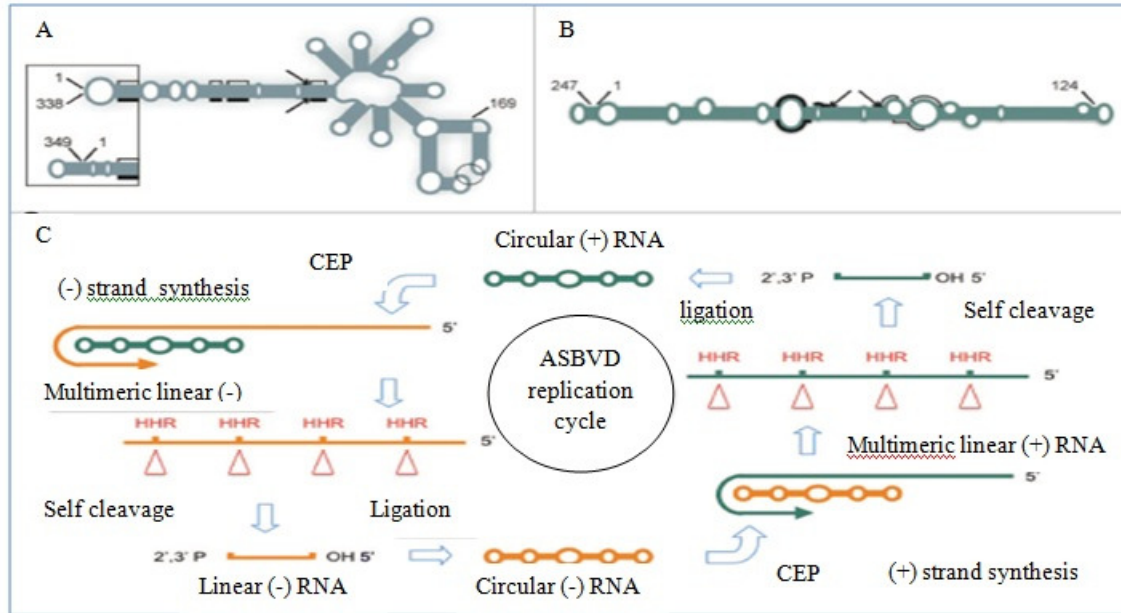


Figure-2:

Structure and replication of Avsunviroidae (Dubeet *et al.*, 2011; Navarro and Flores, 2000; Navarro *et al.*, 2000) (A) Schematic representation of the consensus secondary RNA structures for PLMVd (A) and ASBVd (B). In both parts, regions that correspond to conserved nucleotides of the hammerhead ribozymes (HHR) are indicated by closed and open bars for the plus and minus strand, respectively. The positions of HHR self-cleavage are indicated by arrows. (A) A dotted line indicated a pseudoknot formed in the PLMVd structure. The boxed area shows two variants of PLMVd that differ by an 11 nt extension (lower variant) in the left terminal hairpin. (C) Replication follows a symmetric rolling-circle mechanism

The EBV and holoendemic Malaria are recognized as important cofactors in endemic Burkitt's lymphoma and their contributions are discussed. Additionally, infection with Chikungunya Fever, a potentially oncogenic arbovirus, was associated with the onset of endemic Burkitt's lymphoma in one study and also with space-time case clusters of the lymphoma. Chikungunya Virus has several characteristics typical of oncogenic viruses. The flavivirus, Hepatitis C, a Class 1 Human Carcinogen, closely related to the arboviruses, Yellow Fever, and Dengue, is also more distantly related to Chikungunya Virus³⁷. Chronic HBV infection triggers oncogenic pathway, stimulate host immune responses and chronic necroinflammatory liver diseases and may results into carcinogenesis³⁸. The HCV is strongly found to link with hepatocellular carcinoma (HCC). Few studies in India have also indicated the relation between HCV and HCC; a report from Delhi noted that 15% of patients with HCC were positive for HCV antibody³⁹. HCV is also found to cause steatosis, which is a chronic form of hepatitis C. The interaction of hepatitis C virus core protein with the lipoprotein secretion pathways causes the characteristic alterations of lipid metabolism observed in hepatitis C virus-related steatosis. Several pathogenic mechanisms are likely involved into the pathogenesis of hepatitis C virus-related steatosis, including hyper-homocysteinaemia, hypoadiponecinaemia and insulin resistance. Steatosis is a major determinant of the liver damage progression in chronic hepatitis C (CHC), and negatively affects

