Negative Sense ssRNA Viruses

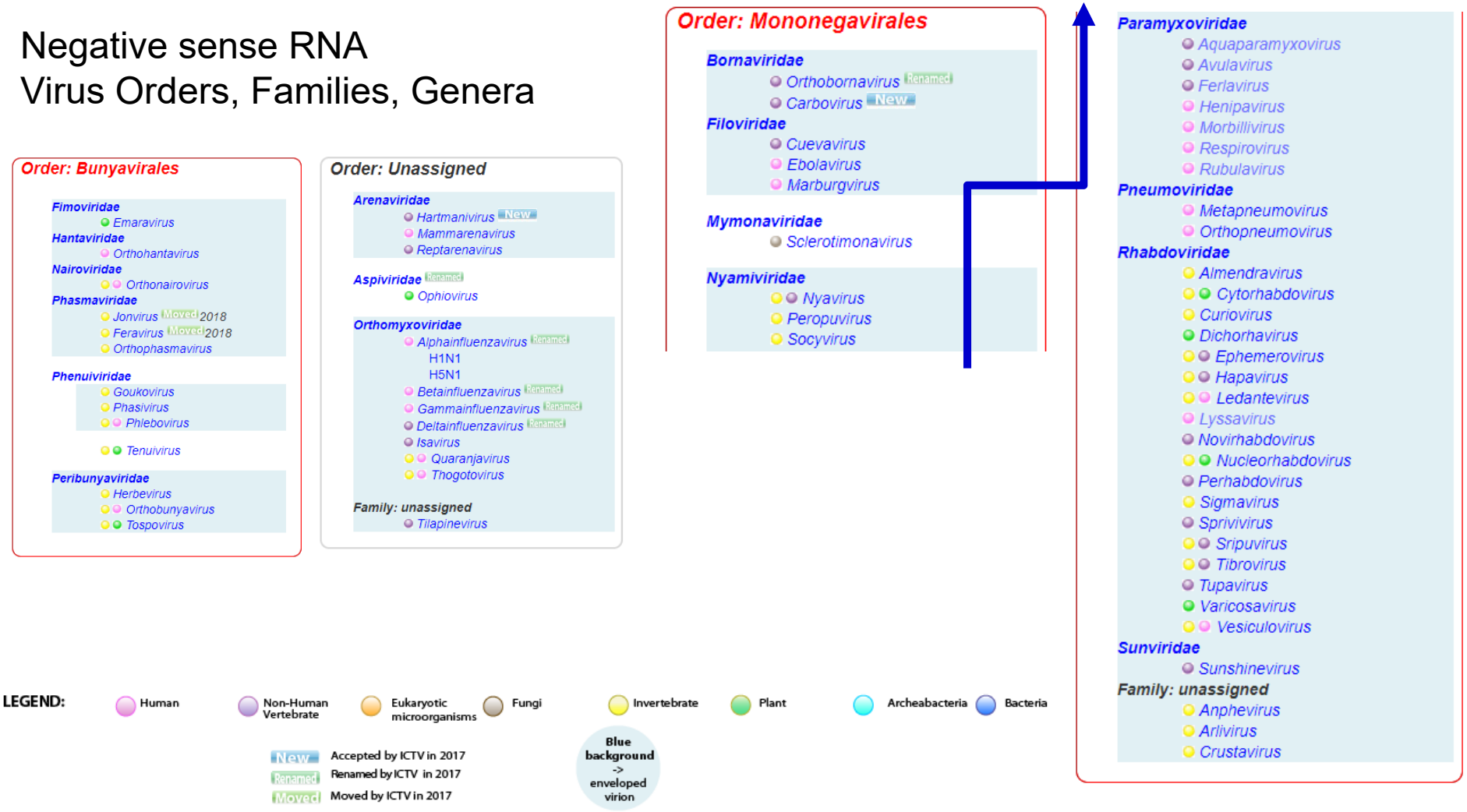
Families and Genera of Plant Viruses – RNA Plant Viruses

Life Cycles of (-) sense ssRNA viruses:

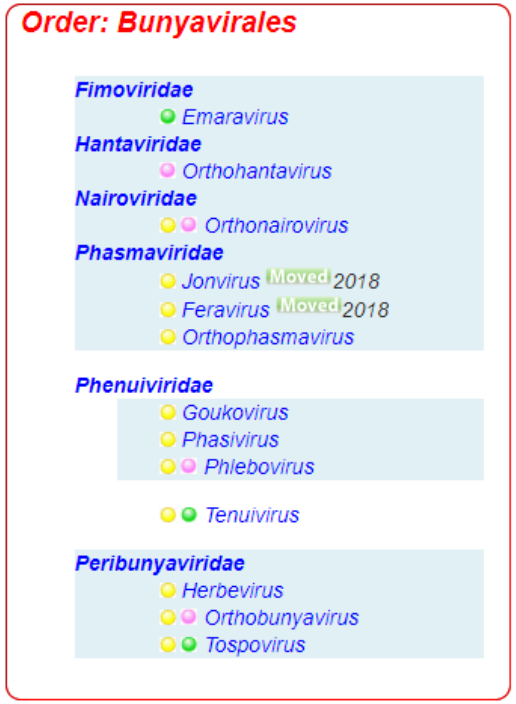
1. **Family: *Rhabdoviridae***
   Genus: *Nucleorhabdovirus*
   Genus: *Cytorhabdovirus*
   combination of several viruses

2. **Family: *Peribunyaviridae***
   Genus: *Tospovirus*
   Tomato spotted wilt virus

