

Sub-viral pathogens

Satellite viruses, virusoids, viroids and the hepatitis δ agent

Characteristics, biological importance and modes of replication of:

- satellite viruses
- virusoids
- viroids
- hepatitis δ agent

(satellite RNAs considered earlier)

A REMINDER of the CHARACTERISTICS of SATELLITE VIRUSES

The first to be discovered and the model example is the satellite virus of tobacco necrosis virus (SV-TNV).

SV-TNV RNA only 0.4×10^5 molecular weight and codes only for the SV capsid protein which is distinct from that of its helper virus

They are mostly plant viruses, just a few satellite viruses exist in animals, e.g. the adeno-associated virus (a replication-defective parvovirus; genus *Dependovirus*).

Satellite viruses

is highly specific and in plants, the presence of satellite virus often modifies symptoms.

In plants, they require some assistance from the helper virus in order to replicate their NA (replicase function).

WHAT ARE the CHARACTERISTICS of VIRUSOIDS?

Virusoids are covalently closed circular single-stranded viroid-like satellite RNA molecules of 300 to 400 nts with a high degree of secondary structure. (e.g. velvet tobacco mottle virus + virusoid)

virus.

They do not code for a protein but become encapsidated along with the RNA of the

There is marked nucleotide sequence homology between some virusoids and some viroids. In short, plant virusoids are encapsidated viroid-like RNAs that seem incapable of autonomous replication.

So far, they are known only in plants - but the hepatitis δ agent has some similarities to both virusoids and viroids

WHAT ARE the CHARACTERISTICS of VIROIDS?

Cause ~12 commercially important diseases of crop plants, best known example is potato spindle tuber viroid (PSTV).

They are absent from healthy plants, are mechanically transmissible, and replicate on introduction into plants.

Comprise a circular single stranded RNA molecule of ~ 1.1 to 1.3×10^5 kDa; equivalent to ~ 246 to 374 nts.

Although not encapsidated, they are extremely stable because a high degree of complementarity gives much double-stranded structure.

They are classified on the basis of nucleotide sequences into two groups (A and B; group B is further sub-divided).

Group A lacks a central conserved region (CCR) and has ribozyme activity (e.g. avocado sunblotch viroid); group B (e.g. potato spindle tuber viroid, group B1) has a CCR but no ribozyme activity.

Their genome contains no information coding for protein (either lack AUG start or GUG inoperative - *in vitro*).

When replicating, they must therefore use host factors that either pre-exist or are induced by viroid infection.

IMPORTANT QUESTIONS ABOUT VIROIDS

What signals do viroid RNA molecules contain (but host RNA molecules do not), that:

- ensure they will be replicated?
- ensure transport to the nucleus (where they are replicated)?
- ensure cell to cell transport (for the spread of infection)?
- induce disease symptoms?

How is viroid RNA is replicated?

How did viroids arise and why do they seem to be restricted to plants?

HOW CAN WE DECIPHER THE SIGNALS CONTAINED in VIROID RNAs

Two approaches have been used:

- sequence comparisons amongst natural variants
- site directed mutagenesis

Sequence comparisons suggest five structural domains or regions

CR

the most highly conserved central domain ~95 nts,

contains an oligo A (15-17 nts

less than 50% homology between otherwise closely related viroids (compared with ~99% in the C domain); a small oligo purine/oligo pyrimidine helix (minimum 3 bps) which is conserved

I = left and T2 = right

slightly conserved sequences to create loops (i.e. unpaired regions)

HOW CAN WE DECIPHER THE SIGNALS CONTAINED in VIROID RNAs

Site directed mutagenesis gives three groups of mutants.

Lethal, i.e. no replication

Most mutations occur in the CC region, effects of some which are lethal can be reversed by further mutations elsewhere.

Replication initially slow

Eventually only wild type sequences are found, i.e. reversion has occurred *in vivo* and revertants out-compete the mutants.

New stable variant

Progeny differ both from the wild type and from the mutant as inoculated but are generated *in vivo* and replicate efficiently, most occur in the P or VM domain.

The results suggest viroids replicate under strong selection pressures for nucleotide sequence.

Almost every nucleotide contributes to the signal leading to replication, i.e. it is a very condensed replicon.