the response rate to the interferon (IFN)-based anti-viral treatment. Recent evidence suggests that steatosis may contribute to liver carcinogenesis, may act as a risk factor for type 2 diabetes and atherosclerosis⁴⁰. Only one human retrovirus, HTLV1 is firmly implicated in the causation of cancer. HTLV-1 is associated with T-cell leukemia. Similar to the AIDS virus, infection of HTLV1 in human requires transformation of infected T cells via sexual intercourse, blood products or breast-feeding³³.

A novel coronavirus has been identified in 2003 as the causative agent of the severe acute respiratory syndrome (SARS) outbreak that has accounted for more than 8000 infected people worldwide. The mechanism of injury caused by SARS-corona virus (SARS-CoV) infection remains unknown. A SARS disease model has been proposed, consisting of three phases: viral replication, immune hyperactivity, and pulmonary destruction⁴¹. SARS pathology of the lung has been associated with diffuse alveolar damage, epithelial cell proliferation, and an increase of macrophages. SARS-CoV genome organization shares some hallmarks of other coronavirus genomes, although some characteristics are unique to SARS-CoV. For example, unlike group II coronaviruses, SARS has no hemagglutinin (HA) esterase (HE) gene, a gene homologous to the HA from influenza C virus⁴².

Many times confections also found to occur in the community.

In one study authors have observed that bacterial pneumonia is often found to be associated with influenza⁴³. WHO estimated that influenza epidemic every year causes severe illness in 3-5 million people and about 2.5-5 lac people died of it⁴⁴. It has been found that *Streptococcus pneumoniae* infection is found confected with viruses like respiratory syncytial virus, Influenza A virus, parainfluenza viruses and adenoviruses. In case of influenza viruses evolutionary changes that occur during adaptation to host cells can alter the natural tendency of viruses to support secondary bacterial infections. All influenza A viruses are zoonotic pathogens, sporadically enters in humans from wild bird reservoirs, either directly or through intermediate hosts such as pigs⁴⁵. Several virological features common in the avian form of these strains act to promote bacterial superinfections. These include low levels of glycosylation on the main surface glycoprotein hemagglutinin (HA), high neuraminidase activity to complement the reduction in receptor binding affinity associated with increased HA glycosylation, and expression of an inflammatory PB1-F2 protein^{46,47}. As these viruses adapt to the mammalian lung, either in humans or in pigs, they tend to lose their disease-associated phenotypes, with increased glycosylation of the surface proteins, lower neuraminidase activity, and either mutation to a non-functional form of PB1-F2 or loss of the active site through introduction of a stop codon and truncation of the resulting protein^{46,48}. In one more study authors have mentioned that H5N1 virus binds primarily to receptors present on bronchiolar and alveolar cells expressing SA- α -2,3-Gal (sialic acid bound to galactose by α -2,3 linkages. Additionally it is found that in ex-vivo condition H5N1 viral replications possible without detectable SA- α -2,3-Gal receptors in cells like nasopharyngeal, adenoid and tonsillar tissue cultures. These studies indicate that viral binding to respiratory tract shows complexity of receptors specificity³⁵. In one's study scientist have performed experiment in ducks to check pathogenicity of H5N1 strains MON3, HK483 and 24 reassortants generated between these two. The study revealed that the PB2, NP, and NS gene segments of MON3 were prerequisite for the high pathogenicity of MON3 in ducks. A set of the PB2, PA, HA, NP, and NS gene segments of MON3 was required to show full pathogenicity in ducks. These data indicate that multigenic factors are responsible for the pathogenicity of MON3 in ducks in compare to HK483. MON3 and reassortants that were lethal to ducks efficiently replicated in the tissues tested, especially in the brain, suggesting a possible correlation between virus growth in the brain and the death of ducks accompanying neurological dysfunction⁴⁹.

