Deleris A, Gallego-Bartolome J, Bao JS, Kasschau KD, Carrington JC, and Voinnet O (2006) Hierarchical action and inhibition of plant Dicer-like proteins in anti-viral defense. *Science* 313(5783): 68–71.
Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, and Imler JL (2006) Essential function *in vivo* for Dicer-2 in host defense against RNA viruses in drosophila. *Nature Immunology* 7(6): 590–597.
Gitlin L, Stone JK, and Andino R (2005) Poliovirus escape from RNA interference: Short interfering RNA-target recognition and implications for therapeutic approaches. *Journal of Virology* 79(2): 1027–1035.
Hamilton A, Voinnet O, Chappell L, and Baulcombe D (2002) Two classes of short interfering RNA in RNA silencing. *EMBO Journal* 21 (17): 4671–4679.
Hamilton AJ and Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286(5441): 950–952.
Himber C, Dunoyer P, Moissiard G, Ritzenthaler C, and Voinnet O (2003) Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. *EMBO Journal* 22(17): 4523–4533.
Li F and Ding SW (2006) Virus counterdefense: Diverse strategies for evading the RNA-silencing immunity. *Annual Review of Microbiology* 60: 503–531.
Li HW, Li WX, and Ding SW (2002) Induction and suppression of RNA silencing by an animal virus. *Science* 296(5571): 1319–1321.
Marathe R, Anandalakshmi R, Smith TH, Pruss GJ, and Vance VB (2000) RNA viruses as inducers, suppressors and targets of post-transcriptional gene silencing. *Plant Molecular Biology* 43(2–3): 295–306.
Matzke MA and Matzke AJ (2004) Planting the seeds of a new paradigm. *PLOS Biology* 2: E133.
Pfeffer S, Sewer A, Lagos-Quintana M, *et al.* (2005) Identification of microRNAs of the herpesvirus family. *Nature Methods* 2(4): 269–276.
Pfeffer S and Voinnet O (2006) Viruses, microRNAs and cancer. *Oncogene* 25(46): 6211–6219.
Segers GC, van Wezel R, Zhang XM, Hong YG, and Nuss DL (2006) Hypovirus papain-like protease p29 suppresses RNA silencing in the natural fungal host and in a heterologous plant system. *Eukaryotic Cell* 5(6): 896–904.
Silhavy D and Burgyan J (2004) Effects and side-effects of viral RNA silencing suppressors on short RNAs. *Trends in Plant Science* 9: 76–83.

Viroids

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Glossary

Catalytic RNA RNA molecules that are able to catalyze, in a protein-free medium, specific reactions involving the formation or breakage of covalent bonds. In nature, these reactions are usually transesterifications (self-cleavage and ligation) affecting the catalytic RNA itself.
Hammerhead structure The conserved secondary/tertiary structure shared by the smallest class of natural ribozymes. Most have been found in one or both strands of certain viroid and viroid-like satellite RNAs where they mediate self-cleavage of multimeric intermediates arising from replication through a rolling-circle mechanism.
Ribozyme RNA motif responsible for the catalytic activity of certain RNA molecules. In nature, they are found embedded within catalytic RNAs.

Introduction

Viroids are the smallest known agents of infectious disease – small (246–401 nt), highly structured, circular, single-stranded RNAs that lack detectable messenger RNA activity. While viruses have been described as 'obligate parasites of the cell's translational system' and supply some or most of the genetic information required for their replication, viroids can be regarded as 'obligate parasites of the cell's transcriptional machinery'. Thus far, viroids are known to infect only plants.

The first viroid disease to be studied by plant pathologists was potato spindle tuber. In 1923, its infectious nature and ability to spread in the field led Schultz and Folsom to group potato spindle tuber disease with several other 'degeneration diseases' of potatoes. Nearly 50 years were to elapse before Diener's demonstration in 1971 that the molecular properties of its causal agent, potato spindle tuber viroid (PSTVd), were fundamentally different than those of conventional plant viruses.

Genome Structure

Efforts to understand how viroids replicate and cause disease without the assistance of any viroid-encoded polypeptides have prompted detailed analysis of their structure. Viroids possess rather unusual properties for single-stranded RNAs (e.g., a pronounced resistance to digestion by ribonuclease and a highly cooperative thermal denaturation profile), leading to an early realization that they might have an unusual higher-order structure.

To date, the complete sequences of 29 distinct viroid species plus a large number of sequence variants have

been determined (Table 1). All are single-stranded circular RNAs containing 246–401 unmodified nucleotides. Theoretical calculations and physicochemical studies indicate that PSTVd and related viroids assume a highly base-paired, rod-like conformation *in vitro* (Figure 1). Pairwise sequence comparisons suggest that the series of short double helices and small internal loops that comprise this so-called 'native' structure are organized into five domains whose boundaries are defined by sharp differences in sequence similarity.

The 'central domain' is the most highly conserved viroid domain and contains the site where multimeric PSTVd RNAs are cleaved and ligated to form circular progeny. The 'pathogenicity domain' contains one or more structural elements which modulate symptom expression, and the relatively small 'variable domain' exhibits the greatest sequence variability between otherwise closely related viroids. The two 'terminal domains' appear to play an important role in viroid replication and evolution. Although these five domains were first identified in PSTVd, apple scar skin viroid (ASSVd) and related viroids also contain a similar domain arrangement. Certain viroids such as *Columnea* latent viroid (CLVd), Australian grapevine viroid (AGVd), and *Coleus blumei* viroid 2 (CbVd 2) appear to be 'mosaic molecules' formed by exchange of domains between two or more viroids infecting the same cell. RNA rearrangement/recombination can also occur within individual domains, leading, in coconut cadang-cadang (CCCVd) and citrus exocortis (CEVd) viroids, to duplications of the right terminal domain plus part of the variable domain. This domain model is not shared by avocado sunblotch (ASBVd) and related viroids.