Negative sense RNA
Virus Orders, Families, Genera



Negative sense RNA Virus Orders, Families, Genera

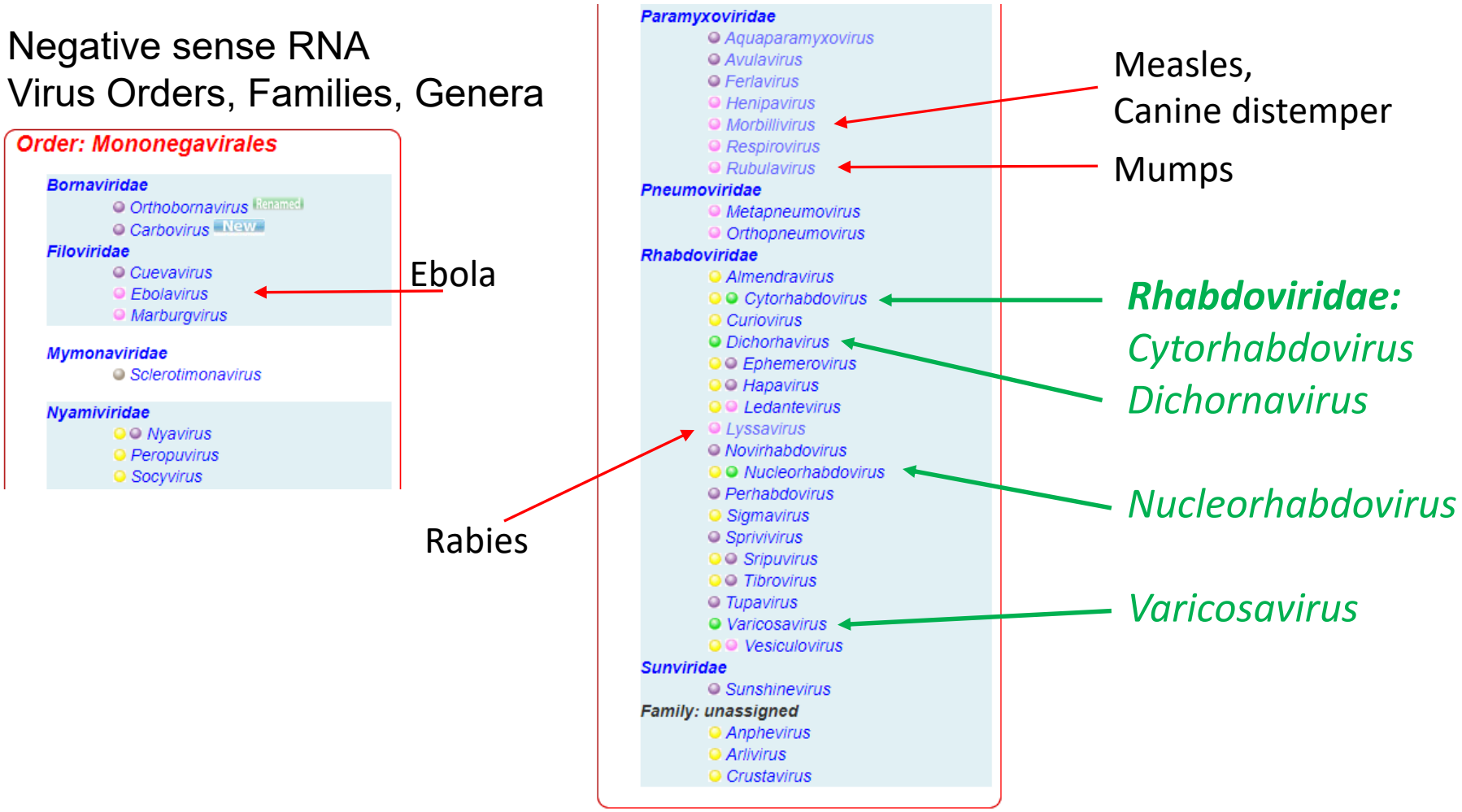


LEGEND: Human; Non-Human Vertebrate; Eukaryotic microorganisms; Fungi; Invertebrate; Plant; Archeabacteria; Bacteria

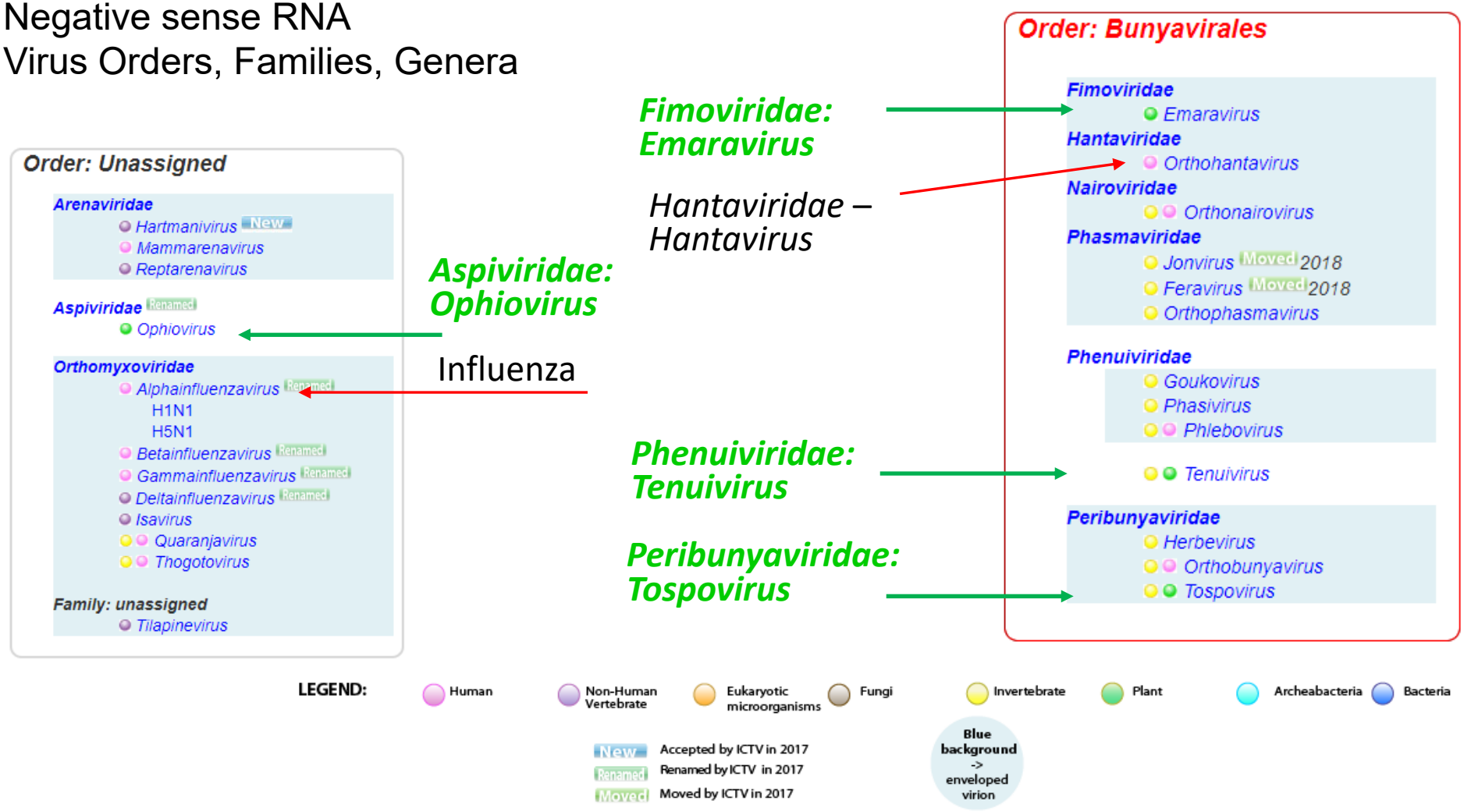
New Accepted by ICTV in 2017
Renamed Renamed by ICTV in 2017
Moved Moved by ICTV in 2017