Vesicular stomatitis disease of livestock's is caused by Vesicular Stomatitis Virus (VSV) containing minus sense single stranded RNA. It is composed of host derived plasma membrane, envelope and ribonucleoprotein core. The envelope contains a transmembrane glycoprotein (G) required to mediate viral entry and exit, large protein (L), Matrix protein (M), Phosphoprotein (P) and nucleocapsid protein (N). After transmission of virus to the animal lesion development occurs. Much is known about VSV in the cell culture, in laboratory animals and its abilities to produce interferon in vitro, the

disease in livestock is not much understood⁵⁰.

Most plant viruses are either rod-shaped or isometric. TMV, Potato Virus Y (PVY), and Cucumber Mosaic Virus (CMV) are examples of a short rigid rod-shaped, a long flexuous rod-shaped, and an isometric virus, respectively. Viruses consist of an inner core of nucleic acid DNA or RNA surrounded by an outer sheath or coat of protein⁵¹. Tobacco Mosaic Virus is the first plant virus studied extensively. The TMV genome contains four open reading frames (ORF). Two ORFs code for an RNA dependent RNA polymerase (RdRp). Other two ORFs code for movement protein and capsid protein. It was proposed that TMV infection is spread quickly from cell to cell via viral replication complexes. Citrus Tristeza Virus (CTV) is found to cause disease in citrus plant worldwide. It has been observed that CTV is largest known plant virus. It contain positive sense single stranded RNA which encodes 12 ORF's that potentially codes for 17 protein products like proteases, methyl transferase and helicase²⁹. One study performed at Finland during 1997 to 2010 on horticulture plants. scientists have detected 8 new viruses belongs to tospoviruses, potexviruses, tymoviruses, ilarviruses and aliexiviruses in greenhouse crops, vegetable crops and garden ornamental plants. 5 new viroids were found in ornamentals and vegetable plant greenhouses⁵². Viruses are particle containing DNA/RNA surrounded by protein coat and/or envelope, pathogenic to animals, plants and even bacteria whereas, viroids are only plant pathogenic very short RNA molecules exerting pathogenicity by means of gene silencing, as they do not contain protein encoding genes.

Many RNA viruses and all viroids exert pathogenesis through RNA silencing. Most viruses have RNA genomes that are replicated and transcribed into messenger RNA by viral RdRps, usually in concert with other viral and host factors. Many eukaryotes also encode putative RdRps that have been implicated in sequence-specific, RNA-triggered gene silencing. Although the viral and cellular RdRps have no sequence homology, they share functional similarities such as copying messenger RNA templates and intercellular spread of the amplified sequences⁵³. Consistent with the antiviral effects of RNA silencing and the involvement of RdRp in at least some RNA silencing, mutation of the *Arabidopsis* RdRp homolog was found to increase the accumulation of cucumber mosaic virus and a potato virus X replicon^{54,55}. This same *Arabidopsis* mutation did not affect the accumulation of several other positive-strand RNA viruses, possibly because these viruses already efficiently inhibit RNA silencing in wild-type plants. Also consistent with a role in virus control, expression of RdRp genes in many plants is greatly stimulated by virus infection. In tobacco, a virus-inducible host RdRp gene with clear antiviral effects is also induced by salicylic acid, which stimulates generalized antiviral and antimicrobial defense responses⁵⁶. Because RNA viruses replicate their genomes through complementary RNA strands, it has been widely suggested that viral dsRNA replication intermediates are primarily responsible for viral induction of RNA silencing.

