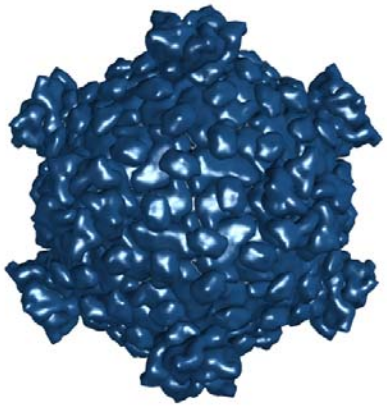
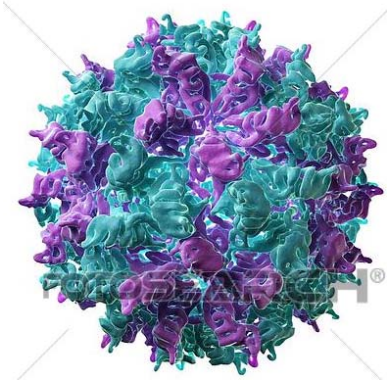
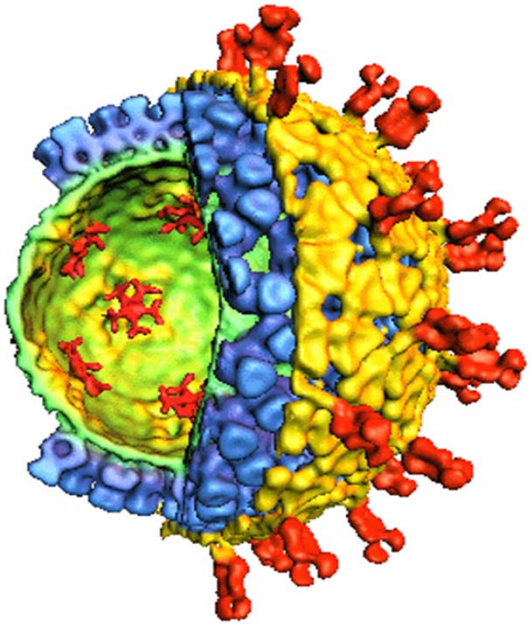
Life Cycle –

dsRNA Viruses And Viroids







Outline:

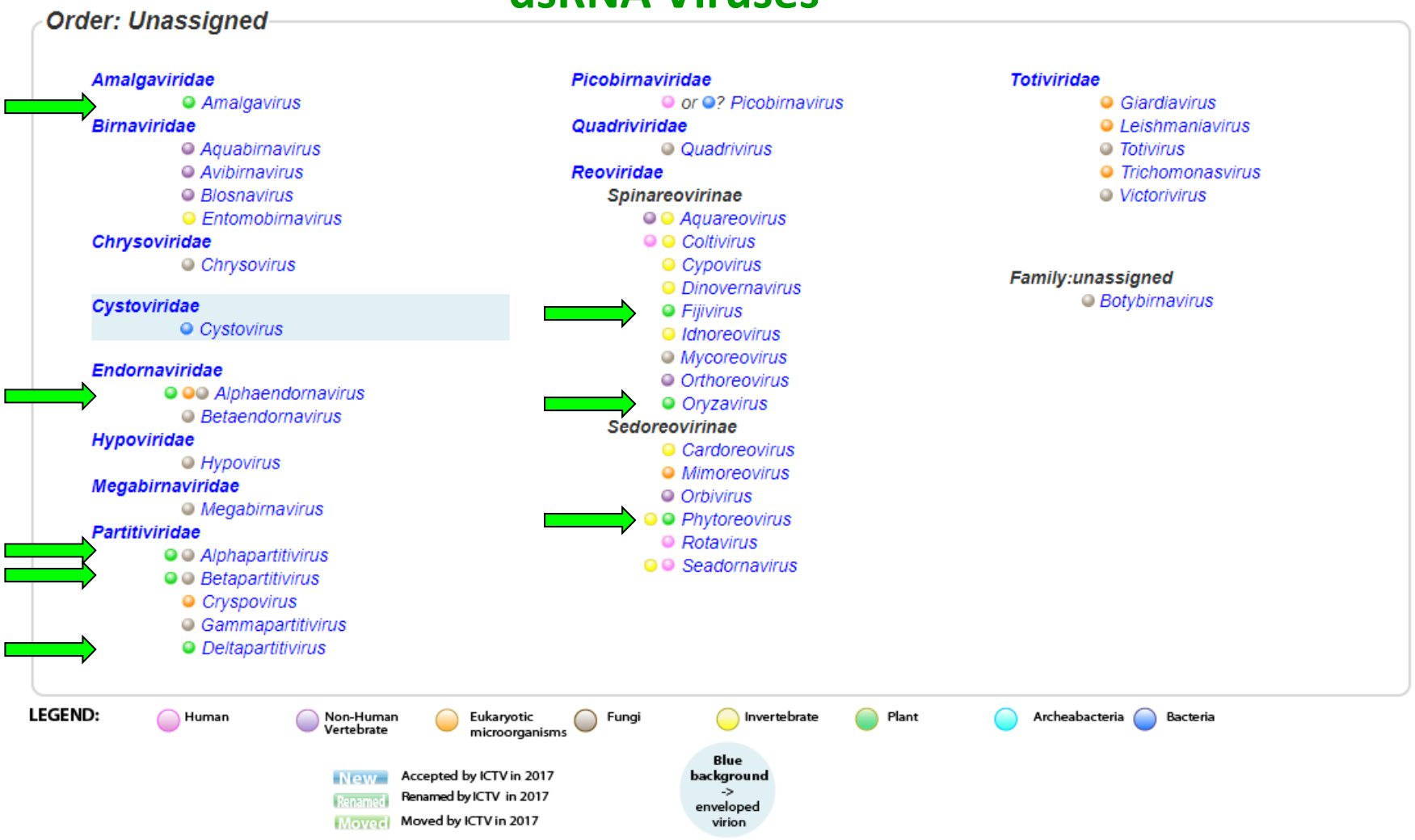
- **dsRNA Virus Families**
- **Life Cycle of dsRNA viruses**

  Family: *Reoviridae*

- **Life Cycle of Viroids**

  Viroids: *Pospiviroidae* and *Avsunvirodae*

dsRNA Viruses

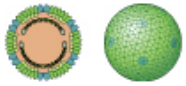


DOUBLE STRAND RNA VIRUSES

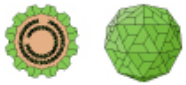
Taxonomy | Virions | Genome stats | Cell Receptors

Vertebrate

Birnaviridae

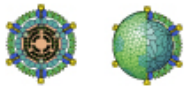


Picobirnaviridae



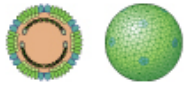
Human

Reoviridae

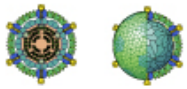


Invertebrate

Birnaviridae



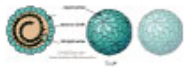
Reoviridae



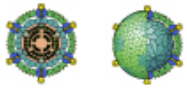
Plants

Endornaviridae

Partitiviridae



Reoviridae



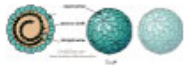
Eukaryotic microorganism

Chrysoviridae

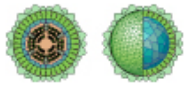
Endornaviridae

Hypoviridae

Partitiviridae



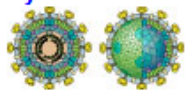
Reoviridae



Totiviridae

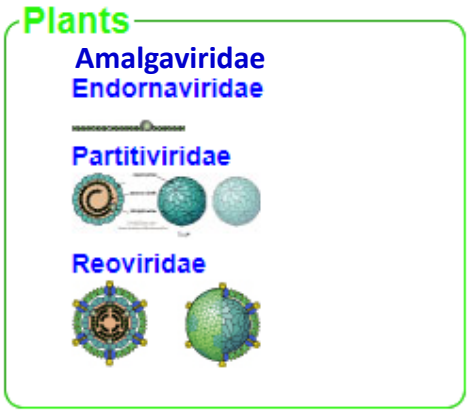
Bacteria

Cystoviridae



Families of dsRNA viruses

- *Amalgaviridae* – 1 segment, Host: plants
- *Endornaviridae* – 1 segment, Host: plants, fungi, oomycetes
- *Partitiviridae* – 4 segments, Host: plants, fungi
- *Reoviridae* – (large family), 10-12 segments,
  Hosts: vertebrates, invertebrates, plants, fungi

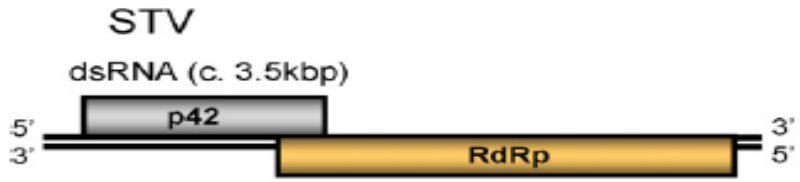


- *Chrysoviridae* – 4 segments, Host: fungi
- *Hypoviridae* – 1 segment, Host: fungi
- *Totiviridae* – 1 or 2 segments, Host: fungi, protozoa,
- *Cystoviridae* – 3 segments, Host: infect bacteria
- *Birnaviridae* – 2 segments, Host: vertebrates, invertebrates
- *Megabirnaviridae* – 2 segments, Host: fungi
- *Picobirnaviridae* – 3 segments, Host: vertebrates
- *Quadriviridae* – 4 segments, Host: fungi

Amalgaviridae -

- No encapsidated virions have been associated with these viruses (virion unknown)

- Linear dsRNA genome of 3.5 kb
- Contains 2 overlapping ORFs.



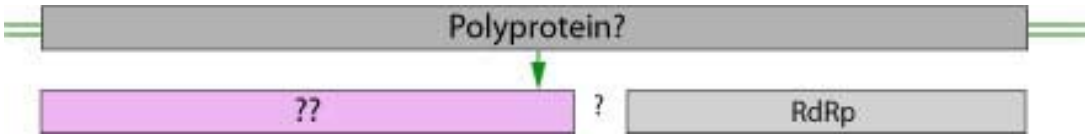
- Not transmitted by mechanical means
- Not transmitted by grafting
- Not transmitted by insects, nematodes, etc…
- Thought to move cell to cell only
- No systemic movement within plant hosts
- Transmitted at high rates by seed

(STV – transmitted through ovule and pollen)

Endornaviridae – (Genus: *Alphaendornavirus*)

Virion:

Genome Organization:



- Hosts: plants, fungi, oomycetes
- Particle: None reported. Probably does not have a capsid.

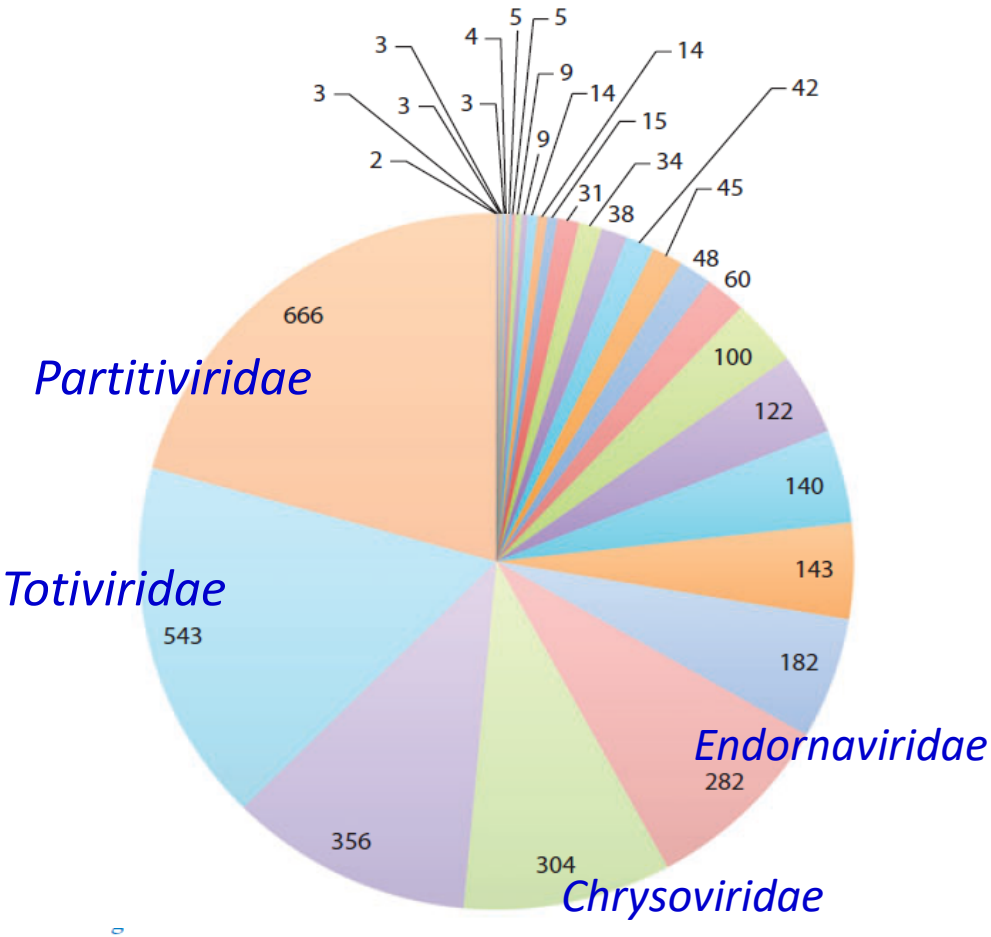
- Linear dsRNA genome (14 kb - 17.6 kb)
- The dsRNA genome is bound to the viral RdRp.