Much less is known about viroid tertiary structure, especially *in vivo* where these molecules almost certainly accumulate as ribonucleoprotein particles. UV-induced

Table 1 Classification of viroids of known nucleotide sequence

Family[a]	*Genus*[a]	*Name*	*Abbreviation*	*Nucleotides*[b]
Pospiviroidae	*Pospiviroid*	Chrysanthemum stunt	CSVd	354–356
		Citrus exocortis	CEVd	368–375 (463–467)
		Columnea latent	CLVd	370–373
		Iresine	IrVd	370
		Mexican papita	MPVd	359–360
		Potato spindle tuber	PSTVd	356–361 (341)
		Tomato apical stunt	TASVd	360–363
		Tomato chlorotic dwarf	TCDVd	360
		Tomato planta macho	TPMVd	359–360
	Cocadviroid	Citrus viroid IV	CVd-IV	284
		Coconut cadang-cadang	CCCVd	246–247 (287–301)
		Coconut tinangaja	CTiVd	254
		Hop latent	HLVd	256
	Hostuviroid	Hop stunt[c]	HSVd	294–303
	Apscaviroid	Apple dimple fruit	ADFVd	306,307
		Apple scar skin[d]	ASSVd	329–334
		Australian grapevine	AGVd	369
		Citrus bent leaf	CBLVd	315,318
		Citrus dwarfing	CDVd	294,297
		Grapevine yellow speckle 1	GVYSVd 1	366–368
		Grapevine yellow speckle 2[e]	GYSVd 2	363
		Pear blister canker	PBCVd	315,316
	Coleviroid	*Coleus blumei* 1	CbVd 1	248–251
		Coleus blumei 2	CbVd 2	301,302
		Coleus blumei 3	CbVd 3	361–364
Avsunviroidae	*Avsunviroid*	Avocado sunblotch	ASBVd	246–251
	Pelamoviroid	Chrysanthemum chlorotic mottle	CChMVd	398–401
		Peach latent mosaic	PLMVd	335–351
	Elaviroid	Eggplant latent	ELVd	332–335

[a]Classification follows scheme proposed by Flores *et al*. (see VIII Report of the International Committee on Taxonomy of Viruses) with minor modifications. The nucleotide sequences of blueberry mosaic, burdock stunt, *Nicotiana glutinosa* stunt, pigeon pea mosaic mottle, and tomato bunchy top viroids are currently unknown; consequently, these viroids have not been assigned to specific genera. Whether apple fruit crinkle and citrus viroid original source should be considered variants or new viroid species of genus *Apscaviroid* is pending.
[b]Sizes of variants containing insertions or deletions arising *in vivo* are shown in parentheses.
[c]Includes cucumber pale fruit, citrus cachexia, peach dapple, and plum dapple viroids.
[d]Includes pear rusty skin and dapple apple viroids.
[e]Formerly termed grapevine viroid 1B.