Viroids contain small, noncoding, and highly structured RNA genomes. How they cause disease symptoms without encoding proteins and why they have characteristic secondary structures are two longstanding questions. Studies have shown that viroids are capable of inducing RNA silencing, suggesting a possible role of this mechanism in the pathology and evolution of these subviral RNAs. In one study authors found that preventing RNA silencing in tobacco, using a silencing suppressor greatly reduces the symptoms caused by the Y satellite of cucumber mosaic virus. Moreover, tomato plants expressing hairpin RNA, derived from potato spindle tuber viroid, developed symptoms similar to those of potato spindle tuber viroid infection. These results provide evidence suggesting that viroids cause disease symptoms by directing RNA silencing against physiologically important host genes. They have also suggested that viroids are significantly resistant to RNA silencing-mediated degradation, suggesting that RNA silencing is an important selection pressure shaping the evolution of the secondary structures of these pathogens⁵⁷. As non-coding RNAs, viroids must incite disease through direct interaction of the genomic RNA (or derivatives thereof) with host cell components. Very limited sequence changes in either the pathogenicity domain or conserved central region can have a dramatic effect on PSTVd symptom expression. For viroids with a branched structure (e.g. CChMVd and PLMVd), changes in a single U-rich tetra loop have a similar effect on symptom expression. Viewed from the host perspective, viroid infection activates the same general plant defense system involving a suite of so-called pathogenesis related proteins (prp) that is activated by many other plant pathogens⁷.

Many reviews have summarized the mechanisms of this process^{58,59}. The key feature is the occurrence of dsRNA that is cleaved by one form of the RNase-III like enzyme Dicer into short double-stranded RNA fragments with single-stranded 30 protruding ends, which are called short interfering RNAs (siRNAs). The siRNAs are then incorporated into a multiprotein complex termed RNA-induced silencing complex (RISC). RISC gets activated and, by a helicase function, the two siRNA strands separate so that the remaining strand guides the sequence-specific cleavage of single-stranded complementary target RNA. In plants, RNA silencing is more complicated because different classes of siRNAs can be distinguished⁶⁰ and silencing is not cell autonomous but can spread from cell to cell and long distance, mediated by an unidentified mobile signal⁶¹.

From the experiment of Sanger *et al.*⁶², it was observed that PSTVd specific chromosomal DNA which is integrated into *Nicotiana tabacum* SRI genome via *Agrobacterium* mediated leaf disc transformation; it becomes methylated as soon as an autonomous viroid RNA directed RNA replication occurred in this plant⁶³. These observations proved that RNA is capable of inducing and directing sequence specific de novo methylation of genomic DNA. DNA methylation is responsible for gene silencing. RNA directed DNA methylation made by viroid results into subsequent plant gene silencing. This phenomenon is known as Transcriptional Gene Silencing (TGS). A recent

review summarizing the evidence that RNA silencing plays a major role in viroid pathogenesis and evolution⁶⁴.

Several scientists have reported that viroids are able to induce Post Transcriptional Gene Silencing (PTGS)⁶⁵. Authors have reported that 25 nt sequence specific to PSTVd can induce PTGS which is also known as RNA interference or RNA silencing^{66,67}. Viroid specific small RNAs of 21-23 nt size were also detected from peach trees and chrysanthemum infected with PLMVd and CChMVd respectively⁶⁸. Chrysanthemums are a common host for two different viroids, CSVd and the CChMVd. These viroids are quite different from each other in structure and function. Authors have reviewed research associated with CSVd and CChMVd that covered disease symptoms, identification, host range, nucleotide sequences, phylogenetic relationships, structures, replication mechanisms, symptom determinants, detection methods, viroid elimination, and development of viroid resistant chrysanthemums, among other studies. Next-generation sequencing-based approaches might provide information on the chrysanthemum transcriptome and how two different viroids regulate it. Chrysanthemum plants are easily transformed, and creating transgenic plants is not difficult. They proposed that the chrysanthemum and these two viroids represent convenient genetic resources for host-viroid interaction studies⁶⁹.

Nuclear as well as chloroplastic viroids can be act as inducers of viroid specific silencing. RNA silencing is nucleotide sequence specific gene regulation, various viroid strains with different nucleotide sequence produces viroid specific small RNAs of different quality in the infected plants, which can target host sequences and may result into severity of expressions of symptoms depending on viroid host combination. For example, tissues with different symptom expressions, characterized by the presence of different predominant ASBVd variants, were found to induce PTGS at differential levels, and detection of the PTGS-associated small interfering RNAs as well as their relative concentration was also related to viroid titer. In contrast, PTGS induced in *Gynura aurantiaca* infected with two closely-related variants of CEVd was not directly related to viroid titer with initiation of symptoms⁷⁰.