Blue background -> enveloped virion

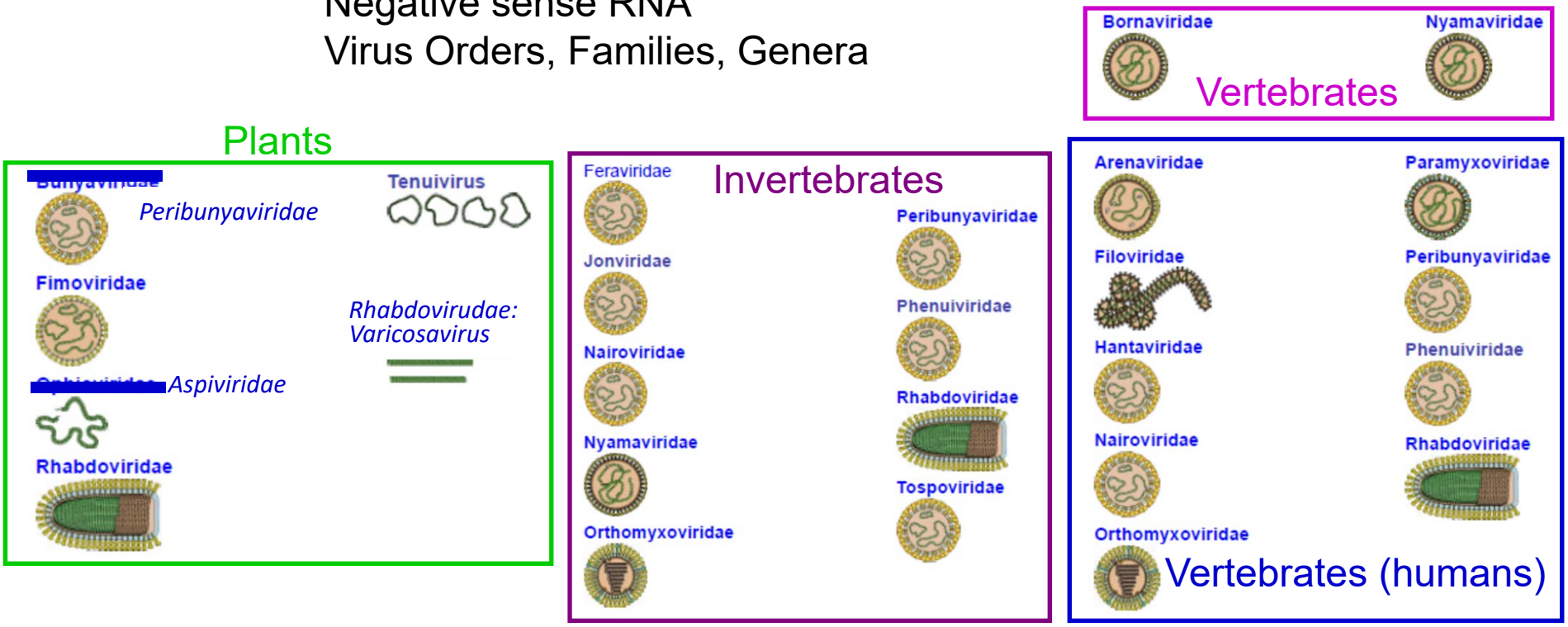
Negative sense RNA Virus Orders, Families, Genera



Negative sense RNA
Virus Orders, Families, Genera



Negative sense RNA
Virus Orders, Families, Genera



Negative-strand RNA viruses are less diverse than (+) sense RNA viruses, BUT are successful pathogens since they can infect a wide range of hosts

Positive sense single stranded viruses have it easy:

the vRNA acts as an mRNA
(5’ - 3’) RNA is translatable

Usually the first protein produced is the RdRp

So what happens if the RNA genome is single stranded but negative sense?