HOW ARE VIROID RNAs REPLICATED?

Most of the viroid RNA (~10,000 molecules per cell) occurs in nucleolus.

Most exists as the circular single-stranded RNA monomers.

Smaller quantities of:

- linear monomers of +ve and -ve sense RNA
- linear oligomers (up to ~6 unit lengths) of +ve and -ve sense RNA
- circular monomers of -ve sense RNA

Several models have been suggested. The symmetric (double) rolling circle model probably best accounts for the experimental evidence.

Parental circular +ve is transcribed to a linear multimeric -ve by RNA polymerase II, followed by cleavage and circularisation.

Circular -ve strands are transcribed by RNA polymerase I (nucleolar) to linear +ve multimers which are cleaved and circularised.

How is site-specific cleavage and ligation achieved?

- signals in RNA must determine sites.
 - oligomers plus RNase T1 will do so *in vitro*.
 - oligomers of group A viroids ribozyme
structure but ligation mechanism unclear.
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WHAT IS THE ORIGIN OF VIROIDS?

Nucleotide sequence analyses suggest viroids, virusoids and satellite RNAs have a common origin

Possibly viroids diverged from virusoids and satellite RNAs when they were both

- viroids acquiring host-dependency
- virusoids and satellite RNAs acquiring dependency on co-infected plants

Viroids and viroid-like satellite RNAs have sequence similarities to:

- Transposable genetic elements
- Mitochondrial plasmids
- Group I introns

But their origins are uncertain.

WHAT ARE the CHARACTERISTICS of the HEPATITIS δ AGENT?

Occurs in association with hepatitis B virus (HBV) infection, estimated 10×10^6 world wide.

Most serious disease seems to result when acquired as a superinfection to existing HBV infection rather than simultaneously. Mortality ~10-fold higher than HBV alone.

Relationship resembles that between plant viruses and their satellite RNAs.

The δ agent genome is a circular single-stranded RNA molecule ~1700 nts with a high degree of base pairing (~70%) - three main genotypes world wide.

It is encapsidated together with the δ antigen as a 19 nm diameter core particle that is surrounded by the HBsAg of the HBV giving an overall diameter of ~36 nm.

The δ agent encodes the δ antigen, which comprises two (nested) proteins (24 kDa, 195 a.a.s and 27 kDa, 214 a.a.s) and there are about 70 copies per δ particle

The two different proteins are formed, because an RNA editing mechanism creates two types of genome and antigenome molecule that differ by just one base at position 1012. The change means a termination codon in the mRNA produced from one form is changed to tryptophan in the other and 19 more a.a.s are read.

WHAT ARE the CHARACTERISTICS of the HEPATITIS δ RNA and HOW is it REPLICATED?

All hepatitis δ RNAs are produced in the host cell nucleus using host RNA polymerase II (that normally transcribes host nuclear DNA to mRNA).

The genomic RNA is negative sense.

Genomic RNA replication also requires the ribozyme function that occurs in both the genomic (-ve) and full length anti-genomic (+ve) RNA molecules.

Transcription using the genomic (-ve) RNA as a template, yields a full-length antigenome (+ve) molecule and a functional mRNA that is shorter than the anti-genome.

The switch from producing mRNA (~600 copies per cell) to producing antigenome is achieved by suppressing a termination/polyadenylation signal.

Full length genomic(-ve) (~300 000 copies per cell) and antigenomic (+ve) (~50 000 copies per cell) molecules are produced by a symmetric (double) rolling circle mechanism.

The ribozyme activity in both strands releases linear monomers that are subsequently ligated to yield circular molecules. The antigenomic (+ve) RNA is an intermediate in the production of genomic (-ve) RNA.

SUMMARY

There are a variety of sub-viral pathogens, which:

- themselves are probably phylogenetically related,
- have close relationships with viruses and,
- cause important agricultural/human disease.

Most, have RNA as their genome and are found in plants. One however, the hepatitis δ agent, has significant pathogenicity in man.

They all have no, or very little protein coding potential.

Replication of several viroids and of hepatitis δ RNA is achieved by a rolling circle mechanism using host RNA polymerase II and their intrinsic ribozyme activity.