It is still remains a question of debate that whether that RNA silencing is involved in viroid pathogenicity or not. Analysis of Sequences of viroid-induced small RNAs in viroid-infected plants have been done by using several viroid-host combinations such as PSTVd-tomato^{71,72}, CEVd-tomato30 and PLMVd-peach⁷³. It was observed that majority of viroid-specific small RNAs are derived from some restricted regions in the viroid molecule. Large-scale nucleotide sequencing analysis on two each of viroids grouped in the family *Pospiviroidae*; HSVd and GYSVd1, and in the family *Avsunviroidae*; CChMVd and PLMVd also confirmed that the large majority of viroid-specific small RNAs of 21 to 24nt are derived from a few specific regions of the plus and minus strand of viroid RNAs shown in center of figure-3^{74,75}.

Two broad classes of plant small RNAs – microRNAs (miRNAs) recognized in 2009 and small interfering RNAs (siRNAs) – differ in several important respects. In Arabidopsis, four different Dicer-Like (DCL) activities and a variety of other are involved in small RNA synthesis. Cleavage of the respective precursor molecules releases different sized small RNAs, and genetic analysis has revealed considerable overlap/redundancy in DCL activity. Viroid-derived siRNAs have been detected in plants infected by several different viroids⁷⁶. PSTVd is known to replicate and accumulate in nuclei; however, PSTVd-specific small RNAs were detected in the cytoplasm but not in the nuclei⁷⁷. The cytoplasmic localization of viroid-specific small RNAs may be the result of the dsRNA replication intermediates and/or highly structured viroid RNA itself are either exported first in the cytoplasm and then cleaved by DCL enzyme(s) such as DCL2 and DCL3 that have been associated with cytoplasmic small RNA biogenesis or cleaved first into small RNAs in the nuclei by DCL enzyme such as DCL1 and then exported into cytoplasm, which is similar to the nuclear processing of plant miRNAs^{78,79}. In plants, furthermore, the small RNAs produced can subsequently be used as primers by RNA dependent RNA polymerase such as RDR6 for converting the target RNA template into dsRNA to produce more substrate for DCL enzyme(s) shown at right in figure-3⁷⁸⁻⁸⁰.

The center panel; Replication of viroid in infected host cells induces viroid-specific RNA silencing. Targeted by RNA silencing, viroid molecule is diced into various lengths of viroid-specific small RNAs consisting of ca. 21–24 nucleotides from several hotspot regions in the molecule, and as a result, a large amount of viroid-specific small RNAs are accumulated in infected plant cells. The right; After induction of viroid-specific RNA silencing, host RNA silencing machineries consist of Dicer-like (DCL) enzyme, RNA-induced silencing complex (RISC), Argonaute (AGO) proteins, RNA-dependent RNA polymerase 6 (RDR6), etc., work co-operatively to protect plants from viroid infection by degrading invaded viroid and amplifying viroid-specific small RNAs (secondary siRNA) to enforce RNA silencing targeting viroid replication. However, accumulation of the viroid-derived small RNAs in the infected host plant, as the side effect, could give rise to inconceivable adverse effects on certain normal host regulation genes in a sequence-specific manner, which may result in viroid pathogenicity. The left; Viroid-specific RNA silencing could be used for the creation of viroid-resistance transgenic plants. By introducing viroid-derived sequence designed to form hairpin or dsRNA after expression in the transgenic plant, the pre-expressed viroid-derived sequence induces artificial viroid-specific RNA silencing and is expected to act as defense against invaded viroid replication.

Scientists did experiments using the chloroplastic viroid PLMVd and wheat germ extract and found that dsRNA

complexes formed by intermolecular base pairing of PLMVd strands can serve as substrate for DCL enzyme(s) and identified the P11 hairpin of PLMVd, which is known to be implicated in its replication, as the domain recognized by DCL enzyme(s), thereby initiating RNA silencing⁸¹. Some in vivo studies have shown that accumulated small RNAs in infected plants form a population of sequences representing the entire PLMVd genome⁶⁸. Analysis of PLMVd-specific small RNA sequence also revealed that the majority of the small RNAs are derived from several regions in the molecule^{73,75}.