- *Amalgaviridae*
- *Endornaviridae*
- *Partitiviridae*
- *Chrysoviridae*
- *Totiviridae*

Found at high frequencies in wild plants (also found in crop plants

Latent infections or Tolerance:

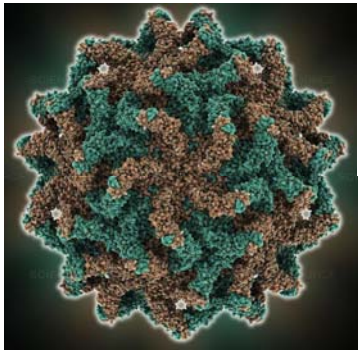
- In wild plants, virus infection often does not correlate with the presence of symptoms.
- Many of them have a persistent lifestyle and do not encode any proteins to aid in their dissemination.
- It is thought that these viruses may have been associated with their wild hosts for a long period on the viral evolutionary timescale.



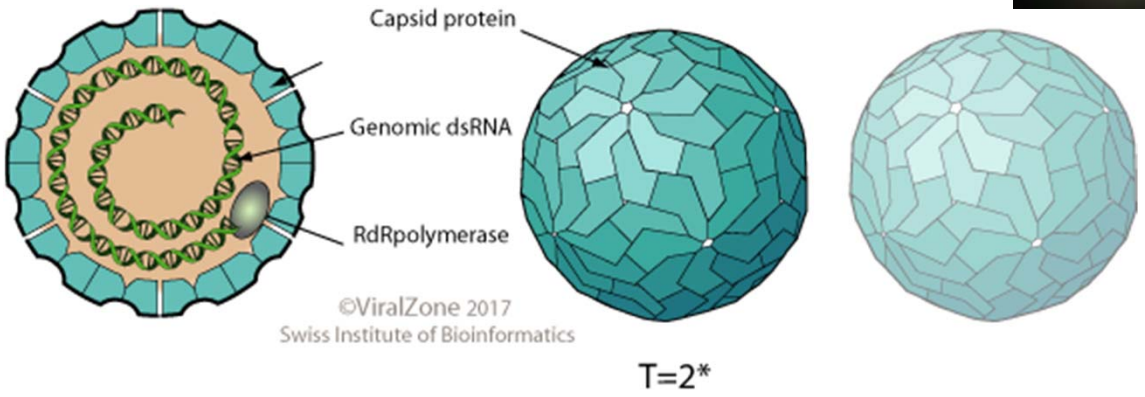
Distribution of plant virus families in wild plants. Numbers indicate the number of individual plants with viruses in the indicated families. Data are pooled from two study sites and are preliminary.

Roosinck 2012 Ann. Rev Plant Genetics 46:357

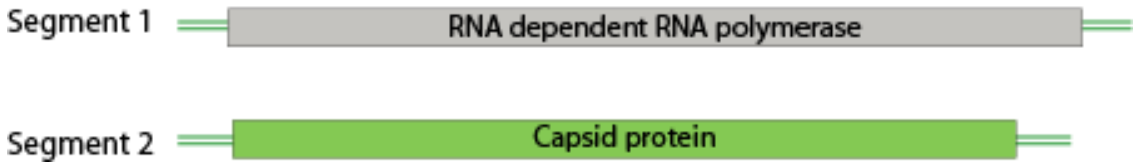
Partitiviridae – (Genus: *Alphapartitivirus*)



Virion:

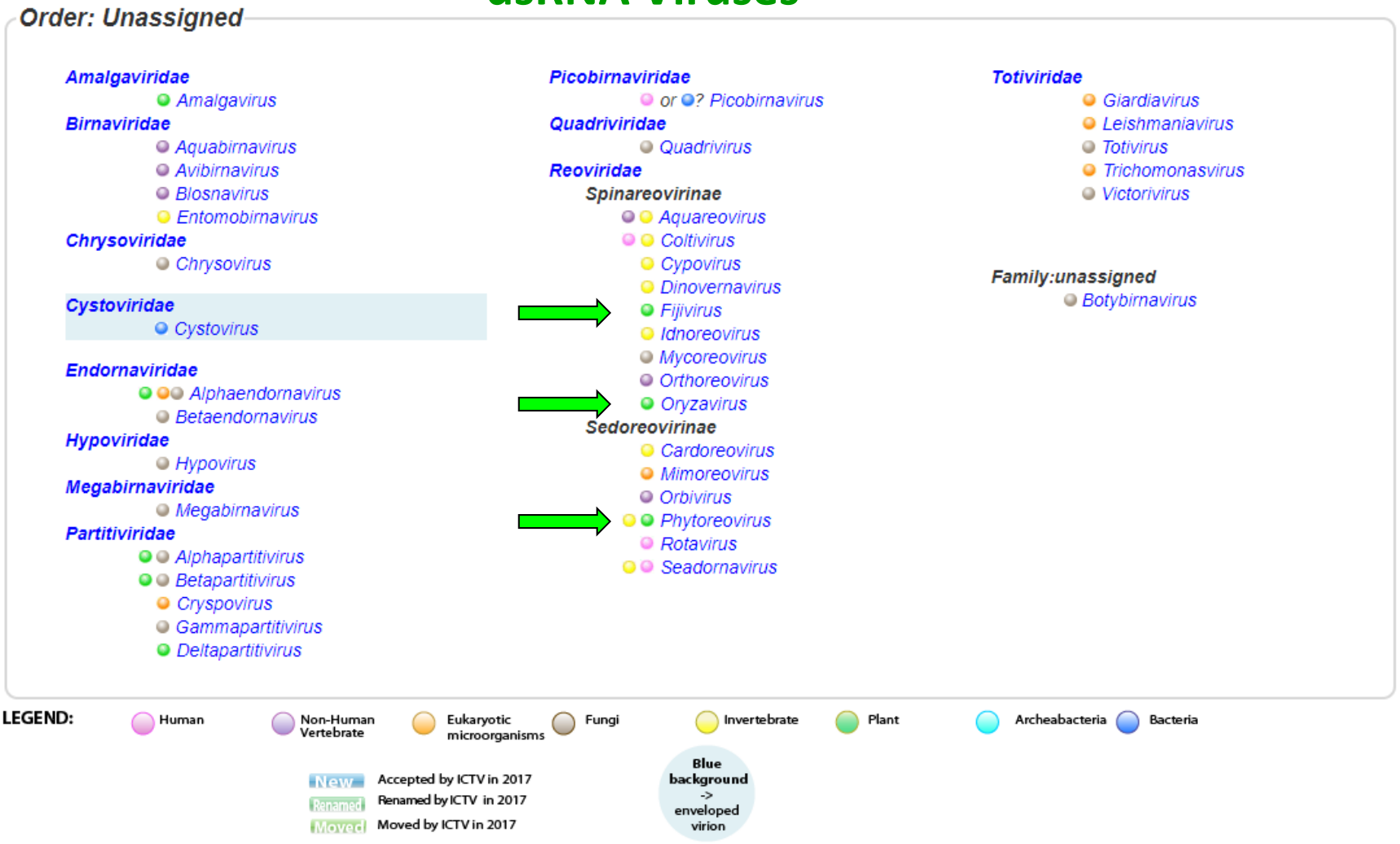


Genome Organization:



Segments are separately encapsidated

dsRNA Viruses



LEGEND: Human; Non-Human Vertebrate; Eukaryotic microorganisms; Fungi; Invertebrate; Plant; Archeabacteria; Bacteria