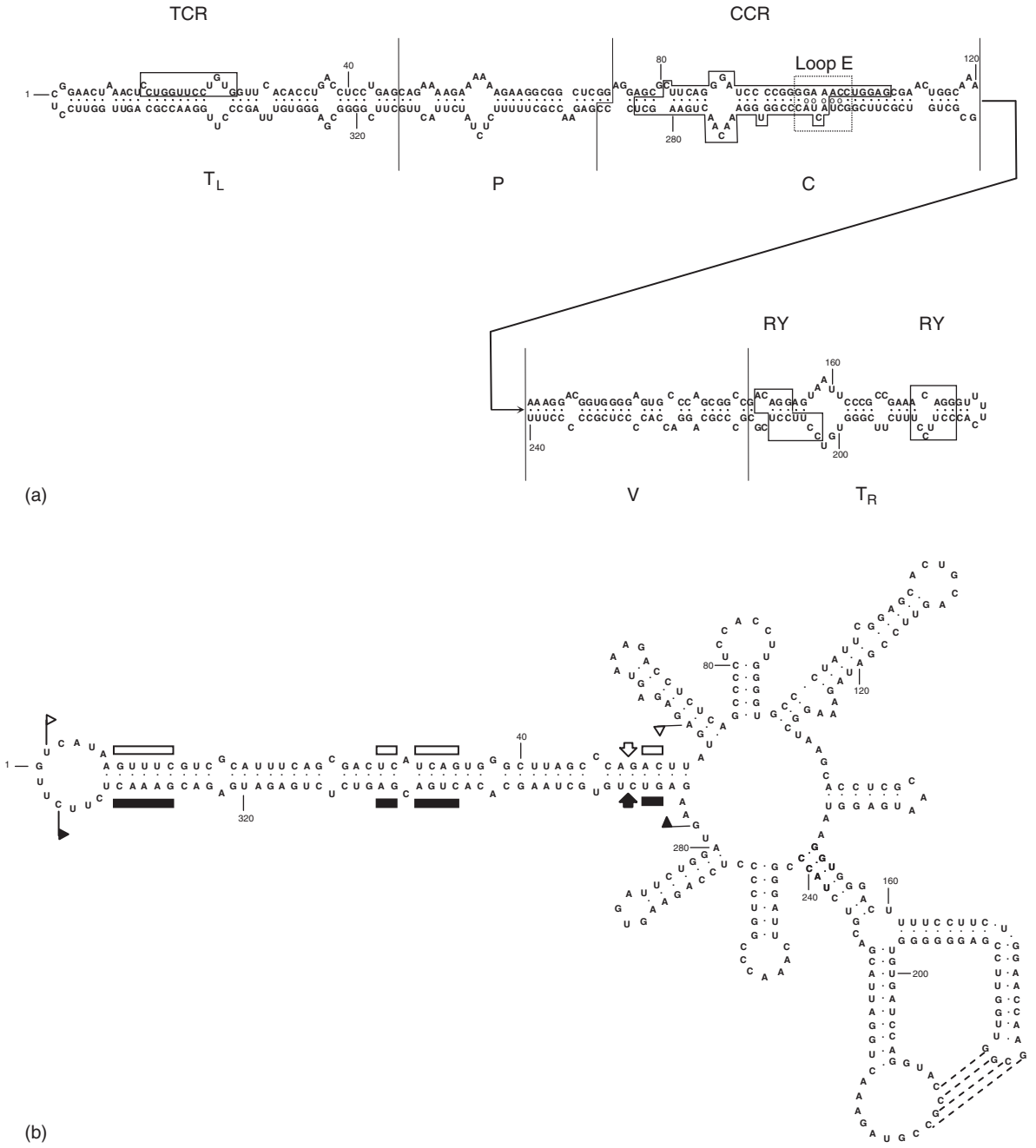

Figure 1 (a) The rod-like secondary structure of PSTVd (intermediate strain) showing the five domains characteristic of members of the family *Pospiviroidae:* the terminal left (T_L), pathogenicity (P), central (C), variable (V), and terminal right (T_R). The central conserved region (CCR) is located within the C domain and contains a UV-sensitive loop E motif with noncanonical base pairs (denoted by circles). The T_L domains of genera *Pospiviroid* and *Apscaviroid* contain a terminal conserved region (TCR), while those of genera *Hostuviroid* and *Cocadviroid* contain a terminal conserved hairpin (not shown). The T_R may also contain 1–2 copies of a protein-binding RY motif. (b) The branched secondary structure of PLMVd (reference variant). Plus and minus self-cleavage domains are indicated by flags, nucleotides conserved in most natural hammerhead structures by bars, and the self-cleavage sites by arrows. Black and white symbols refer to plus and minus polarities, respectively. Nucleotides involved in a pseudoknot are indicated by broken lines. Redrawn with modifications from Gross HJ, Domdey H, Lossow C, *et al*. (1978) Nucleotide sequence and secondary structure of potato spindle tuber viroid. *Nature* 273: 203–208; Hernández C and Flores R (1992) Plus and minus RNAs of peach latent mosaic viroid self-cleave *in vitro* through hammerhead structures. *Proceedings of the National Academy of Sciences, USA* 89: 3711–3715; Bussière F, Ouellet J, Côté F, Lévesque D, and Perreault JP (2000) Mapping in solution shows the peach latent mosaic viroid to possess a new pseudoknot in a complex, branched secondary structure. *Journal of Virology* 74: 2647–2654.

cross-linking of two nucleotides within a loop E motif in the central domain of PSTVd provided the first definitive evidence for such tertiary interactions. Similar UV-sensitive structural elements have also been discovered in a number of other RNAs including 5S eukaryotic rRNA, adenovirus VAI RNA, and the viroid-like domain of the hepatitis delta virus genome. Loop E forms during the conversion of multimeric PSTVd RNAs into monomers. The ability of ASBVd-related RNAs to undergo spontaneous self-cleavage mediated by hammerhead ribozymes as well as the presence of pseudoknots critical for infectivity in some other members of the *Avsunviroidae* (**Figure 1**) provide additional evidence for the functional importance of viroid tertiary structure.

Classification

Based upon differences in the structural and functional properties of their genomes, viroids species are assigned to one of two taxonomic families (see **Table 1**). Members of the family *Pospiviroidae* (type member PSTVd) have a rod-like secondary structure that contains five structural–functional domains and several conserved motifs. Most members of the family *Avsunviroidae* (type member ASBVd), in contrast, appear to adopt a branched conformation, and multimeric RNAs of all family members behave as catalytic RNAs and undergo spontaneous self-cleavage (**Figure 1**). Differences in their sites of replication also support this classification scheme; that is, PSTVd and ASBVd replicate in the nucleus and the chloroplast, respectively, and the same appears to occur for other members of each family. Each family is subdivided into genera according to certain demarcating criteria. Groups of sequence variants that show >90% sequence identity in pairwise comparisons and share some common biological property are arbitrarily defined as viroid species. *In vivo*, each viroid species is actually a 'quasispecies', that is, a collection of closely related sequences subject to a continuous process of variation, competition, and selection. There is phylogenetic evidence for an evolutionary link between viroids and other viroid-like subviral RNAs (**Figure 2**).

Host Range and Transmission

All viroids are mechanically transmissible, and most are naturally transmitted from plant to plant by man and his tools. Individual viroids vary greatly in their ability to infect different plant species. PSTVd can replicate in about 160 primarily solanaceous hosts, while only two members of the family Lauraceae are known to support ASBVd replication. HSVd has a particularly wide host range that includes herbaceous species as well as woody perennials. Many natural hosts are either vegetatively propagated or crops that are subjected to repeated grafting or pruning operations. PSTVd, ASBVd, and CbVd1 are vertically transmitted through pollen and/or true seed, but the significance of this mode of transmission in the natural spread of disease is unclear. PSTVd can be encapsidated by the coat protein of potato leafroll virus (PLRV, a polerovirus) as well as velvet tobacco mottle virus (VTMoV, a sobemovirus), and epidemiological surveys suggest that PLRV facilitates viroid spread under field conditions.

Commonly used techniques for the experimental transmission of viroids include the standard leaf abrasion methods developed for conventional viruses, 'razor slashing' methods in which phloem tissue in the stem or petiole is inoculated via cuts made with a razor blade previously dipped into the inoculum, and, in the case of CCCVd, high-pressure injection into folded apical leaves. Viroids can also be transmitted by either plant transformation or 'agroinoculation' during which a modified *Agrobacterium tumefaciens* Ti plasmid is used to introduce full-length viroid-complementary DNA into the potential host cell. Either technique can overcome the marked resistance of some hosts to mechanical inoculation. Identification of the molecular mechanism(s) that determine viroid host range remains an important research goal.

Symptomatology

Viroids and conventional plant viruses induce a very similar range of macroscopic symptoms. Symptom expression is usually optimal at the same relatively high temperatures (30–33 °C) that promote viroid replication. Stunting and leaf epinasty (a downward curling of the leaf lamina resulting from unbalanced growth within the various cell layers) are considered the classic symptoms of viroid infection. Other commonly observed symptoms include vein clearing, veinal discoloration or necrosis, and the appearance of localized chlorotic/necrotic spots or mottling in the foliage. Symptoms may also be expressed in flowers and bark, and fruits or tubers from viroid-infected plants may be abnormally shaped or discolored. Viroid infection of certain citrus rootstock/scion combinations may result in tree dwarfing (**Figure 3**). Viroid infections are often latent and rarely kill the host.

Viroid infections are also accompanied by a number of cytopathic effects – chloroplast and cell wall abnormalities, the formation of membranous structures in the cytoplasm, and the accumulation of electron-dense deposits in both chloroplasts and cytoplasm. Metabolic changes include dramatic alterations in growth regulator levels.