For years, it was believed that viroids induce disease by interacting with an unknown host factor like protein, thereby disrupting normal cell function. This hypothesis was proposed in the mid- 1980s when a region of the viroid genome was identified as the virulence-modulating (VM) region; i.e., changes of nucleotide within the region of PSTVd have dramatic effects on the virulence⁸². Degradation directed by siRNA requires a minimum sequence identity of about 19 nt between the siRNA and the target RNA^{83,84}. The pathogenicity of viroids is generally determined by the nucleotide sequence within regions of the viroid genome⁸⁵. i.e. PSTVd virulence modulating (VM) region with nucleotides no. 45–68^{82,14}. A BLAST search with the full-length sequence of PSTVd-RG1 revealed numerous sequences from several plant species that have 19–20-nt identities with the PSTVd genome⁵⁷. Almost all of these 19–20-nt sequences correspond to the A plus G-rich VM region of PSTVd, suggesting that small RNAs derived from this region of PSTVd may target the silencing of host regulatory genes.

In one study scientists have performed sequencing of AGVd and GYSVd 1 isolated from grape wine trees in Tunisia. Their data confirmed the worldwide spread of both viroids for the first time⁸⁶. In one study authors have performed sampling of grape wines near to hop gardens in northern Bohemia for detection of HSVd-DNA. They found 70% grape wine samples infected with HSVd. The study shows potential danger for hop cultivation⁸⁷.

Authors have studied sequence similarity of viroids with genomes of prokaryotes and eukaryotes with nucleotide BLAST search tool. Study includes prokaryotes (*Acetobacteracetii*, *Bordetella bronchiseptica*, *Borrelia garinii*, *Brucella abortus* biovar, *Brucella melitensis*, *Brucella melitensis*, *Brucellaisuis* etc.), fungi (*Magnaporthe grisea*), plants (*Oryzasativa Arabidopsis thaliana*, *Aspergillus fumigatus*, *Medicago truncatula* etc) and animals (*Trypanosoma cruzi*, *Entamoeba histolytica*, *Caenorhabditis briggsae*, *Drosophila melanogaster*, *Anopheles gambiae*, *Strongylocentrotus purpuratus* and Sea urchin-*Cionaintestinalis*, *Xenopus tropicalis*, *Bos Taurus*, *Mus musculus*, *Rattus norvegicus*, *Homo sapiens*) genomes. Nucleotide BLAST search does not find significant similarity of viroid genome with other genomes⁸⁸.