New — Accepted by ICTV in 2017

Renamed — Renamed by ICTV in 2017

Moved — Moved by ICTV in 2017

Blue background -> enveloped virion

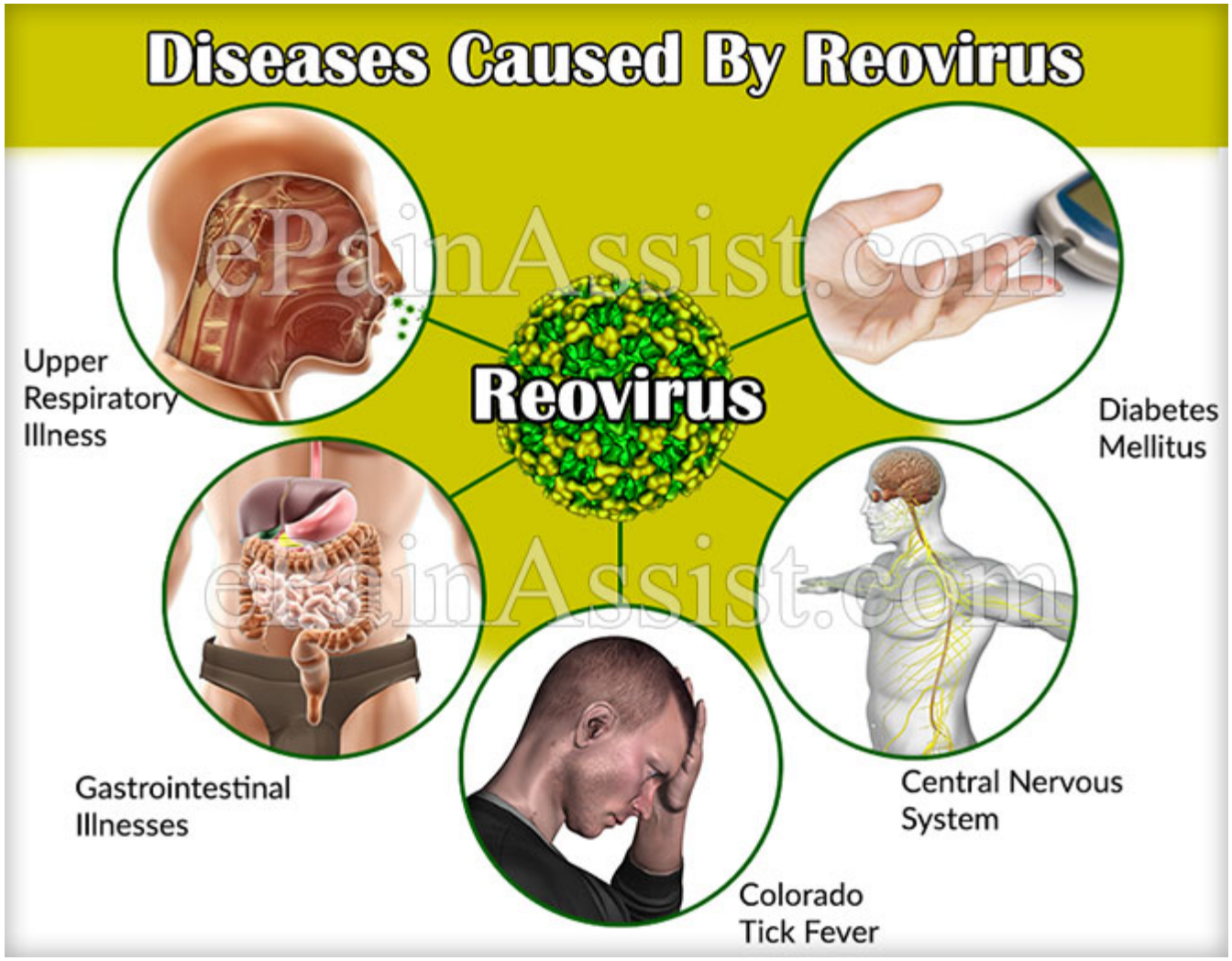
Reoviridae

REO = Respiratory Enteric Orphan virus

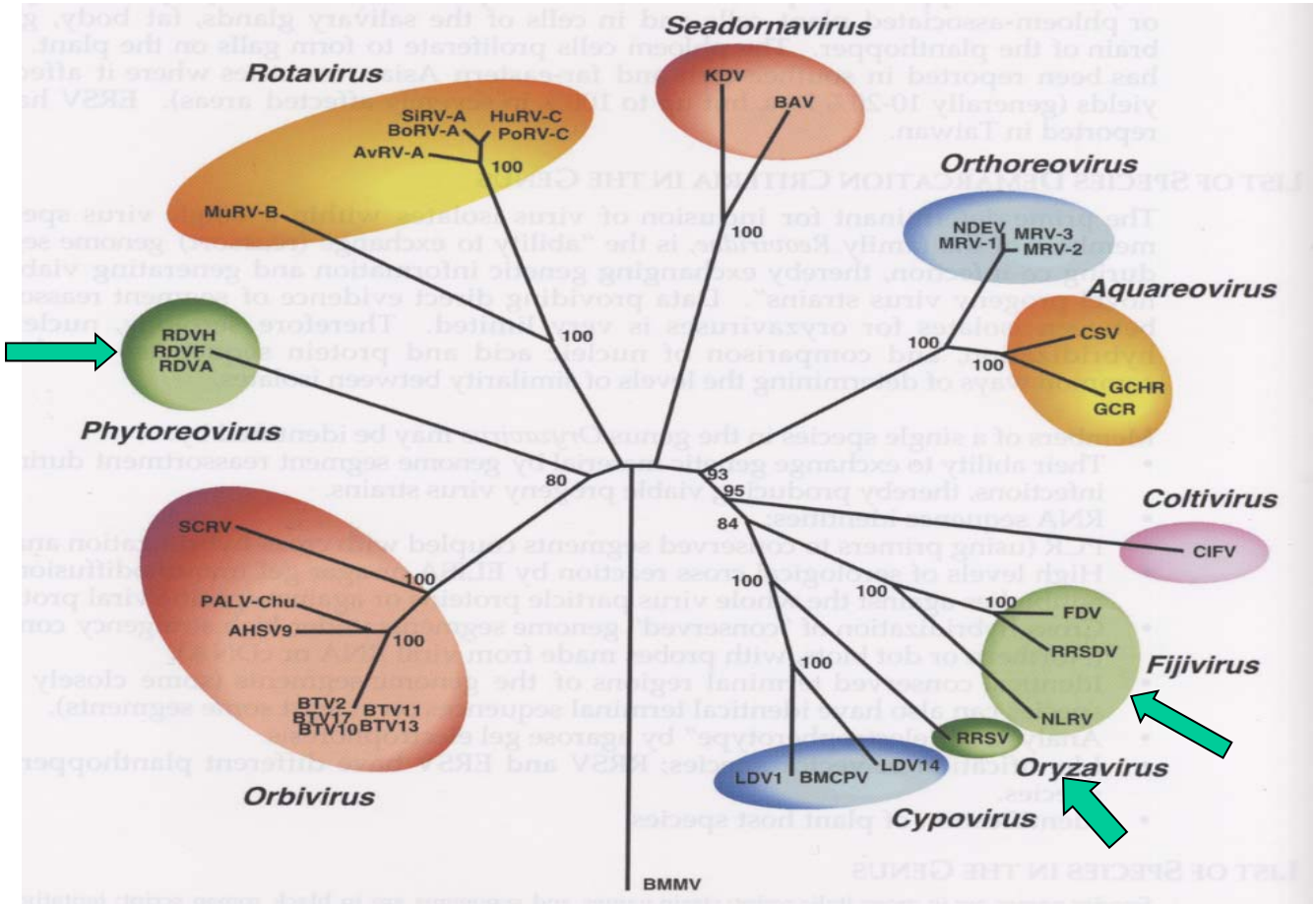
15 Genera:

Plant-Infecting

Genera	Hosts	No. Species	Example
Fijivirus	**Plants, Insects**	**8**	***Fiji disease virus***
Oryzavirus	**Plants, Insects**	**2**	***Rice ragged stunt virus***
Phytoreovirus	**Plants, Insects**	**3**	***Rice dwarf virus***

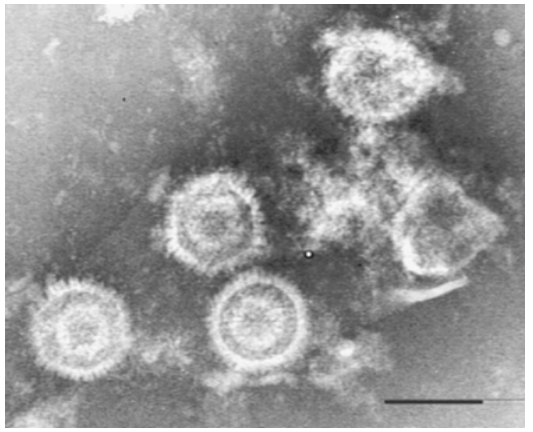

Diseases Caused By Reovirus
Reovirus
Upper Respiratory Illness
Diabetes Mellitus
Gastrointestinal Illnesses
Central Nervous System
Colorado Tick Fever
ePainAssist.com

Phylogenetic Relationships of the Reoviridae Genera

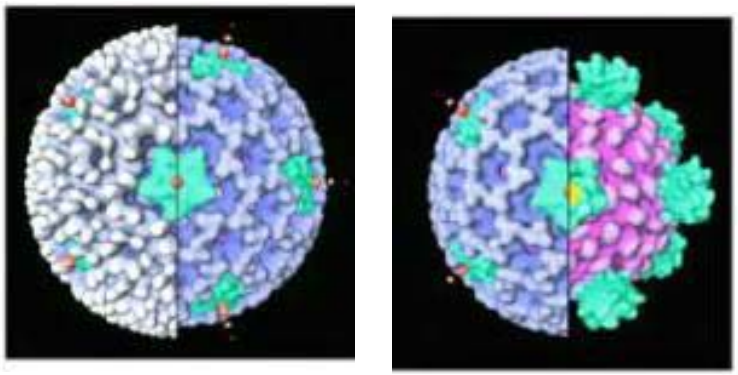


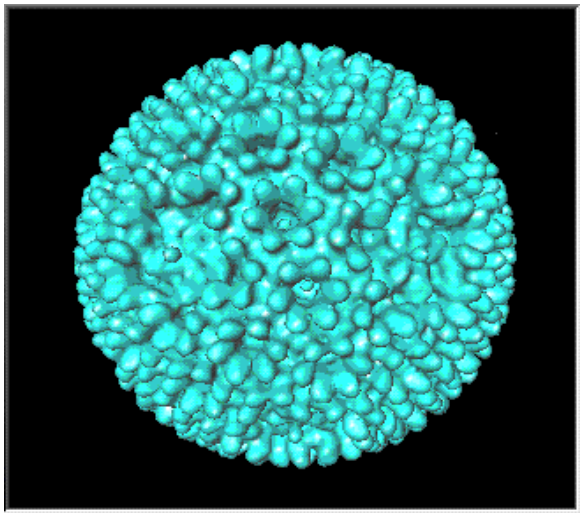
Reovirus Particle Morphology

- Particle – has double (2) or triple (3) shells
- No lipid envelope
- Particle comprised of 8 or more proteins
- Well-studied T=13 structure
- May or may not have surface projections
- 50 nm core is transcriptionally active

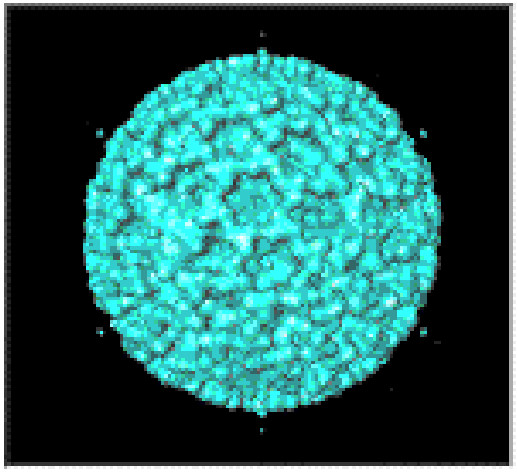


Example of Reovirus
with 3 shells:

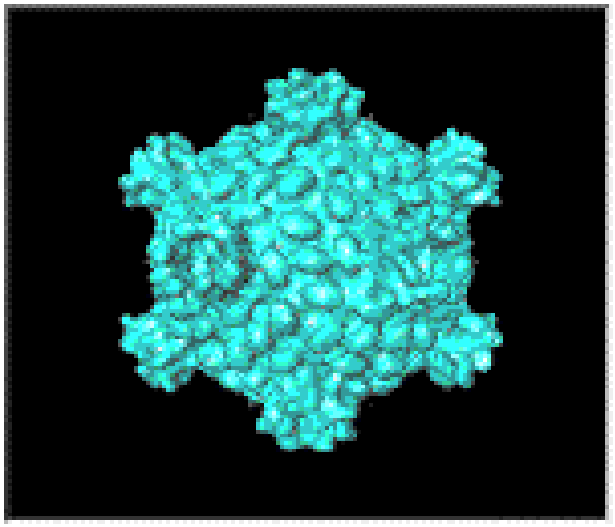




Virion



Infectious Subvirion Particles (ISVPs)

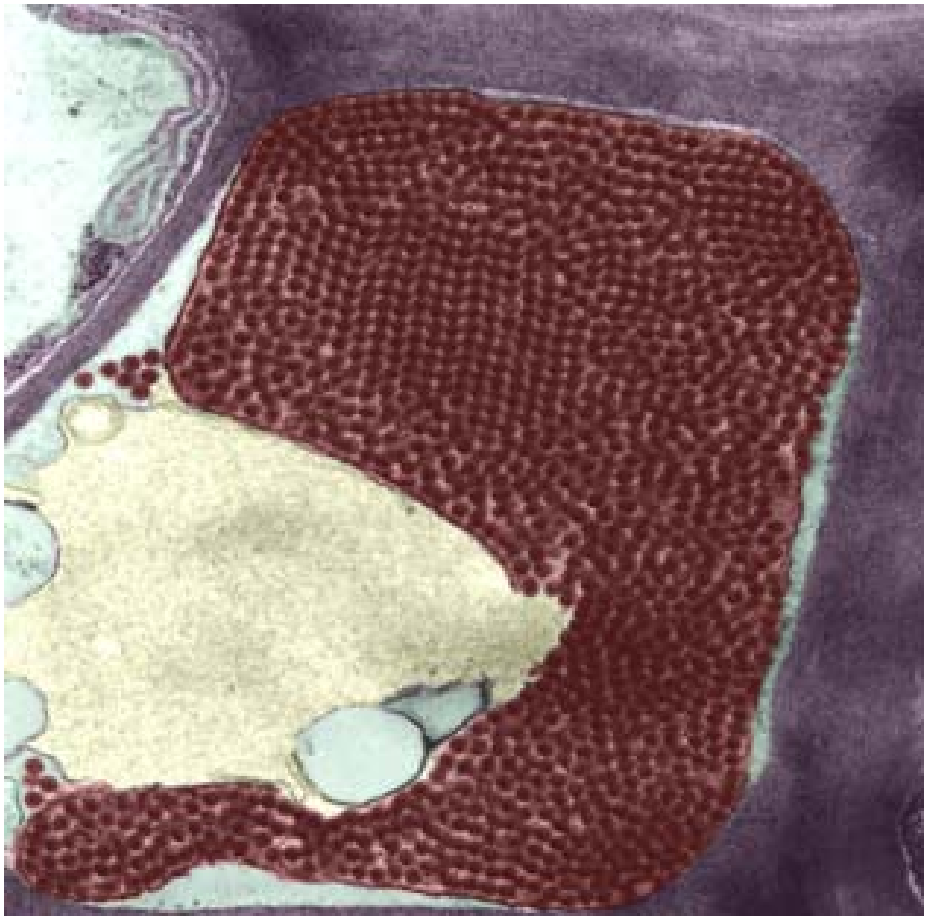


Core

Transcriptionally active

Dryden, K.A., G. Wang, M. Yeager, M.L. Nibert, K.M. Coombs, D.B. Furlong, B.N. Fields, and T.S. Baker. 1993. *J. Cell Biol.* **122**:1023-1041.