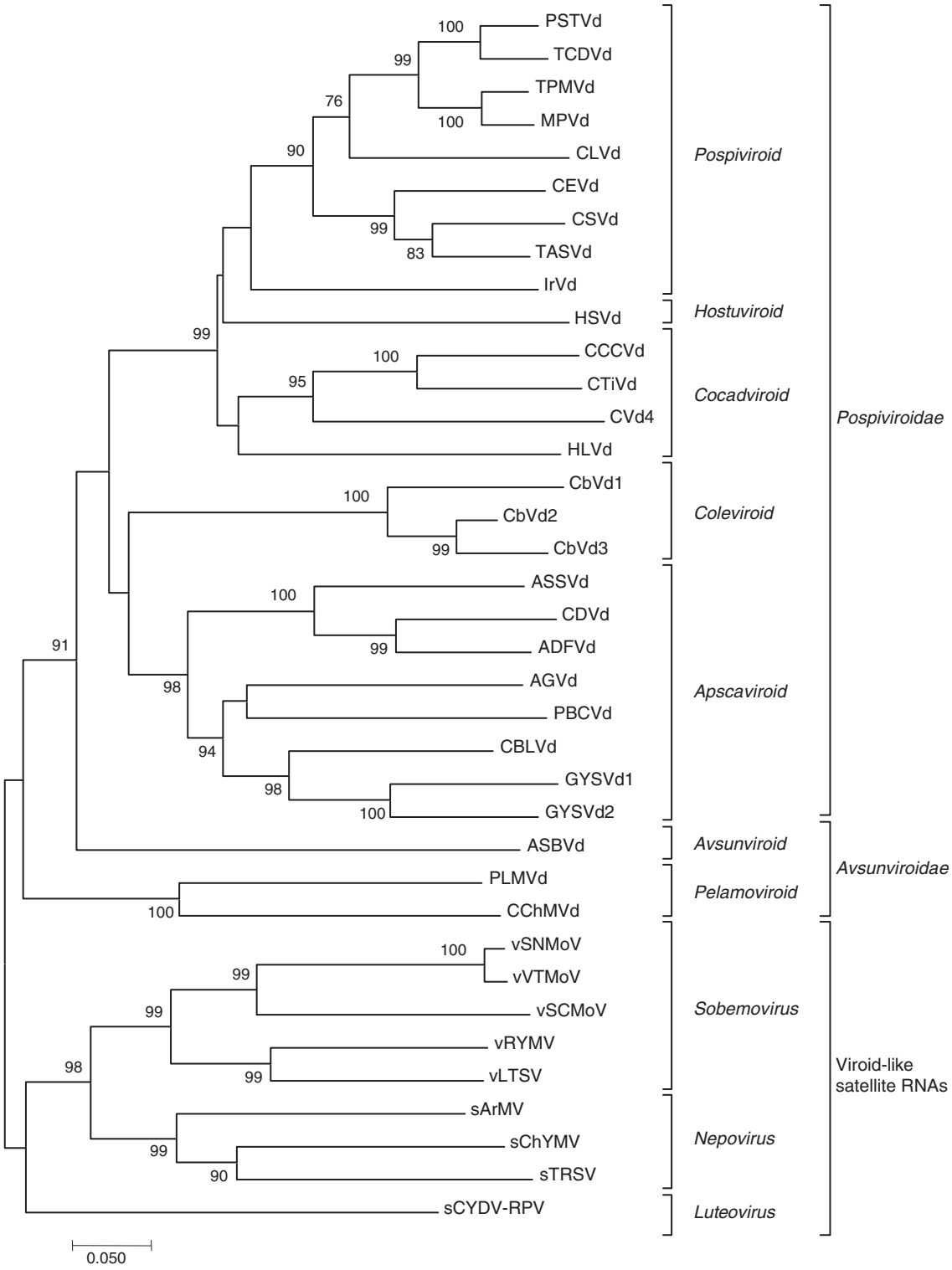

Figure 2 Neighbor-joining phylogenetic tree obtained from an alignment manually adjusted to take into account local similarities, insertions/deletions, and duplications/rearrangements described in the literature for viroid and viroid-like satellite RNAs. Bootstrap values were based on 1000 random replicates (only values >70% are shown). Viroid abbreviations are those used in **Table 1**. Viroid-like satellite RNAs: lucerne transient streak virus (sLTSV); rice yellow mottle virus (sRYMV); subterranean clover mottle virus (sSCMoV); *Solanum nodiflorum* mottle virus (sSNMoV)); velvet tobacco mottle virus (sVTMoV); tobacco ringspot virus (sTRSV); *Arabis* mosaic virus (sArMV); chicory yellow mottle virus (sChYMV); cereal yellow dwarf virus-RPV (sCYDV-RPV). Adapted from Elena SF, Dopazo J, de la Peña M, Flores R, Diener TO, and Moya A (2001) Phylogenetic analysis of viroid and viroid-like satellite RNAs from plants: A reassessment. *Journal of Molecular Evolution* 53: 155–159.