the vRNA cannot be a mRNA
Host ribosomes cannot translate (3’ - 5’) RNA

NEGATIVE STRAND RNA VIRUSES

1. Virions contain molecules of RNA dependent RNA polymerase

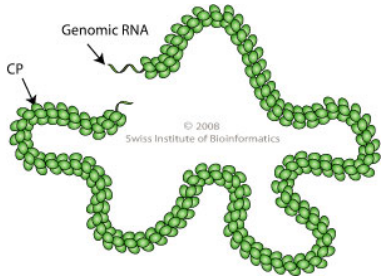
2. Upon infection these polymerases initiate RNA synthesis from viral RNA (vRNA).

    vRNA is transcribed by RdRp to produce a (+)sense mRNA

3. Host ribosomes read the viral (+) sense RNA to produce viral proteins needed for replication

Plant-Infecting ss (-) sense RNA virus families:

- Most ss (-) sense RNA plant viruses are enveloped (exception are viruses in the genera *Ophiovirus, Tenuivirus*)

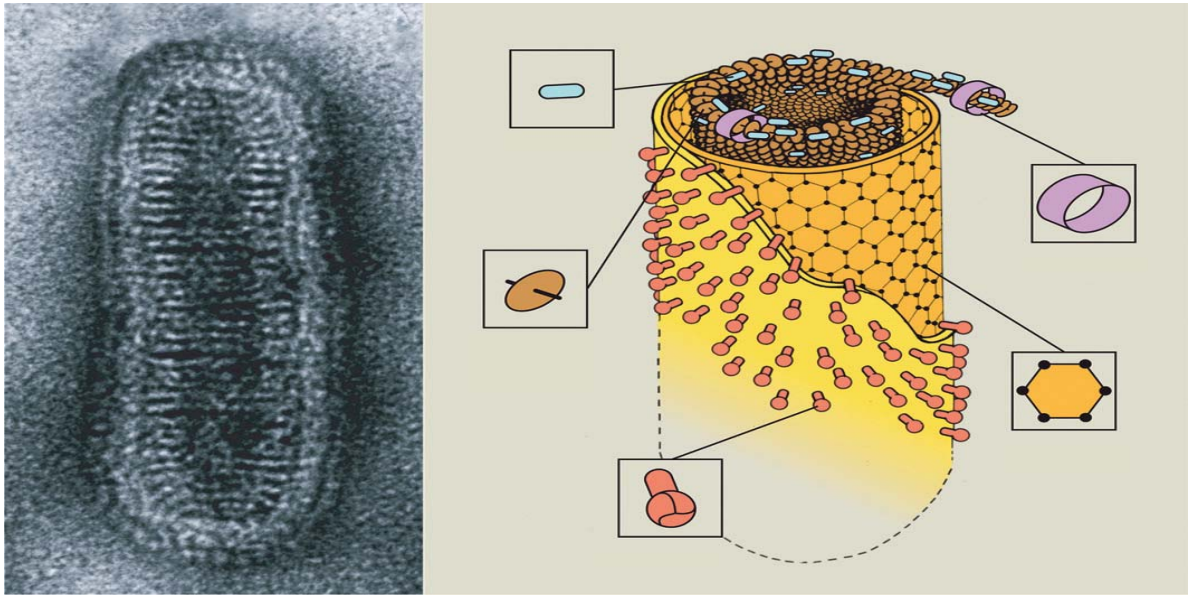


- All plant-infecting (-) strand RNA viruses infect and replicate in their invertebrate vectors

- Unlike many +RNA viruses, the RNA alone is not infectious

Family *Rhabdoviridae*

Virions – enveloped, bacilliform shape with a diameter of 60-100 nm and a length of 100- 430 nm



Family: *Rhabdoviridae*

4 genera that infect plants:

Genus *Cytorhabdovirus*,
aphid or leafhopper vectors
Genus *Nucleorhabdovirus*,
aphid or leafhopper vectors
Genus *Dichorhavirus*
mite vector

Replicate in insect vector and plants

Genus *Varicosavirus*
fungal vector

?Replication in vector?

Virus is transmitted via spores of *Olpidium brassicae* (Chytridomycetes)

Nucleorhabdoviruses:

- Undergo nucleocapsid assembly in the nucleus, morphogenesis through the inner nuclear membrane, and accumulation is in the perinuclear spaces.

Cytorhabdoviruses:

- Assembly, morphogenesis, and accumulation occurs in the cytoplasm

Inclusion of a Nucleorhabdovirus

Viral inclusion (arrow) in the nucleus of an infected epidermal cell of *Nicotiana* hybrid stained with Azure A. The smaller staining body is the nucleolus. Bar - 10 µm.