Unidentified Phytoreovirus in a plant cell



Plant Reoviruses:

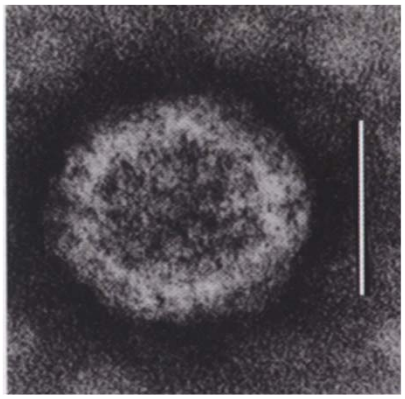
3 genera: ***Phytoreovirus***
Fijivirus
Oryzavirus

- Elicit tumors arising from abnormal phloem development
- Transmitted by leafhoppers or planthoppers
- Viruses multiply in their Hemipteran vectors

Genera vary in:

- No. of genome segments
- 5’ and 3’ termini sequences
- Protein coding sequences
- Inner capsid structure
- Family of insect vector

Family *Reoviridae*



Rice gall dwarf virus

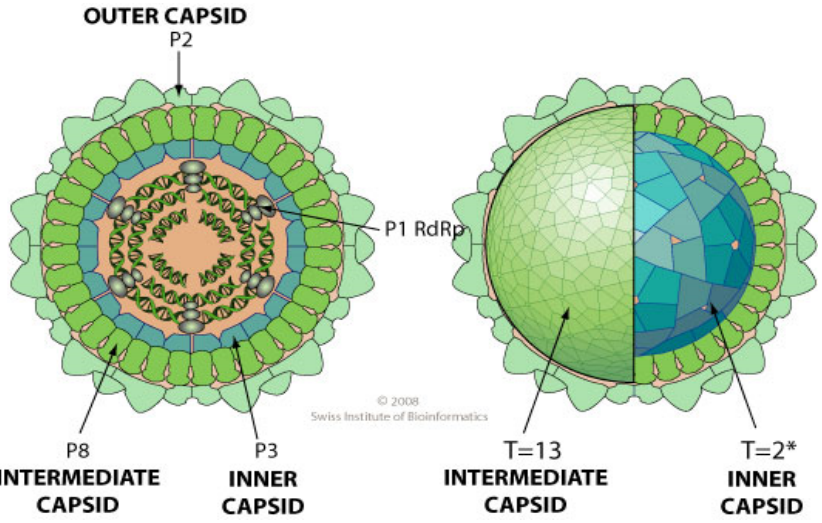
Phytoreovirus:

type species: *Wound tumor virus*,

- 12 dsRNAs; total genome 25,749 bp
- transmitted by leafhoppers
- No spikes on inner capsid

70 nm dia. Double shelled isometric particles

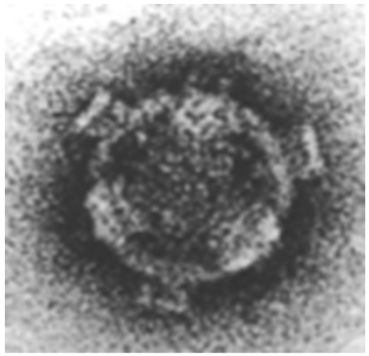
Consist of an outer capsid layer
(780 molecules of protein)
and an inner capsid layer ("core")
(120 molecules of protein)



Family *Reoviridae*

Genus Oryzavirus

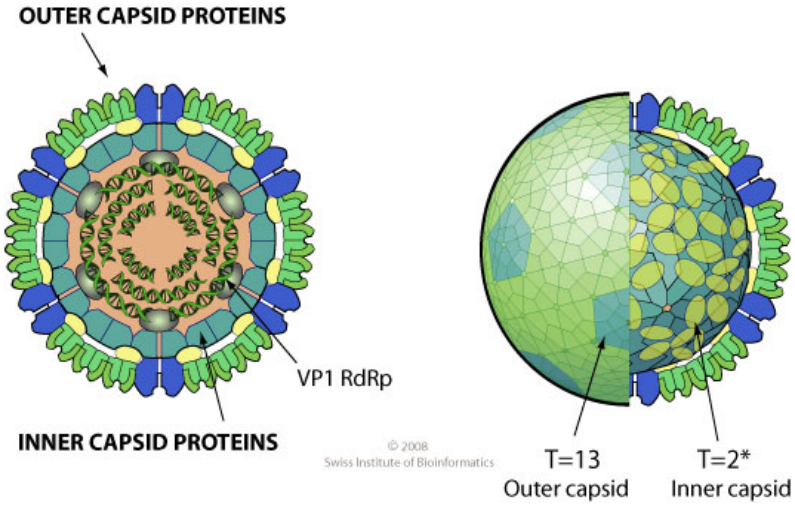
type species – *Rice ragged stunt virus*
-10 dsRNAs, total genome 26,066 bp
- transmitted by leafhoppers (*Cicadellidae*)
- spikes on inner capsid layer



Maize rough dwarf virus (*Fijivirus*)

Double shelled particle

Inner layer has spikes

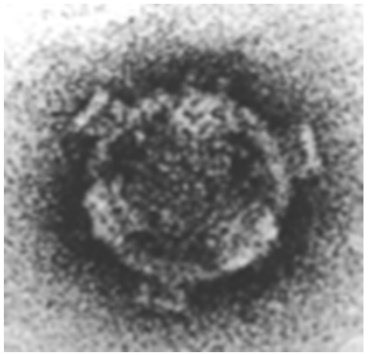


Family *Reoviridae*

Genus *Fijivirus*

type species: *Fiji disease virus*,

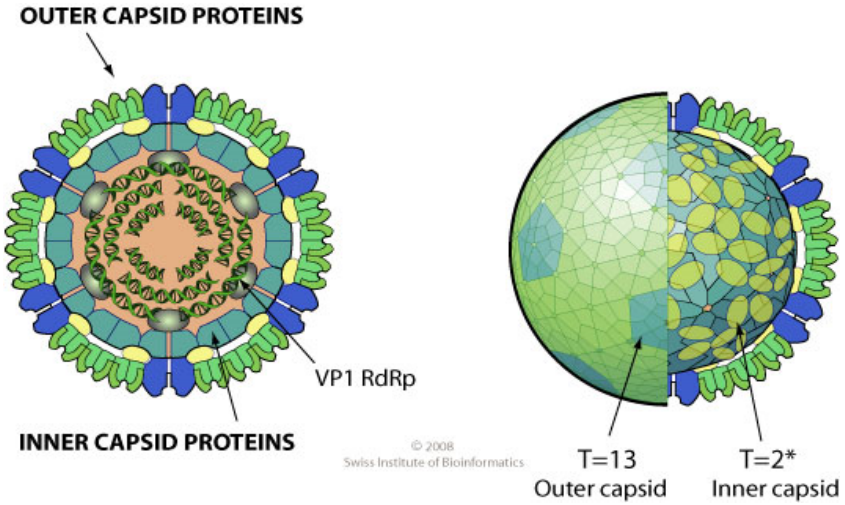
- 10 dsRNAs, total genome 28,699 bp
- transmitted by planthoppers (*Delphacidae*)
- spikes on inner capsid layer



Maize rough dwarf virus (*Fijivirus*)

Double shelled particle

Inner layer has spikes



Plant Reoviruses

- Transmitted by either leafhoppers or planthoppers
- These viruses replicate in the cells of the salivary glands, fat body, gut and brain of the insect vectors

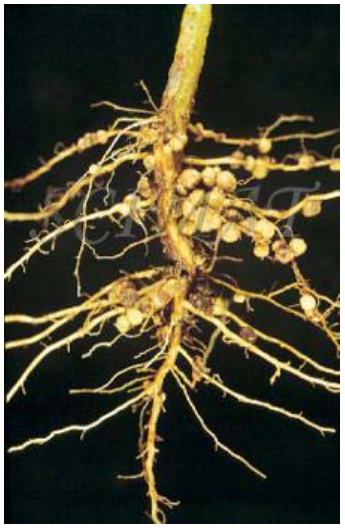
Families of *Hemiptera*

- *Cicadellidae* leafhoppers
- *Delphacidae* planthopper

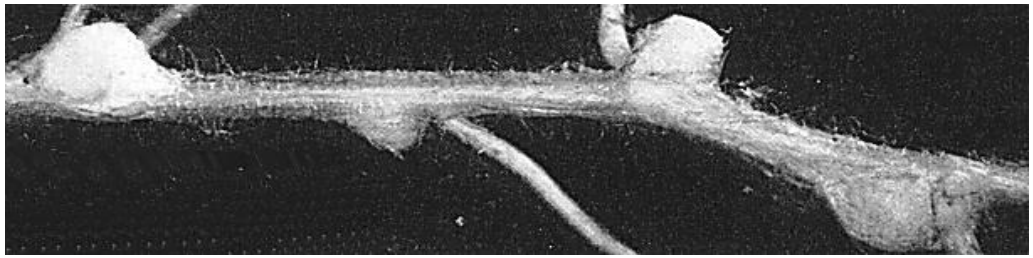
Leafhopper vector

Nilaparvata lugens, brown planthopper, vector of RGSV.

Genus *Phytoreovirus*, type species *Wound tumor virus*



- 3 species
- Name derived from the fact that infected plants develop phloem-derived galls (tumors) at wound sites, notably at the emergence of lateral roots.
- Replicate in leafhopper vector and plants
- Transmitted by leafhoppers (*Cicadellidae*)

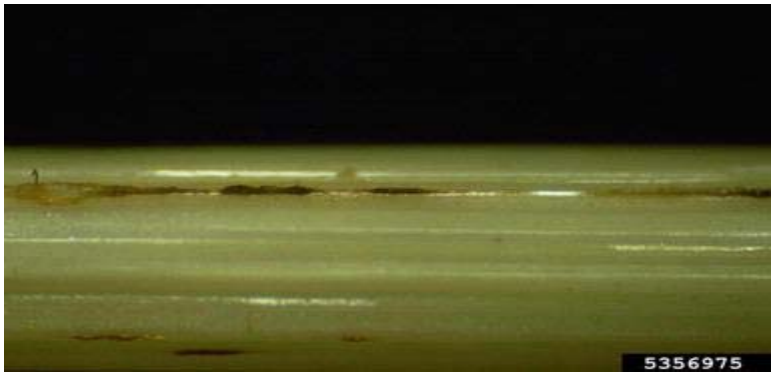


Galls caused by *Wound tumor virus* (WTV)

Genus *Fijivirus*
type species *Fiji disease virus* (FDV)

- Fijiviruses induce hypertrophy of the phloem (both expansion and multiplication of cells) resulting in vein swellings, galls (enations or tumors) derived from phloem cells, especially on the backs of leaves;
- Suppress flowering, cause plant stunting, increase production of side shoots, induce a dark green coloration, and plant death.
- Transmitted by planthoppers (*Delphacidae*)
- All species replicate in planthoppers,

Symptoms of *Rice black streaked dwarf virus* (RBSDV) in rice



Genus *Oryzavirus*
type species: *Rice ragged stunt virus*

- Oryzaviruses replicate in fibrillar viroplasms within the cytoplasm of phloem or phloem associated plant cells. The phloem cells proliferate to form galls on the plant.
- Transmitted by planthoppers (*Delphacidae*). Viruses replicate in insect fat bodies, salivary glands, gut and brain cells.
- All species (2) replicate in plants (grasses) and planthoppers

Symptoms of Rice ragged stunt virus (RRSV) in rice

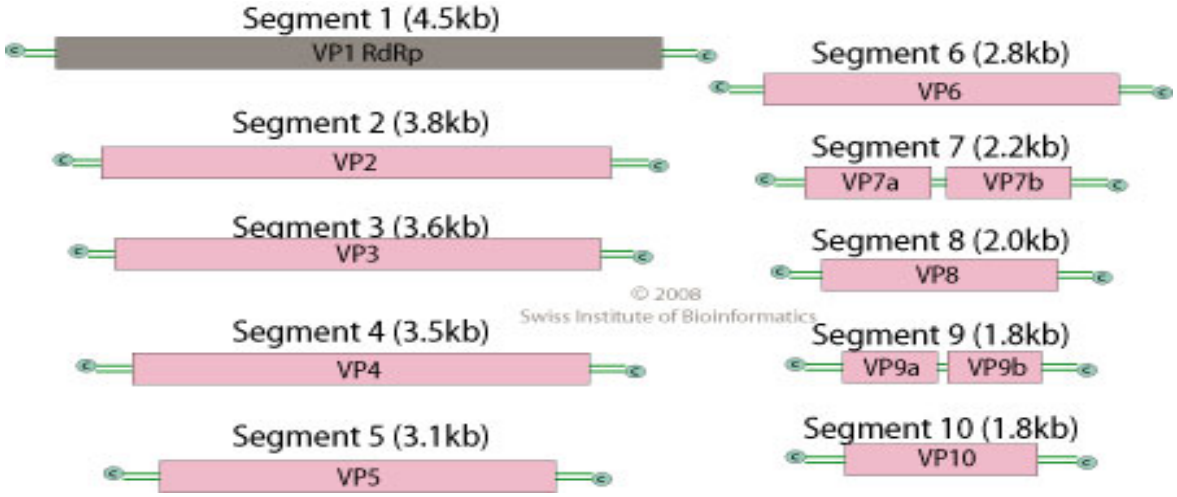
Genome Organization and Replication of Plant Reoviruses

Strategies Employed:

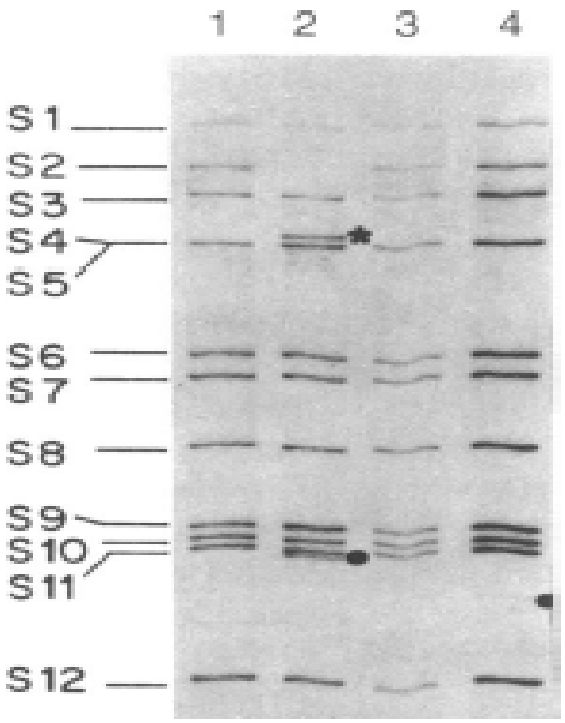
- Segmented genomes (10-12)
- most of the dsRNA segments are monocistronic