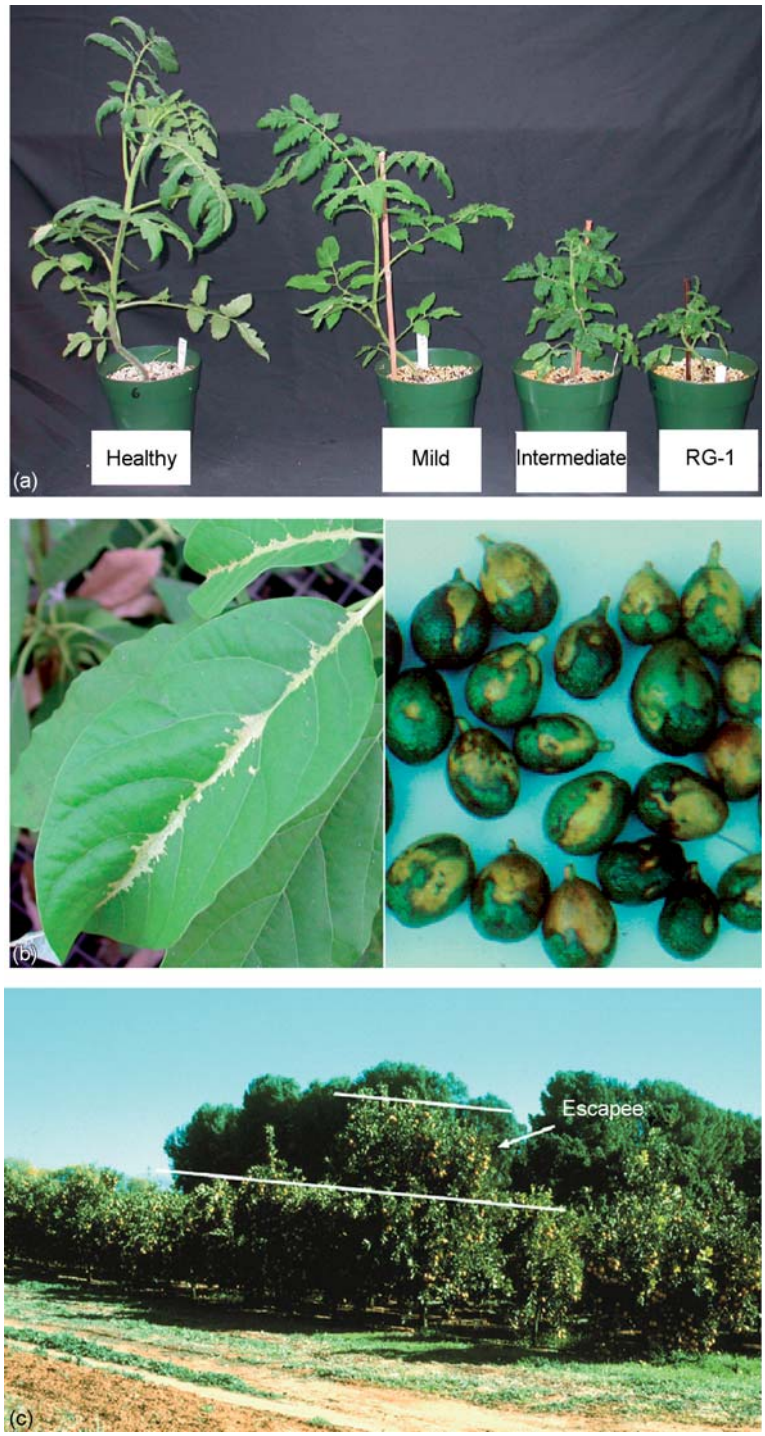

Figure 3 (a) Symptoms of PSTVd infection in Rutgers tomato approximately 4 weeks after inoculation of cotyledons with PSTVd strains causing mild, intermediate, and severe symptoms. (b) Symptoms of ASBVd infection in avocado fruits and leaves. (c) Viroid-induced dwarfing of citrus growing on susceptible rootstocks: All trees in the block were graft-inoculated with CDVd shortly after transfer to the field; only one tree (right foreground) escaped infection. Note the difference in height.

Geographic Distribution

Although PSTVd, HSVd, CEVd, and ASBVd are widely distributed throughout the world, other viroids have never been detected outside the areas where they were first reported. Several factors may contribute to this variation in distribution pattern. Among the crops most affected by viroid diseases are a number of valuable woody perennials such as grapes, citrus, various pome and stone fruits, and hops. Propagation and distribution of improved cultivars is

highly commercialized, with the result that many cultivars are now grown worldwide. The international exchange of plant germplasm also continues to increase at a rapid rate. In both instances, the large number of latent (asymptomatic) hosts facilitates viroid spread.

Epidemiology and Control

Viroid diseases pose a potential threat to agriculture, and several are of considerable economic importance. Ready transmission of PSTVd by vegetative propagation, foliar contact, and true seed or pollen continues to pose a serious threat to potato germplasm collections and breeding programs. Coconut cadang-cadang has killed over 30 million palms in the Philippines since it was first recognized in the early 1930s. While many viroids were first detected in ornamental or crop plants, most viroid diseases are thought to result from chance transfer from endemically infected wild species to susceptible cultivars. Several lines of circumstantial evidence are consistent with this hypothesis:

1. The experimental host ranges of several viroids include many wild species, and these wild species often tolerate viroid replication without the appearance of recognizable disease symptoms.
2. Although co-evolution of host and pathogen is often accompanied by appearance of gene-for-gene vertical resistance, no useful sources of resistance to PSTVd infection have been identified in the cultivated potato.
3. Viroids and/or viroid-related RNAs closely related to TPMVd and CCCVd have been detected in weeds and other wild vegetation growing near fields containing viroid-infected plants.

Growers and plant pathologists are unlikely to have simply overlooked diseases with symptoms as severe as those of chrysanthemum stunt or cucumber pale fruit, two diseases first reported after World War II. Large-scale monoculture of genetically identical crops and the commercial propagation/distribution of many cultivars are two comparatively modern developments which would facilitate the development of serious disease problems following the chance transfer of viroids from wild hosts to cultivated plants. Viroid diseases may also arise by transfer between cultivated crop species. For example, pears provide a latent reservoir for ASSVd; likewise, while HSVd infections of grapes are often symptomless, this viroid causes severe disease in hops. In both instances, the two crops are often grown in close proximity.

Because no useful sources of natural resistance to viroid disease are known, diagnostic tests continue to play a key role in efforts to control viroid diseases. Since viroids lack a protein capsid, the antibody-based techniques used to detect many plant viruses are not applicable. Tests based upon their unique molecular properties have largely supplanted biological assays for viroid detection. Problems with viroid bioassays include the length of time required for completion (weeks to years) and difficulties in detecting mild or latent strains. Several rapid (1–2 day) protocols involving polyacrylamide gel electrophoresis (PAGE) under denaturing conditions take advantage of the circular nature of viroids. Using these protocols, nanogram amounts of viroid can be unambiguously detected without the use of radioactive isotopes. In recent years, diagnostic procedures based upon nucleic acid hybridization or the polymerase chain reaction (PCR) are being widely used. The simplest methods involve the hybridization of a nonradioactively labeled viroid-complementary DNA or RNA probe to viroid samples that have been bound to a solid support followed by colorimetric or chemiluminescent detection of the resulting DNA–RNA or RNA–RNA hybrids. Such conventional 'dot blot' assays can detect picogram amounts of viroids using clarified plant sap or tissue prints rather than purified nucleic acid as the viroid source. PCR-based protocols are finding increasing acceptance in those cases where either this level of sensitivity is inadequate or a number of closely related viroids are present in the same sample.

Molecular Biology

Although devoid of messenger RNA activity, viroids replicate autonomously and cause disease in a wide variety of plants. Much has been learned about the molecular biology of viroids and viroid–host interaction over the past 25 years, but the precise nature of the molecular signals involved remains elusive. A series of questions first posed by Diener summarizes the many gaps in our current understanding of the biological properties of these unusual molecules:

1. What molecular signals do viroids possess (and cellular RNAs evidently lack) that induce certain DNA-dependent RNA polymerases to accept them as templates for the synthesis of complementary RNA molecules?
2. What are the molecular mechanisms responsible for viroid replication? Are these mechanisms operative in uninfected cells? If so, what are their functions?
3. How do viroids induce disease? In the absence of viroid-specified proteins, disease must arise from direct interaction(s) of viroids (or viroid-derived RNA molecules) with host-cell constituents. Infections by PSTVd and ASBVd induce RNA silencing (see below).
4. What determines viroid host range? Are viroids restricted to higher plants, or do they have counterparts in animals?
5. How did viroids originate?

