Encyclopedia

Viroids: Definition and features

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Viroids are plant-restricted parasites that represent a remarkable model system to analyze many aspects of host-pathogen interactions at the genomic level . As the smallest known agents of infectious disease (247-401 nucleotides, nt), they have a highly structured, single-stranded circular naked and non-coding RNA genome. Although the list of known diseases caused by viroids and molecular characterization of the causative agents has expanded since they were discovered, their origin,evolution, and interaction with host genetic machinery to induce symptoms or escape the defensive system remains unclear.

Viroids were discovered by Dr. Theodor Diener while undergoing efforts to identify the cause of a potato spindle tuber disease. In the first instance, the disease was believed to be caused by a virus. Experiments designed to identify the hypothetical presumed virus yielded unexpected results. Observations revealed that most of the infectious agent present in extracts from diseased plants did not sediment into a pellet when subjected to a centrifugal force that was enough to sediment all known viruses. Density gradient centrifugation and polyacrylamide gel electrophoresis confirmed that the infectious agent was an unconventional particle. This peculiar particle was sensitive to treatment with ribonuclease (no infectivity), and insensitive to deoxyribonuclease, phenol, chloroform, n-butanol or ethanol treatments. In 1967 it become evident that the agent of the potato spindle disease was not a virus, so the term “viroid” was proposed for this free infectious RNA[2][3][4]

Viroids are plant restricted parasites which might lead to conditions that cause significant losses in agricultural crops. Viroids consist of single-stranded, circular and low molecular weight RNA (246-496 nt), they do not possess a protein or membrane shell, however, and given their complex secondary structure they have unusual properties such as resistance to ribonuclease digestion and denaturation. Viroids do not encode any protein to provide specific functions, their mechanism of systemic infection is based on sequestering the nucleic acid synthesis machinery of the host cell and interacting with genetic host factors, and replicating autonomously by rolling circle replication mechanisms (rates of replication may exceed rates of degradation).

The replication intermediates involve dsRNA and are processed in three basic steps: the synthesis of long strings (multiple units) mediated by host DNA-dependent RNA polymerases, which are then processed to monomers and finally ligated into circular units Reference. The most intriguing questions which remain concerning viroid diseases are:1) how do they replicate autonomously in host plants (molecular basis of viroid host range) evading defense mechanisms against infection, 2) how do viroids spread systemically (what host factors participate in specific steps of the replication cycle and trafficking) and 3) how do viroids cause disease symptoms (what are the targets of viroid pathogenicity determinants) without enconding proteins.

Classification Scheme

Comparative analyses of the primary and secondary structures have allowed the classification of viroids into two families; these families exhibit significant differences in their secondary structures, replication pathways, and subcellullar localization[5]

Pospiviroidae family (most viroids) is an acronym derived of potato spindle tuber viroid, the representative species type. Viroids that belong to this family adopt a quasi-rod or rod-like secondary structure, with

dsRNA regions separated by unpaired internal single chain loops, in which five structural domains can be distinguished (refernce). The Central Conserved Region (CCR) that contains conserved sites among species from the same genus, and plays a role in viroid replication/processing, the left and right terminal domains (T L , T R) related to duplication and movement of the viroid, the variable domain (V) that is the most different among viroid species from the same genus, and the pathogenicity domain (P) containing structural elements that contribute substantially to the regulation of symptom expression [6]

Within these domains, there are three which are conserved among species: 1) CCR, formed by two opposing series of nucleotides which are flanked by repeated reverse sequences (the lower and upper branch), 2) Terminal Conserved Region (TCR),located on the upper branch of the left terminal domain, and 3) Terminal Conserved Hairpin (TCH), which is also located on the left terminal domain. The sequence of the CCR, and the presence or absence of TCR and TCH (both regions do not co-existsimultaneously) served to group viroid species of this family into five genera (the type of CCR serves to define the genus). The species are primarly defined on the basis of their primary structure. An arbitrary level of 90% sequence identity is accepted to separate species from strains [7]

Avsunviroidae family.-The three conserved motifs mentioned above are not present in four viroids species that belong to the second family termed Avsunviroidae, that is named after the type species, ASBVd. The classification of this species is based on the G+ C composition and secondary structure predictions, on the morphology of the hammerhead ribozyme (HHRz) and on basis of the LiCl insolubility. These species exhibit the property that the strands of both polarities are able to undergo auto- cleavage by hammerhead ribozymes, and further, two of them (ASBVd, PLMVd) adopt clearly branched secondary structures and tertiary structure elements that help to stabilize the structure.

The classification of viroids into two families is also an important endorsement from another perspective that is linked to replication. Pospiviroidae family members replicate and accumulate in the nucleus and the nucleolus following an asymmetric rolling-circle mechanism, and the host enzymes that could be involved in the replication of the members of this family are enzymes having nuclease activity specifically members of the RNase III family, an enzyme different from the onlyligase characterized in plants, and the RNA-dependent DNA polymerase II [8]; while Avsunviroidae family members replicate and accumulate in the chloroplast and replication proceeds via symmetric rolling-circle mechanism using a nucleus-encoded plastid (NEP)-like RNA polymerase, act as self-catalyzing ribozymes performed by hammerhead motifs contained in strands of both polarities, and have been postulated to also possess the property of self-ligation [9]

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Keywords

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