  (= one ORF per segment)
- All transcription occurs in the viral core
- No known nuclear component to infection cycle – all replication is cytoplasmic

Genome organization of a Fijivirus:

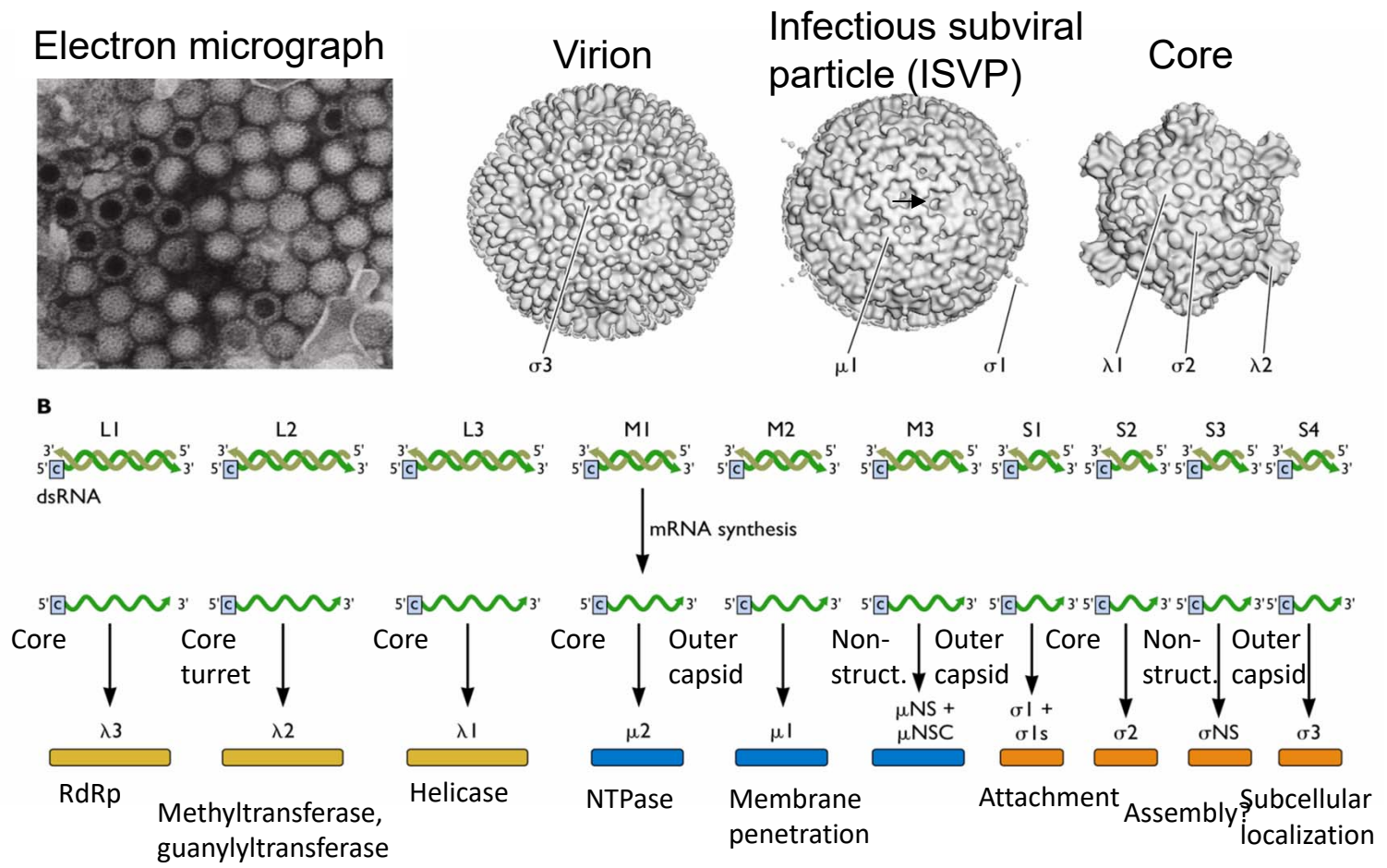


dsRNAs visible By PAGE



- Segmented dsRNA linear genome.
- Contains 10 segments coding for 12 proteins
- Segments size range from 1.4 to 4.5 kb
- One copy of each dsRNA per particle

Structure and Genome Organization (*Mammalian orthoreovirus 3*)



Modified from Flint et al., Principles of Virology 2nd Ed., ASM Press

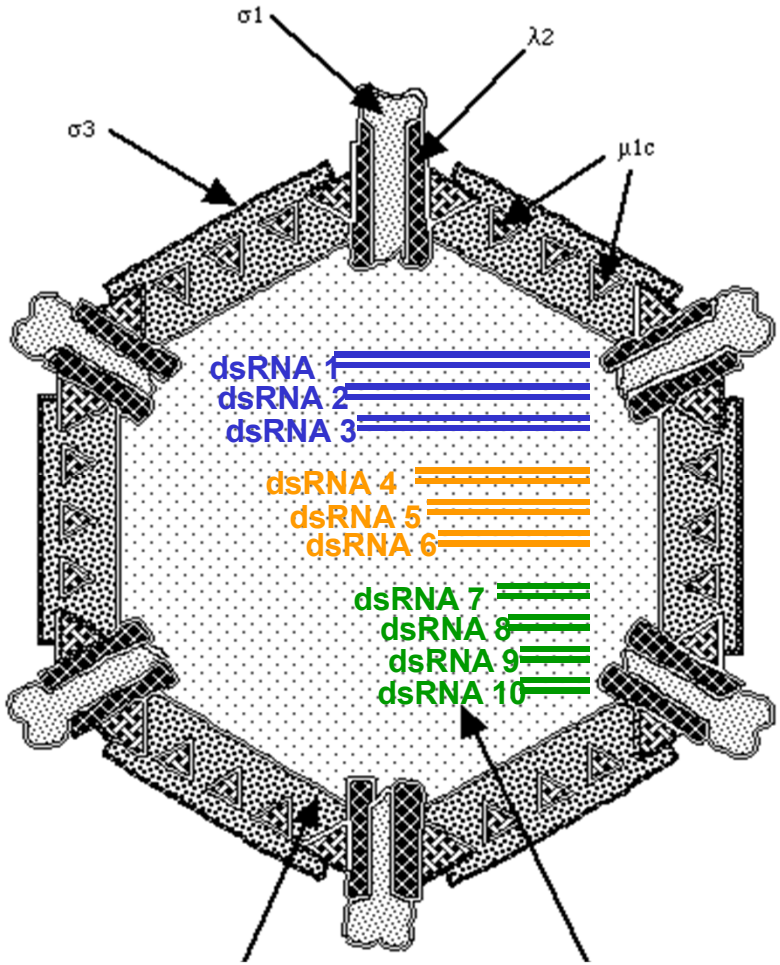
Replication occurs in the cytoplasm:

1. Virus enters the cytoplasm.

2. Transcription of the dsRNA genome by viral polymerase occurs inside the virion, the genome is never exposed to the cytoplasm.

3. This (+)RNA (plus strand transcript) is used as the template for translation.

4. (+)RNAs are encapsidated in virion particles, inside which they are transcribed to give RNA (-) molecules with which they become base-paired to produce dsRNA genomes.

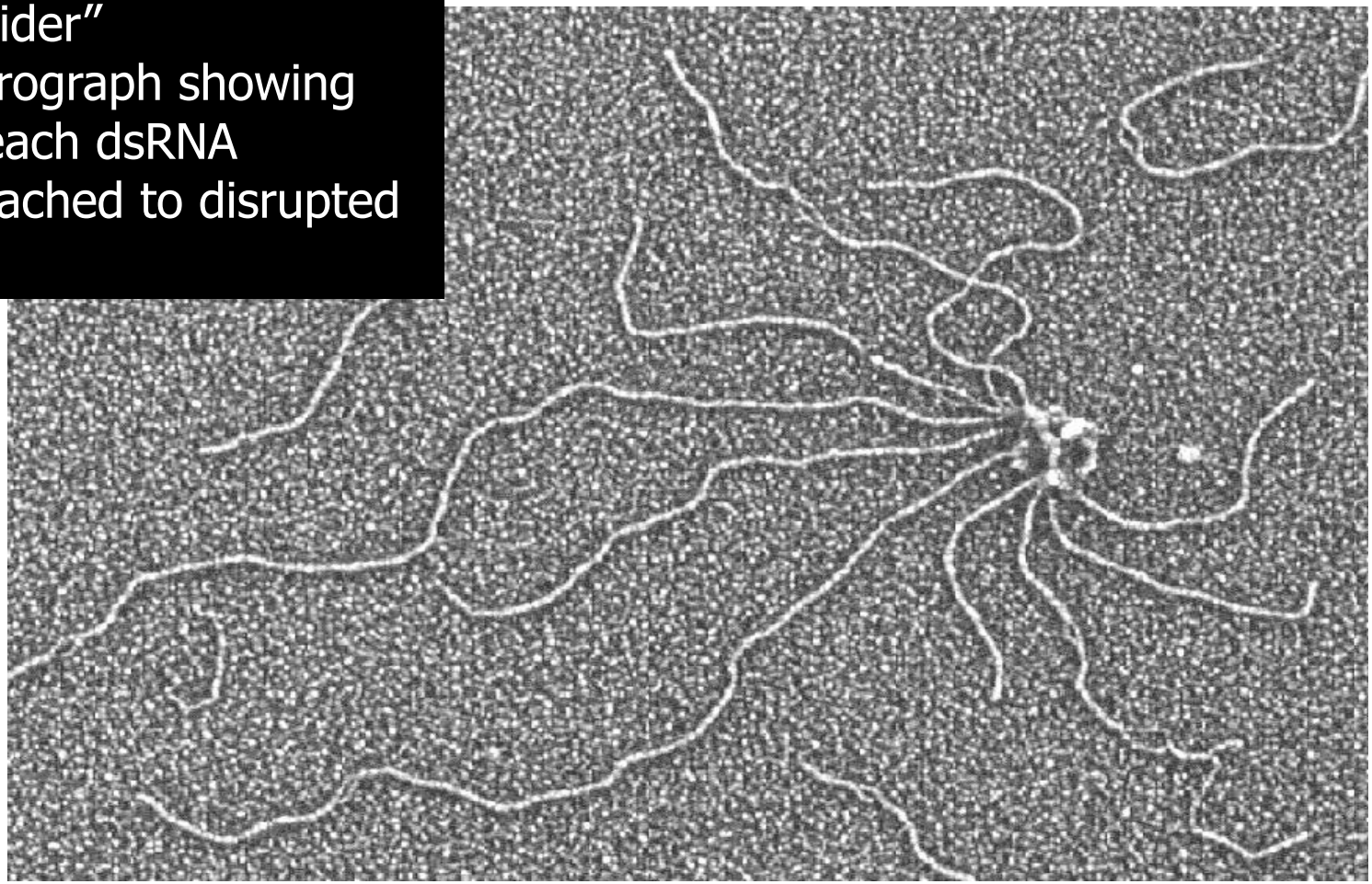


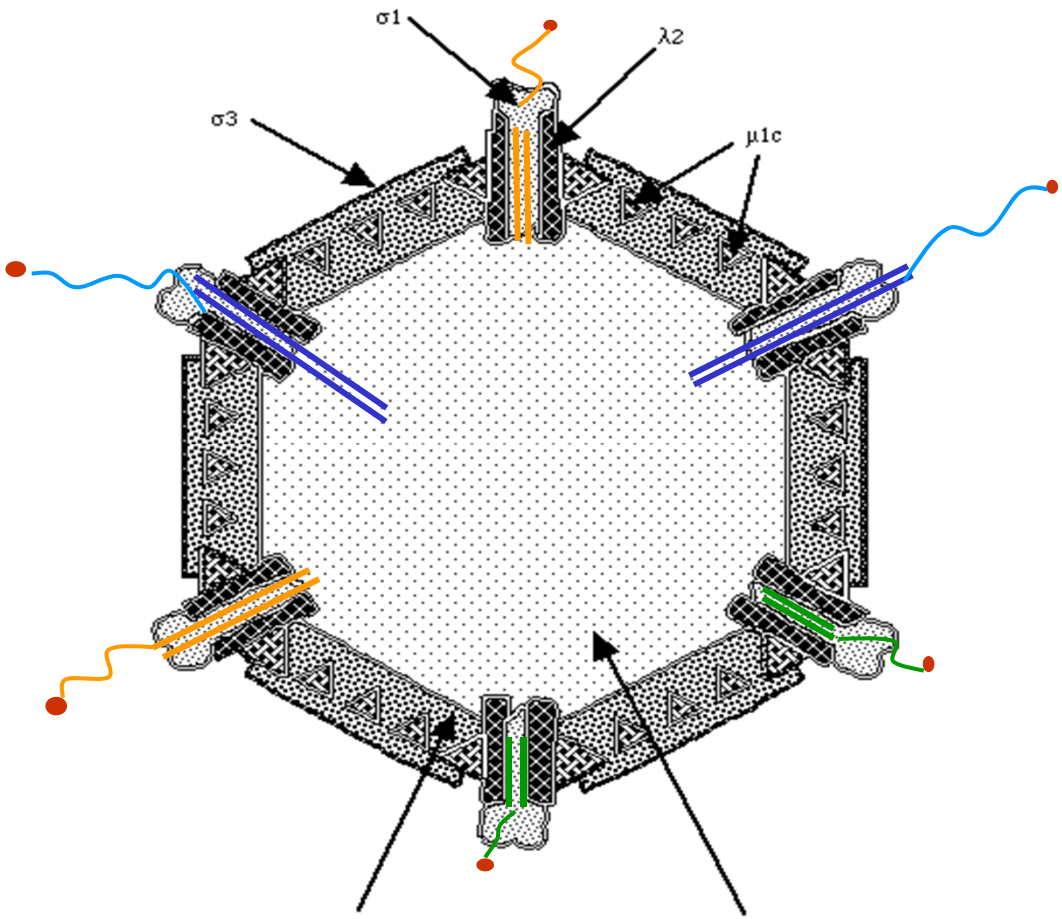
- A Reovirus virion contains exactly one segment of each of the 10-12 segments of dsRNA that constitute the viral genome, encapsidated in a single complex virus particle comprised of 6-8 proteins

- The core is biochemically active with RNA dependent RNA polymerase (RdRp), capping enzyme, and helicase enzyme.