Replication

A variety of multimeric plus- and minus-strand RNAs have been detected by nucleic acid hybridization in viroid-infected tissues. Based on their analysis, viroid replication has been proposed to proceed via a 'rolling circle' mechanism that involves reiterative transcription of the incoming plus circular RNA to produce a minus-strand RNA template. ASBVd and related viroids utilize a symmetric replication cycle in which the multimeric minus strand is cleaved to unit-length molecules and circularized before serving as template for the synthesis of multimeric plus strands. PSTVd and related viroids utilize an asymmetric cycle in which the multimeric minus strand is directly transcribed into multimeric plus strands. In both cases, the multimeric plus strands are cleaved to unit-length molecules and circularized.

A diversity of host-encoded enzymes have been implicated in viroid replication. Low concentrations of α-amanitin specifically inhibit the synthesis of both PSTVd plus and minus strands in nuclei isolated from infected tomato, strongly suggesting the involvement of DNA-dependent RNA polymerase II, transcribing an RNA template, in the replication of PSTVd and related viroids. In nuclear extracts, transcription of the PSTV plus strand by RNA polymerase II starts in the left terminal loop; furthermore, incubation of active replication complexes containing CEVd with a monoclonal antibody directed against the carboxy-terminal domain of RNA polymerase II results in the immunoprecipitation of both CEVd plus- and minus-strand RNAs. Mature PSTVd plus strands accumulate in the nucleolus and the nucleoplasm, while *in situ* hybridization indicates that minus-strand RNAs are confined to the nucleoplasm. The identity of the polymerase(s) responsible for replication of members of the family *Avsunviroidae* in the chloroplast is less certain. ASBVd synthesis is resistant to tagetitoxin, strongly indicating the involvement of a nuclear-encoded chloroplastic RNA polymerase. Initiation sites for both ASBVd plus- and minus-strand synthesis have been mapped to the AU-rich terminal loops of their respective native structures.

In vitro evidence indicates that specific cleavage of multimeric PSTVd plus-strand RNAs requires (1) rearrangement of the conserved central region to form a branched structure containing a GNRA tetraloop and (2) the action of one or more host-encoded nucleases. Other less-efficient processing sites can also be used *in vivo*. Plus- and minus-strand RNAs of ASBVd and related viroids, in contrast, undergo spontaneous self-cleavage through hammerhead ribozymes to form linear monomers (**Figure 4**). Addition of certain chloroplast proteins acting as RNA chaperones facilitates this hammerhead ribozyme-mediated self-cleavage reaction. The final step in viroid replication is the ligation of linear monomers to form mature circular progeny. Plant cells are known to contain RNA ligase activities which can act upon the 5′ hydroxyl and 2′,3′ cyclic phosphate termini formed by either cleavage pathway.

Movement

Upon entering a potential host cell, viroids must move to either the nucleus (*Pospiviroidae*) or chloroplast (*Avsunviroidae*) before beginning replication. Available data suggest that PSTVd enters the nucleus as a ribonucleoprotein complex formed by the interaction of cellular proteins with specific viroid sequence or structural motifs. VirP1, a bromodomain-containing protein isolated from tomato, has a nuclear localization signal and binds to the terminal right domain of PSTVd. Proteins such as TFIIIA and ribosomal protein L5 that bind to the loop E motif may also be involved in viroid transport into the nucleus. How ASBVd or other members of the family *Avsunviroidae* enter and exit the chloroplast is currently unknown.

To establish a systemic infection, viroids leave the initially infected cell – moving first from cell to cell and then long distances through the host vasculature. Upon injection into symplasmically isolated guard cell in a mature tomato leaf, fluorescently labeled PSTVd RNA does not move. Injection into interconnected mesophyll cells, in contrast, is followed by rapid cell-to-cell movement through the plasmodesmata. Long-distance movement of viroids, like that of nearly all plant viruses, occurs in the phloem where it follows the typical source-to-sink pattern of photoassimilate transport. Viroid movement in the phloem almost certainly requires formation of a ribonucleoprotein complex, possibly involving a dimeric lectin known as phloem protein 2 (PP2), the most abundant protein in phloem exudate. Movement of PSTVd in the phloem appears to be sustained by replication in supporting cells and is tightly regulated by developmental and cellular factors. For example, *in situ* hybridization reveals the presence of PSTVd in vascular tissues underlying the shoot apical meristem of infected tomato, but entry into the shoot apical meristem itself appears to be blocked. Another important control point for PSTVd trafficking is the bundle sheath–mesophyll boundary in the leaf. By disrupting normal pattern of viroid movement, it may be possible to create a plant that is resistant/immune to viroid infection.

Pathogenicity

Sequence comparisons of naturally occurring PSTVd and CEVd variants as well as infectivity studies with chimeric viroids, constructed by exchanging the pathogenicity domains of mild and severe strains of CEVd, have clearly shown that the pathogenicity domain in the family *Pospiviroidae* contains important determinants of symptom expression. Symptom expression is also affected by the