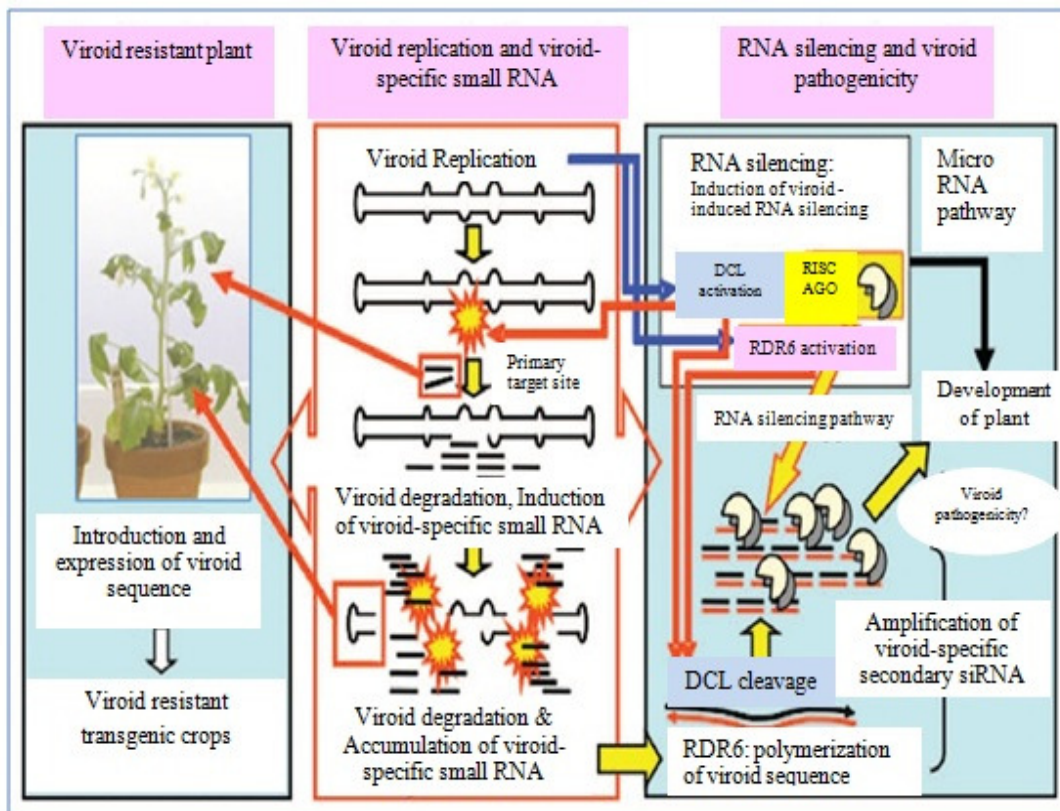


Figure-3

Schematic representation of viroid replication, viroid-specific small RNAs, RNA silencing and viroid-resistant transgenic plant (Voynet, 2008; xie and Qi, 2008; Dillin, 2003)

PSTV dis a quarantine pathogen was identified in plants of *Solanumjasmminoides* and *S. rantonnetii* in Italy in one study. Molecular characterization of four *S. jasmminoides* and one *S. rantonnetii* PSTVd isolates showed limited sequence variability. A tissue-printing hybridization method for detecting PSTVd in ornamental *Solanaceae* was successfully tested. Authors have first time reported PSTVd in Italy and this was the first report of PSTVd naturally infecting *S. rantonnetii*⁸⁹.

In one survey and molecular detection of two citrus viroids affecting commercial citrus orchards, scientists have observed that citrus trees were acting as symptomless carriers of CEVd and HSVd as citrus tree samples are found to be CEVd and HSVd positive by reverse transcription polymerase chain reaction. In one interesting study authors have noted that citrus viroids like CEVd, HSVd and CVd III/IV can induce resistance to *Phytophthora* infection. To know the reason, they have extracted phenolic acids, salicylic acid and flavone. There was no considerable concentration change found for phenolic acids and salicylic acid in viroid and *Phytophthora* infected plants. But flavone was detected in slightly higher concentration in infected plants⁹⁰. Molecular and biological characterization of AGVd Iranian isolate was performed. It contains wide host range and cause stunting, leaf deformation, mottling and vein clearing upon mechanical inoculation of the viroid infectious

DNA. Predicted secondary structure of the AGVd-Ir showed a difference from the predicted structure of the type isolate in the viroid pathogenicity domain⁹¹.

Conclusion

Unlike other microorganisms viruses have only dark sides for living entities. The speed at which new viruses are emerging (Norwalk virus, Rotavirus, Parvovirus, Enterovirus, Ebolavirus, Hantavirus, Sabiavirus, Sin nombre virus, Bocavirus, Chikungunya virus etc.) and old viruses are reemerging (Dengue fever virus, Measles virus, O'nyong'nyong fever virus, Mumps virus, Monkey pox virus, Avian influenza A by H5N1 etc.), it has become a matter of high concern. Yet we have no much more medications to fight against viral diseases. We have to go under deeper insights of viral pathogenesis, which can improve our knowledge to reduce viral epidemics and for preparing antiviral agents. On the other hand viroids are emerging as potent plant pathogen. Future research will reveal more details about how viroids RNAs are generated. Molecular biology, plant pathology and bioinformatics together can answer many questions regarding viroid mode of action. In future we will have clear picture of the biological function of these unusual non-coding RNAs. Perhaps, we may have answered our question that why do viroids infect to higher plants only.

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