Modified From Alan Cann by BIH

Reovirus “spider”
electron micrograph showing one end of each dsRNA molecule attached to disrupted viral core.



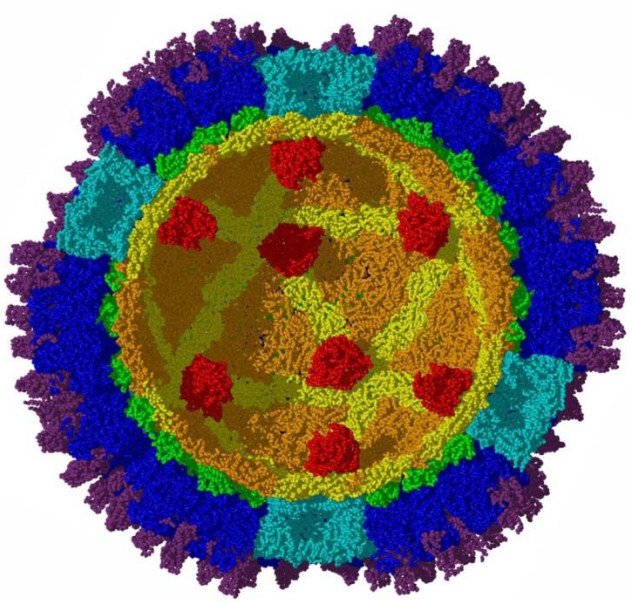


Expression of Viral Proteins:

- mRNAs of viral proteins are transcribed at the transcription complexes at each of 12 vertices of the icosahedral particles.
- The dsRNA genome is never completely uncoated - prevents activation of cellular defenses in response to presence of dsRNA
- The viral polymerase synthesizes a capped mRNA from each dsRNA segment. This capped mRNA is translocated to the cell cytoplasm where it is translated.

Modified From Alan Cann by BIH

Cryo-EM image of the inside of a Reovirus showing virus “factories” (ie transcription complexes)

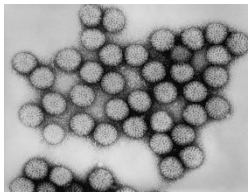


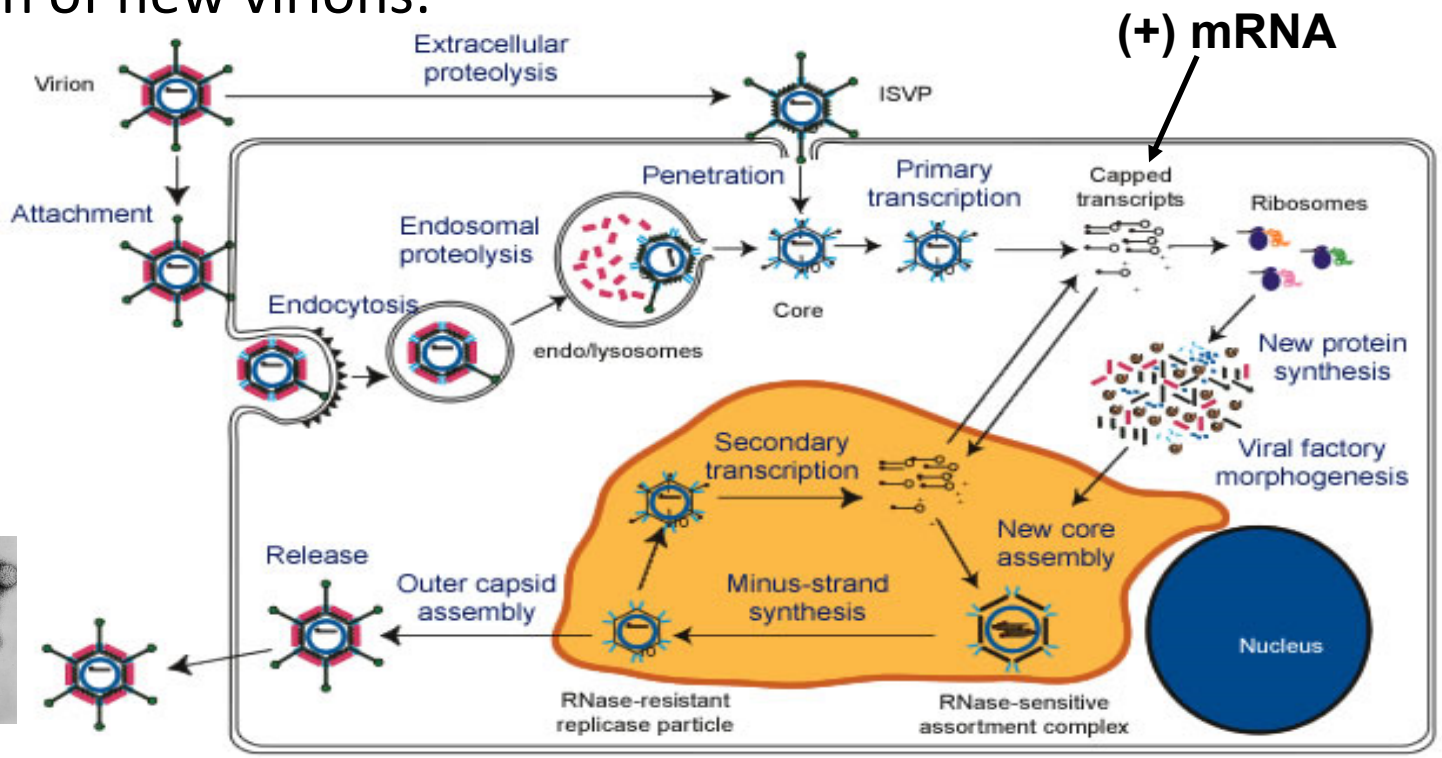
Human Reovirus

Cross section of a reovirus shows features down to 7.6-angstrom resolution
Red = "factories" where raw materials from a host cell's interior are converted into mRNA which then instruct the cell to begin manufacturing more viruses.

Structure determined by Cryo-electron Microscopy by T. Baker, Purdue University

Formation of new virions:





Reoviruses replicate in the cytosol of infected cells. Following penetration of the cellular membrane, viral cores begin transcribing the viral genome segments. The viral genome segments encode the viral proteins (structural and nonstructural). The nonstructural protein mNS forms the matrix of viral factories where new cores assemble and begin secondary rounds of transcription. The viral cores are coated with the outer capsid proteins m1, s3, and s1 to form intact virions that are released following cell lysis.

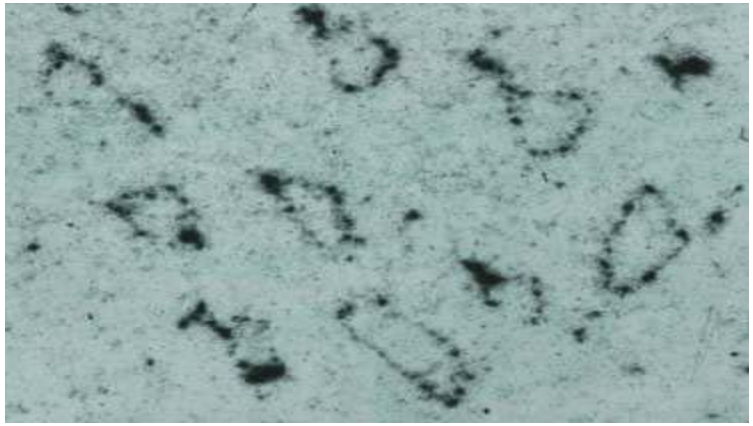
Good (short) lecture on Reovirus replication:

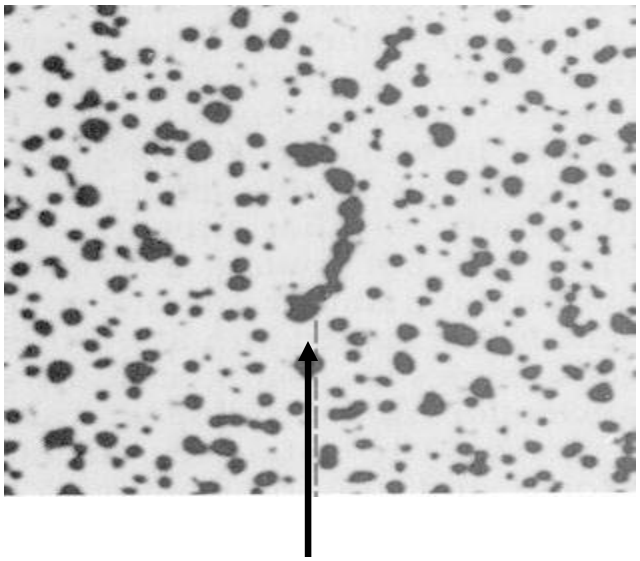
https://www.youtube.com/watch?v=NKGy3xuEKQM

Use of Reoviruses to kill cancer cells in humans:

http://www.youtube.com/watch?v=nsoP4SPi2jY

Replication of Viroids

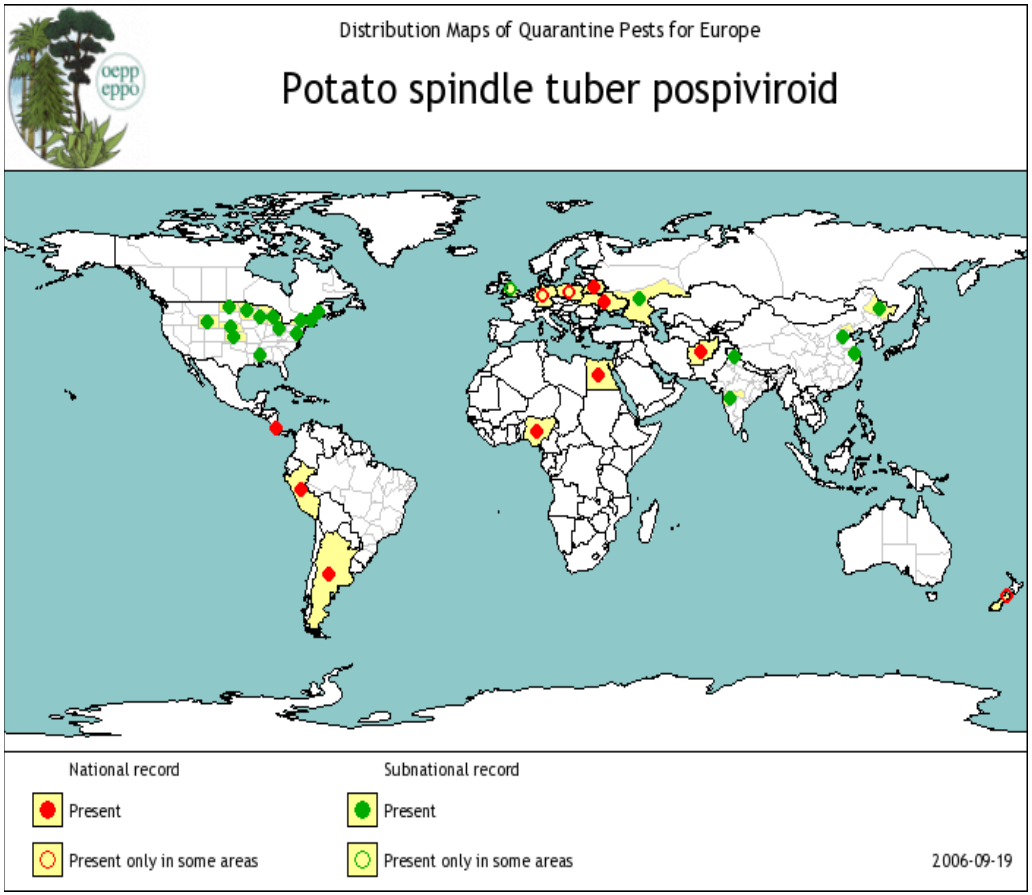




240bp *Potato spindle tuber viroid* –
magnified 440,000 times

Symptoms of viroid diseases:

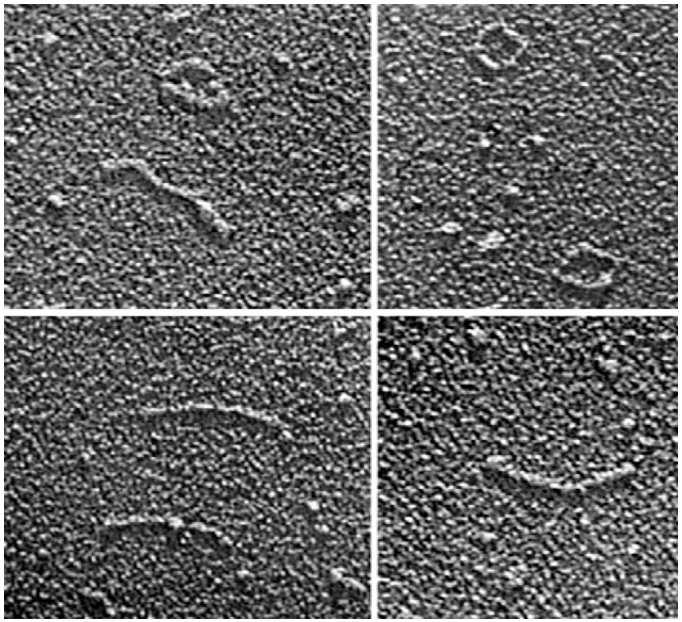
A: *Potato spindle tuber viroid* in tomato, **B**: *Avocado sun blotch viroid*,
C: *Chrysanthemum stunt viroid*, **D:** *Chrysanthemum chlorotic mottle viroid*

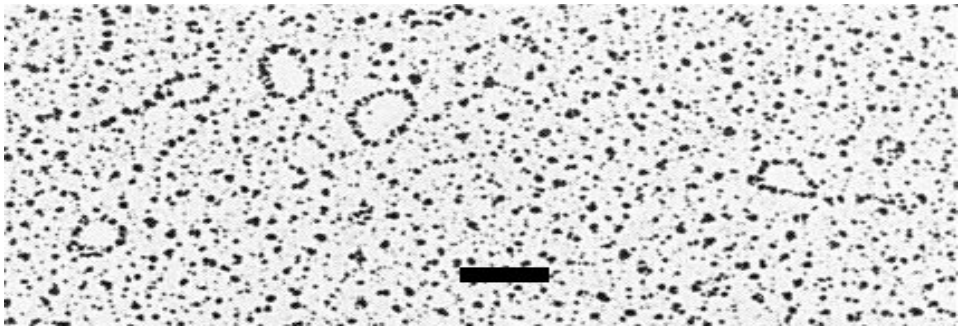


Effect on potato yields

Subviral Agents: Viroids

- **Viroids** are **low-molecular-weight, covalently closed, single-stranded, circular RNAs (246 to 375 nucleotides)**
- Exist in their native state as **highly base-paired, rod-like structures** 50 nm long,





Viroid Replication

- **Replication is autonomous**

  viroids do not depend on the presence of a helper virus

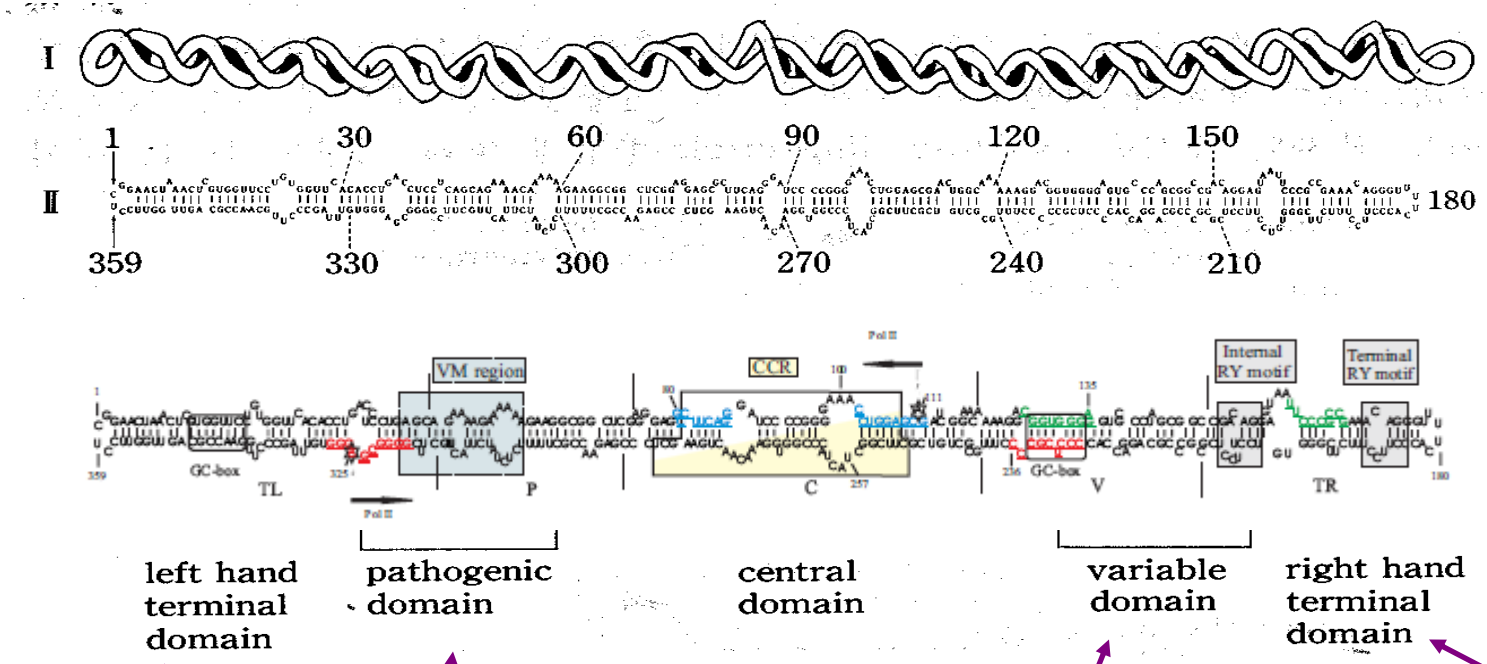
- **Viroids lack mRNA activity**

  Since viroids do not code for any polypeptide, they must use pre-existing host nucleic acid synthesizing enzymes. No proteins are encoded.

- **Viroids lack a coat protein.**

  The high degree of base-pairing accounts for the stability of the particle despite the lack of a coat protein.

Pospiviroidae:



Viroid Replication

• Viroids replicate via an RNA template and the replication involves **rolling-circle mechanism.**

→RNA strands complementary to viroid RNA are found in infected plants.

→ Minus strand *Potato spindle tube viroid* (PSTVd) can exist as a tandem multimer of several unit-length monomers.

• Some viroids have a ribozyme activity (a ribozyme is a catalytic RNA molecule, in this case RNA cleavage is the ribozyme activity)

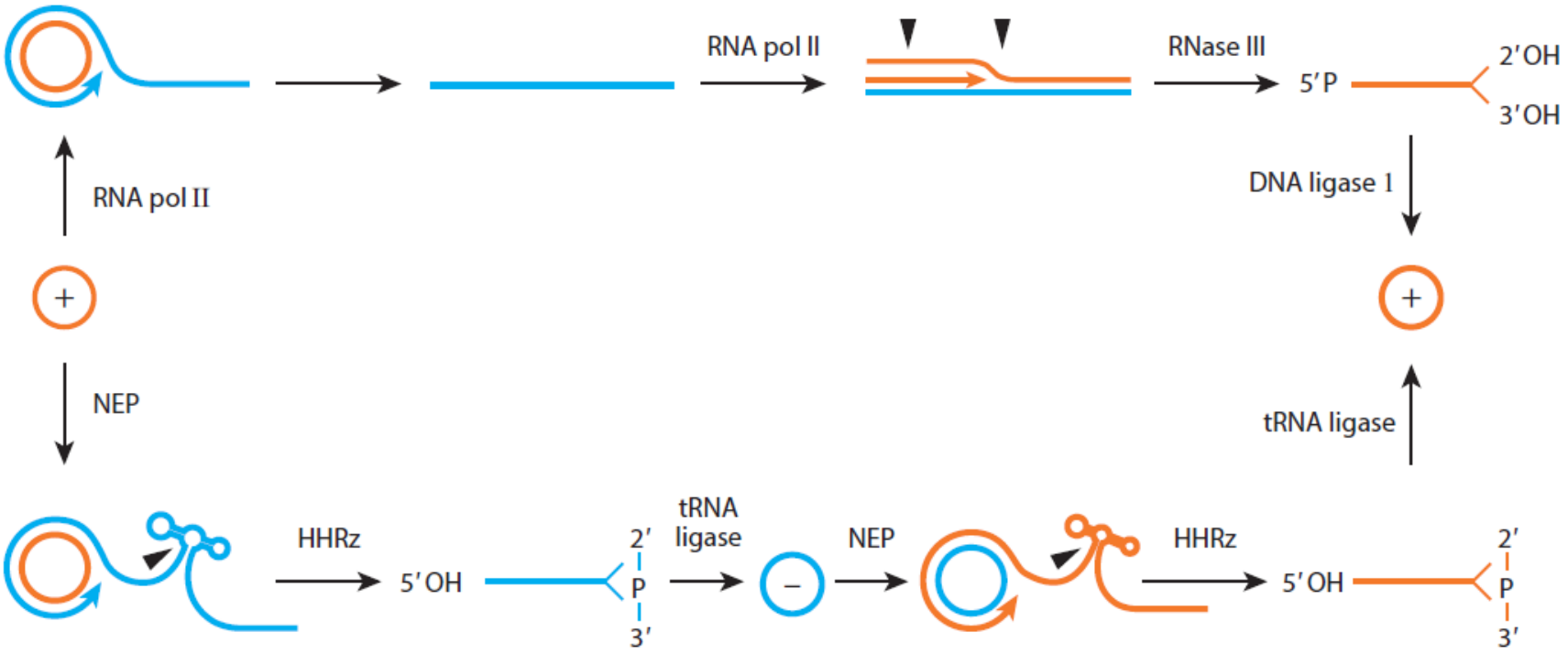
Pospiviroidae:

- Replicate in the plant **nucleolus**,
- Host RNA polymerase II makes both plus and minus strands.
- Replicated through doubled-stranded intermediates.
- **Asymmetric** replication (1 rolling circle)