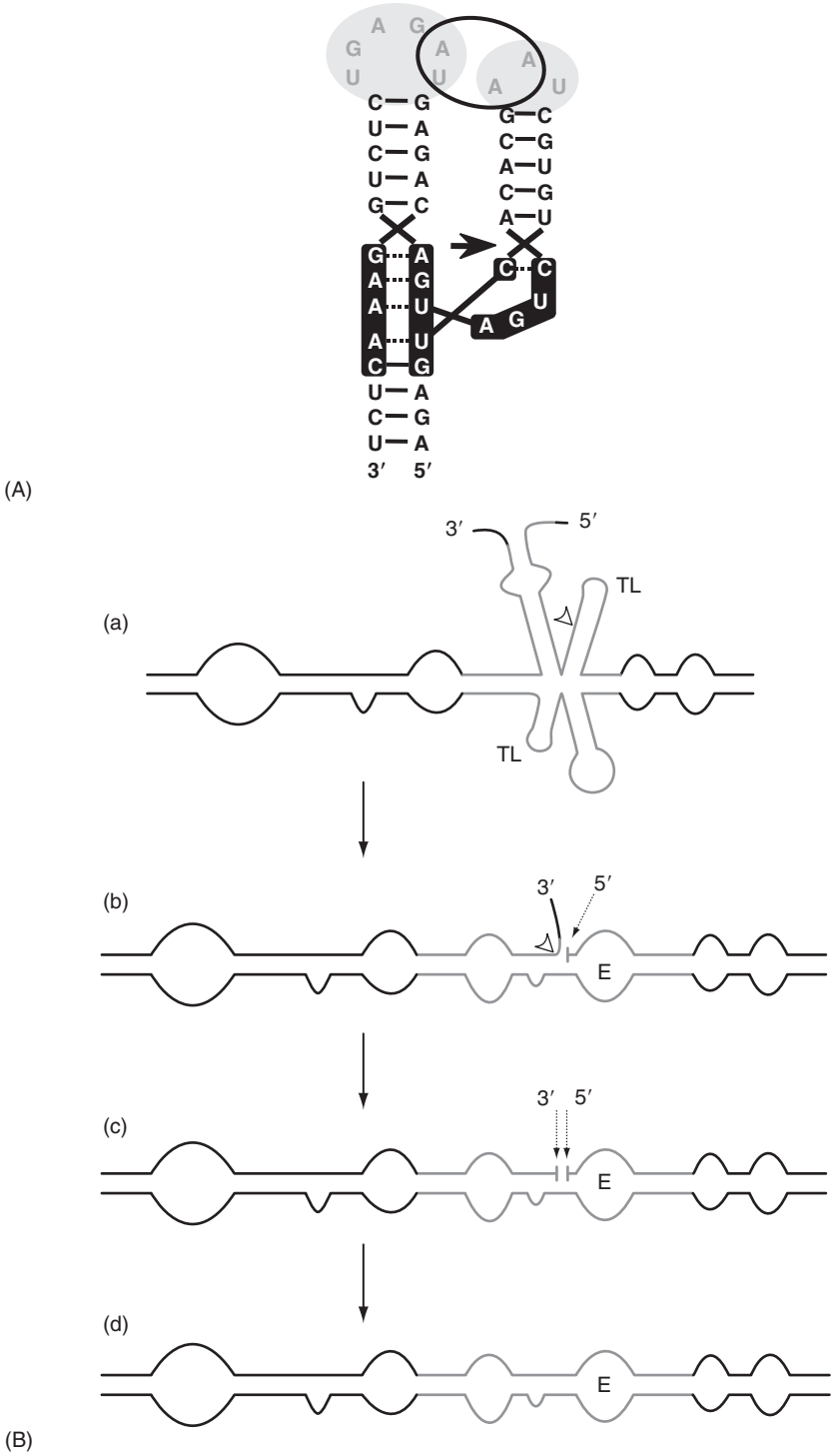

Figure 4 Cleavage of multimeric viroid RNAs requires rearrangement of the native structure. (A) During transcription, the strands of both polarities of members of the family *Avsunviroidae* can fold into hammerhead structures (here illustrated for the hammerhead of the PLMVd plus RNA) and self-cleave accordingly. Nucleotides conserved in most natural hammerhead structures are on a black background, and the self-cleavage site is denoted by an arrow. A circle delimits the presumed tertiary interaction between terminal loops enhancing the catalytic activity. Watson-Crick and noncanonical base pairs are represented by continuous and discontinuous lines, respectively. After self-cleavage, the RNA adopts a new conformation favoring ligation (or self-ligation). (B) Processing of a longer-than-unit-length plus PSTVd RNA transcript in a potato nuclear extract. The central conserved region of the substrate for the first cleavage reaction (a) contains a tetraloop (denoted TL). After dissociation of the 5′ segment from the cleavage site, the new 5′ end refolds and is stabilized by formation of a UV-sensitive loop E (b), while the 3′ end partially base-pairs with the lower strand. Single-stranded nucleotides at the 3′ end are then cleaved between positions 95 and 96 (c), and ligation of the 5′ and 3′ termini (d) results in formation of mature circular progeny. From Baumstark T, Schröder ARW, and Riesner D (1997) Viroid processing: Switch from cleavage to ligation is driven by a change from a tetraloop to a loop E conformation. *EMBO Journal* 16: 599–610.

rate of viroid replication, and sequence changes in the variable domain have been shown to regulate progeny titers in infected plants. Studies with TASVd revealed the presence of a third pathogenicity determinant in the left terminal loop. Also, a single U/A change position 257 in the central domain of PSTVd results in the appearance of severe stunting and a 'flat top' phenotype. In the family *Avsunviroidae,* determinants of pathogenicity have been mapped to either a tetraloop capping a hairpin stem in chrysanthemum chlorotic mottle viroid (CChMVd) or an insertion that folds into a hairpin also capped by a tetraloop in peach latent mosaic viroid (PLMVd).

The ability of novel viroid chimeras to replicate and move normally from cell to cell implies certain basic similarities between their structures *in vitro* and *in vivo* but provides no information about the nature of the molecular interactions responsible for symptom development. Until recently, it was widely assumed that the mature viroid RNA was the direct pathogenic effector. Just like viruses, however, viroid replication is also accompanied by the production of a variety of small (21–26 nt) RNA molecules. The role of these small interfering RNAs (siRNAs) in viroid pathogenicity is not yet clear, but the inverse relationship between accumulation levels of the mature viroid RNAs and the corresponding siRNAs for members of the family *Avsunviroidae* suggest that the latter may regulate the titer of the former. Also, recovery of tomato plants from the symptoms of severe PSTVd infections is preceded by the accumulation of PSTVd-specific siRNA.

Viroid infections are accompanied by quantitative changes in a variety of host-encoded proteins. Certain of these are 'pathogenesis-related' proteins whose synthesis or activation is part of a general host reaction to biotic or abiotic stress, but others appear to be more specific. In tobacco, PSTVd infection results in the preferential phosphorylation of a host-encoded 68 kDa protein that is immunologically related to an interferon-inducible, dsRNA-dependent mammalian protein kinase of similar size. The human kinase is differentially activated by PSTVd strains of varying pathogenicity *in vitro*, while infection of tomato by intermediate or severe strains of PSTVd induces the synthesis of PKV, a dual-specificity, serine/threonine protein kinase. Broad changes in host gene expression following PSTVd infection have been detected by complementary DNA macroarray analysis.

Host Range

Possibly as a result of its involvement in the cleavage/ligation of progeny RNA, nucleotides in the central domain of PSTVd and related viroids appear to play an important role in determining host range. For example, a single nucleotide substitution in the loop E motif results in a dramatic increase in the rate of PSTVd replication in tobacco. The biological properties of CLVd also suggest that this domain contains one or more host-range determinants. CLVd appears to be a natural mosaic of sequences present in other viroids; phylogenetic analysis (see **Figure 2**) suggests that it can be considered to be a PSTVd-related viroid whose conserved central domain has been replaced by that of HSVd. Like HSVd (but not PSTVd or related viroids), CLVd can replicate and cause disease in cucumber.

Origin and Evolution

Much of the early speculation about viroid origin involved their possible origin as 'escaped introns' (i.e., descent from normal host RNAs). More recently, however, viroids have been proposed to represent 'living fossils' of a precellular RNA world that assumed an intracellular mode of existence sometime after the evolution of cellular organisms. The presence of ribozymes in members of the *Avsunviroidae* strongly supports this view.

The inherent stability of viroids and viroid-like satellite RNAs (structurally similar to viroids but functionally dependent on helper viruses) which arises from their small size and circularity would have enhanced the probability of their survival in primitive, error-prone RNA self-replicating systems and assured their complete replication without the need for initiation or termination signals. Most viroids (but not satellite RNAs or random sequences of the same base composition) also display structural periodicities with repeat units of 12, 60, or 80 nt. The high error rate of prebiotic replication systems may have favored the evolution of polyploid genomes, and the mechanism of viroid replication (i.e., rolling-circle transcription of a circular template) provides an effective means of genome duplication.

Viroids and viroid-like satellite RNAs all possess efficient mechanisms for the precise cleavage of their oligomeric replication intermediates to form monomeric progeny. PSTVd and related viroids appear to require proteinaceous host factor(s) for cleavage, but others (members of the family *Avsunviroidae* and viroid-like satellite RNAs) contain ribozymes far smaller and simpler than those derived from introns. Thus, ASBVd and the other self-cleaving viroids may represent an evolutionary link between viroids and viroid-like satellite RNAs. No viroid is known to code for protein, a fact that is consistent with the possibility that viroids are phylogenetically older than introns.

Phylogenetic evidence for an evolutionary link between viroids and other viroid-like subviral RNAs has been presented by Elena *et al.* (see **Figure 2**). Among several subviral RNAs possibly related to viroids is carnation small viroid-like RNA, a 275 nt circular molecule with self-cleaving hammerhead structures in both its plus and minus strands that has a DNA counterpart. This novel retroviroid-like element shares certain features with both viroids and a small RNA transcript from newt.

See also: Hepatitis Delta Virus; Origin of Viruses; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Quasispecies; Ribozymes; Satellite Nucleic Acids and Viruses.

Further Reading

Baumstark T, Schröder ARW, and Riesner D (1997) Viroid processing: Switch from cleavage to ligation is driven by a change from a tetraloop to a loop E conformation. *EMBO Journal* 16: 599–610.

Bussière F, Ouellet J, Côté F, Lévesque D, and Perreault JP (2000) Mapping in solution shows the peach latent mosaic viroid to possess a new pseudoknot in a complex, branched secondary structure. *Journal of Virology* 74: 2647–2654.

Diener TO (1979) *Viroids and Viroid Diseases.* New York: Wiley-Interscience.

Ding B, Itaya A, and Zhong X (2005) Viroid trafficking: A small RNA makes a big move. *Current Opinion in Plant Biology* 8: 606–612.

Elena SF, Dopazo J, de la Peña M, Flores R, Diener TO, and Moya A (2001) Phylogenetic analysis of viroid and viroid-like satellite RNAs from plants: A reassessment. *Journal of Molecular Evolution* 53: 155–159.

Flores R, Hernandez C, Martinez de Alba AE, Daros JA, and DiSerio F (2005) Viroids and viroid–host interactions. *Annual Review of Phytopathology* 43: 117–139.

Flores R, Randles JW, Owens RA, Bar-Joseph M, and Diener TO (2005) Viroids. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball AL (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1145–1159. San Diego, CA: Elsevier Academic Press.

Gross HJ, Domdey H, Lossow C, *et al*. (1978) Nucleotide sequence and secondary structure of potato spindle tuber viroid. *Nature* 273: 203–208.

Hadidi A, Flores R, Randles JW, and Semancik JS (eds.) (2003) *Viroids.* Collingwood: CSIRO Publishing.

Hernández C and Flores R (1992) Plus and minus RNAs of peach latent mosaic viroid self-cleave *in vitro* through hammerhead structures. *Proceedings of the National Academy of Sciences, USA* 89: 3711–3715.

Hull R (2002) *Matthews' Plant Virology,* 4th edn. New York: Academic Press.

Virus Classification by Pairwise Sequence Comparison (PASC)

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Glossary

Demarcation A mapping of ranges of pairwise distances into taxonomic categories.

Introduction

Virus classification is very important for virus research. It is also an extremely difficult task for many virus families. Traditionally, virus classification relied on properties such as virion morphology, genome organization, replication mechanism, serology, natural host range, mode of transmission, and pathogenicity. Yet viruses sharing the above properties can reveal tremendous differences at the genome level. For example, classification of many phages is currently based on presence, structure, and length of a tail, and this approach has been shown not to correlate with genomic information, leading to a very difficult situation and hundreds of unclassified phages.

Molecular virus classification based on virus sequences has been used increasingly in recent years, thanks to the growing number of viral sequences available in the public sequence databases. The most commonly used sequence comparison methods include multiple sequence alignment and phylogenetic analysis. Another molecular classification method that has drawn more and more attention from virologists is pairwise sequence comparison (PASC). In this article, we briefly describe various sequence comparison methods, introduce the PASC tool, and compare it with other methods.

Sequence Comparison Methods

A universal approach to compare biological sequences, in a sense of producing meaningful results at various levels of divergence, is in the realm of sequence alignment. An alignment is an arrangement of residues of two or more sequences in a way that reveals their possible relatedness, with space characters inserted into the sequences to indicate single-residue insertions and deletions. A variety of algorithms and programs are available to suit a wide range of problems requiring sequence alignments as parts of their solutions. Depending on the specifics of a problem, different types of algorithms or their combinations may work best. Most alignment algorithms can be broadly categorized by the scope of their application on sequences (local vs. global), or by the number of sequences involved (pairwise vs. multiple).

Each pairwise alignment can be viewed as an array of per-residue operations transforming one sequence to the other. These operations are substitutions (called matches