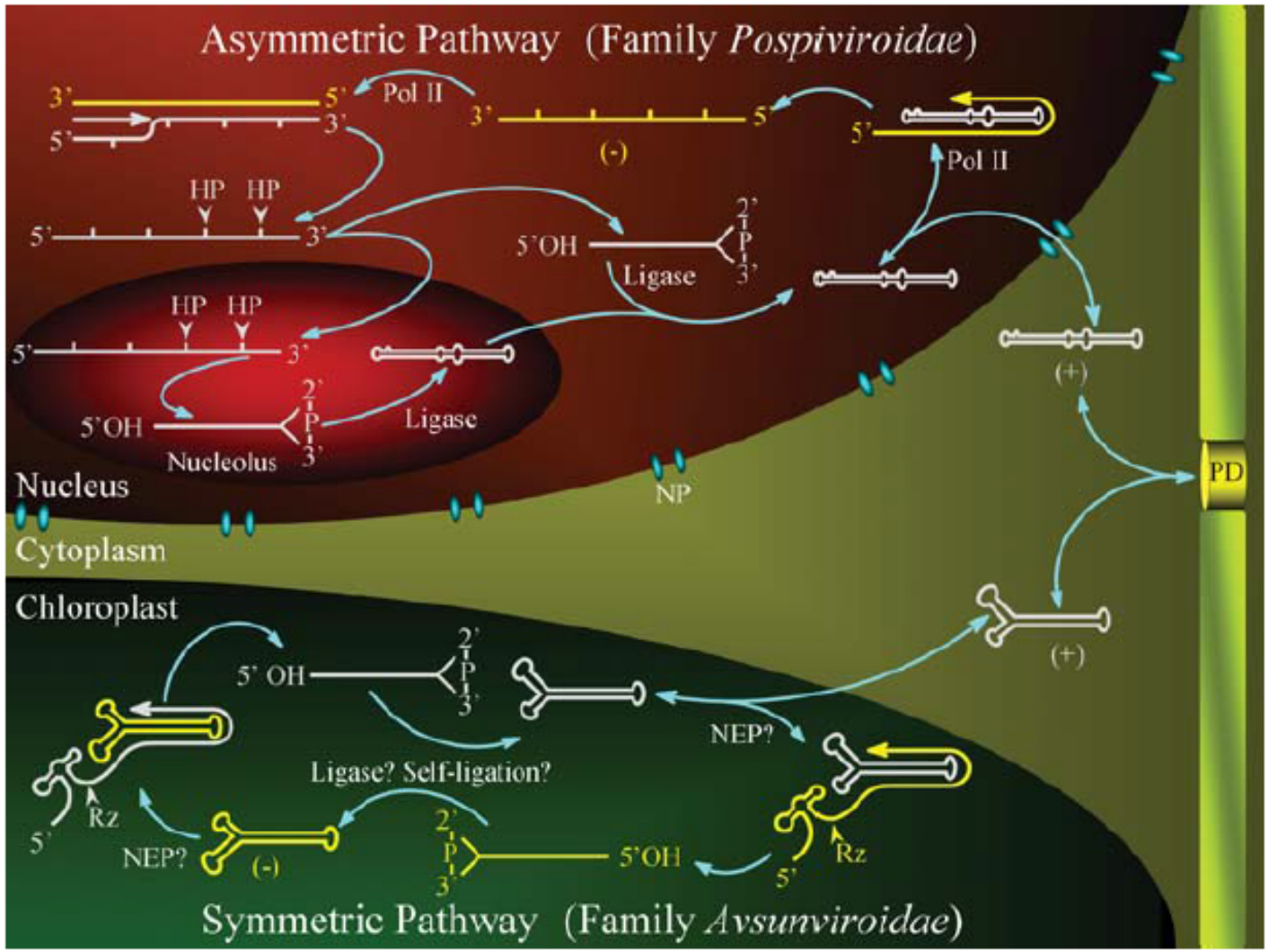
Asunviroidae:

- Accumulates and replicate in the **chloroplasts**
- Presumably use chloroplast RNA polymerase
- Replicated through doubled-stranded intermediates.
- **Symmetric** replication (2 rolling circles)

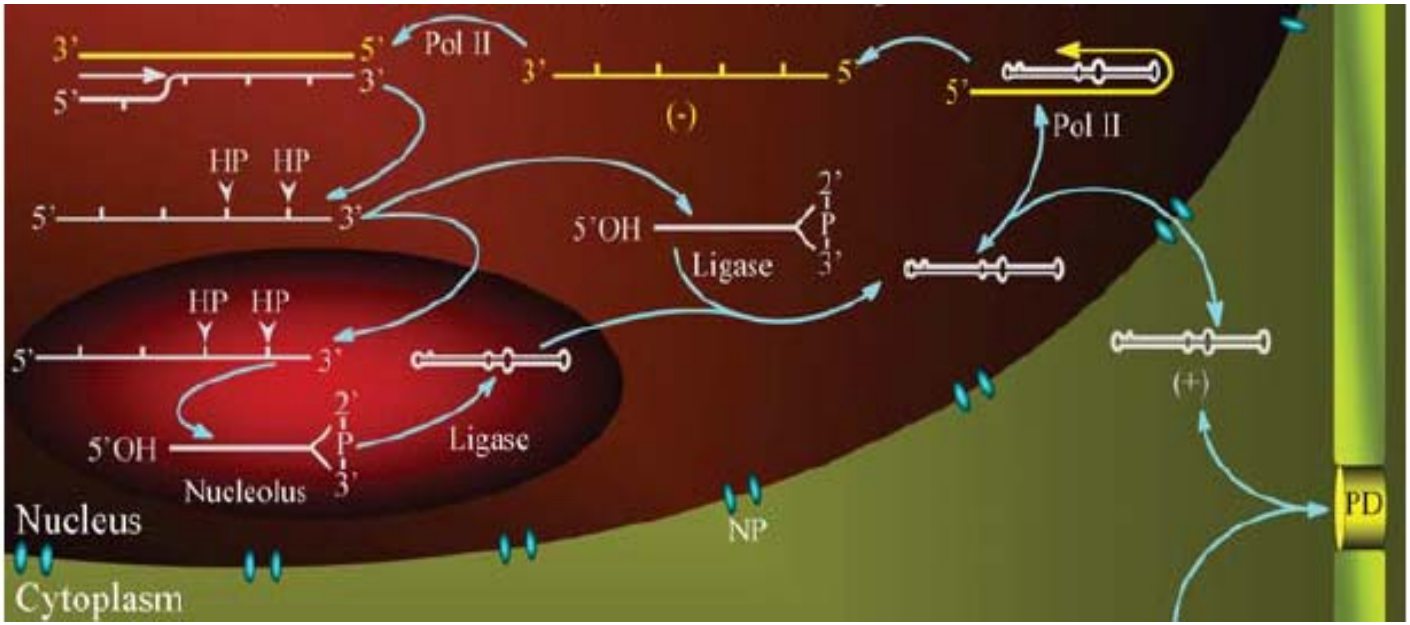
Asymmetric Pathway (Pospiviroids)



Symmetric Pathway (Asunviroids)


Asymmetric Pathway (Family *Pospiviroidae*)
Pol II
(-)
Pol II
HP HP
5'OH
Ligase
HP HP
5'OH
Ligase
Nucleolus
(+)
PD
NP
Nucleus
Cytoplasm
Chloroplast
5' OH
(+)
NEP?
Ligase? Self-ligation?
Rz
NEP?
(-)
5'OH
Rz
Symmetric Pathway (Family *Avsunviroidae*)

Asymmetric Pathway



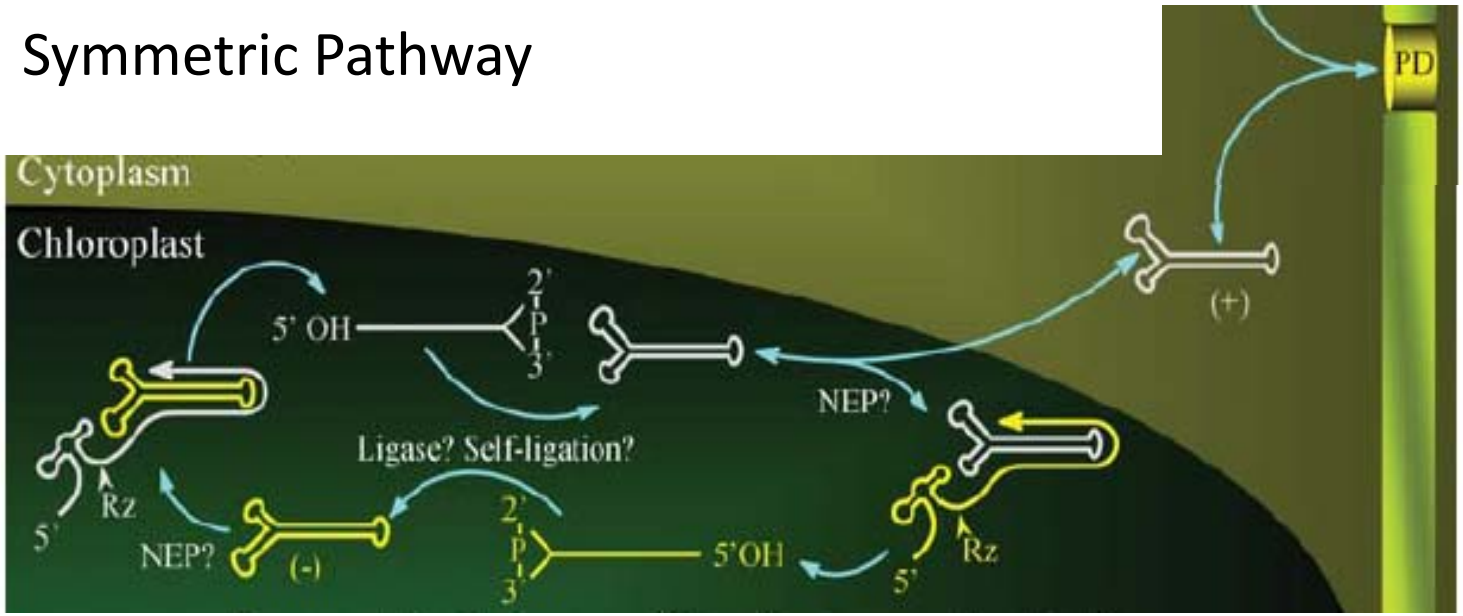
Pospiviroidae

Mechanism:
One rolling circle

RNA polymerase, RNase, and RNA ligase required for replication are host encoded.

White lines indicate plus (+) strands and and yellow lines indicate minus (-) strands, Pol II refers to RNA polymerase II and NEP to nuclear-encoded RNA polymerase. PD and NP are abbreviations for plasmodesmata and nuclear pores, respectively.

Symmetric Pathway



Asunviroidae

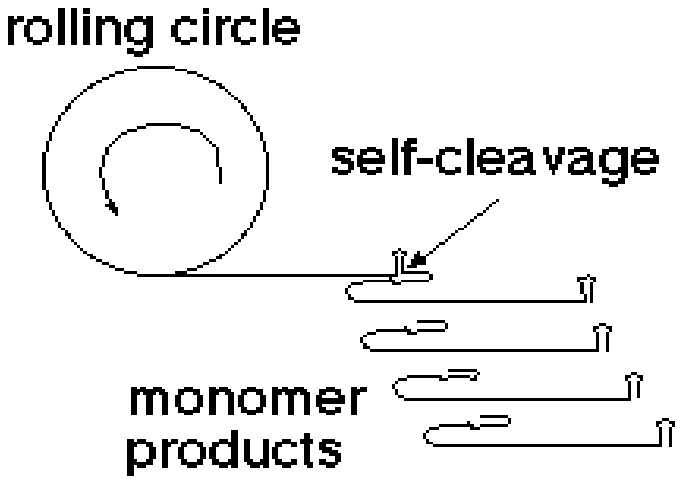
Mechanism:
Two rolling circles

RNA polymerase, and RNA ligase are host encoded.

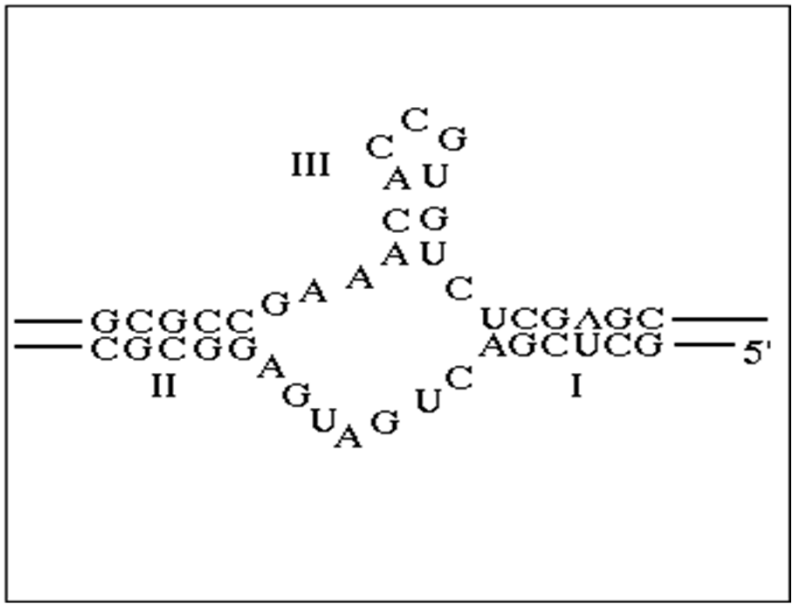
RNase activity is by hammerhead ribozymes

White and yellow lines indicate + and - strands, respectively.
Cleavage sites are marked by arrowheads. Self-cleavage mediated by hammerhead ribozymes (Rz).
Pol II refers to RNA polymerase II and NEP to nuclear-encoded RNA polymerase (DdRp).
PD and NP are abbreviations for plasmodesmata and nuclear pores, respectively.

Self-cleavage of Asunviroidae RNAs



Secondary structure of some viroids' RNA forms a "hammerhead", that chelates a magnesium, and forms a self-cleaving structure



www.escience.ws/ b572/L27/L27.htm

Detail of the cleavage site

www.escience.ws/ b572/L27/L27.htm

Movement of Viroids:

- Within the cell –
  unclear but a nuclear receptor has been identified

- Cell to cell – unclear mechanism but it is rapid (for PSTVd)

- Long Distance – replication in phloem parenchyma, can form ribonucleoprotein complexes with host phloem protein 2 (a lectin that moves rapidly through plasmodesmata into the phloem in the direction of source to sink (with photo-assimilates)

Many basic questions regarding viroids remain to be answered:

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.

4. What determines viroid host range? Are viroids restricted to higher plants, or do they have counterparts in animals?