Family *Rhabdoviridae* *Genus – Nucleorhadbovirus*

Ex. *Sonchus yellow net virus* (SYNV)
common in Florida

Example of some hosts:
Bidens pilosa – often in mixed infections with
Bidens mottle virus

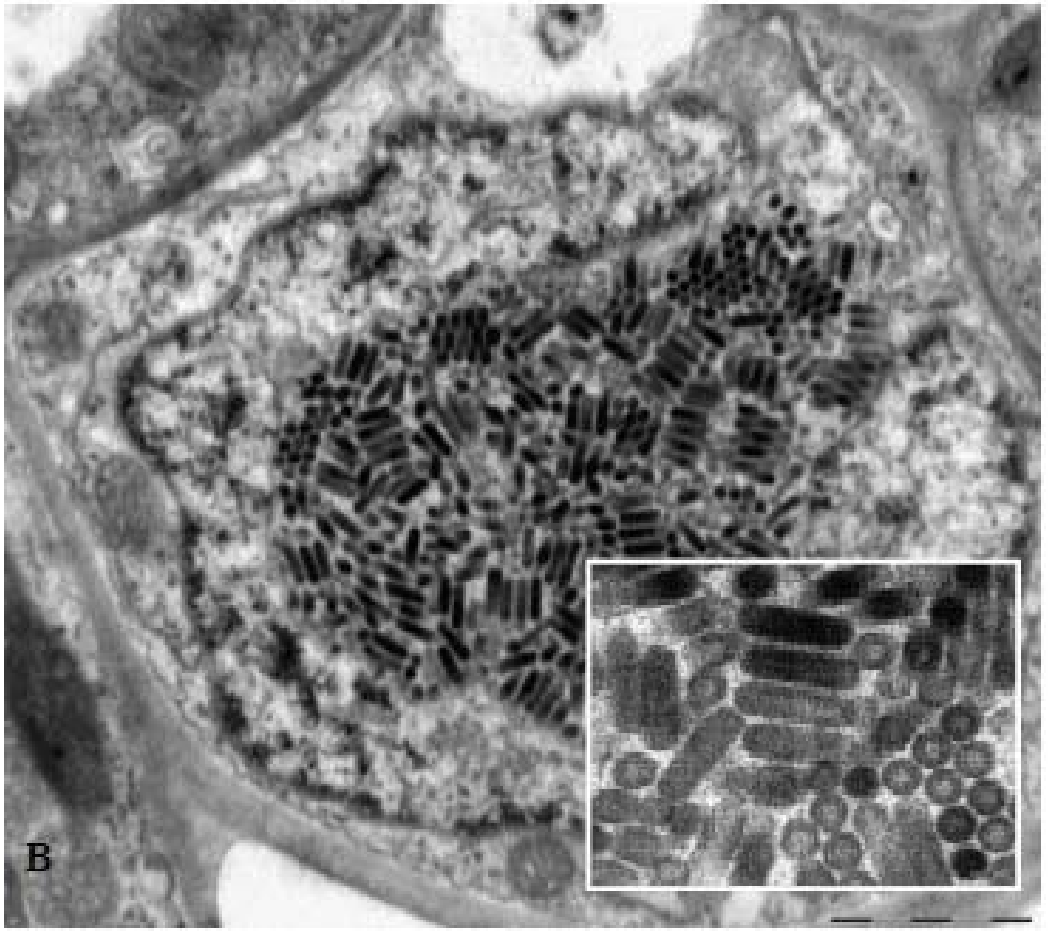
Sonchus oleraceus - vein clearing, yellow patches between veins

SYNV symptoms in *B. pilosa*

SYNV symptoms in *S. oleraceus*

Nucleorhabdovirus:

Sonchus yellow net virus (SYNV)
Thin section of an infected plant cell with cluster of virions in the nucleus.



Family *Rhabdoviridae* *Genus – Cytorhabdovirus*

Ex. *Strawberry crinkle virus* (SCV)

Hosts:

Species of *Fragaria*

Several species of aphids (insect vector)

Symptoms of SCV on strawberry

General Steps in the Replication Cycle of (-) Sense RNA Viruses

1. Disassembly
2. Transcription – production of (+) sense RNA
3. Translation of proteins
4. Replication – production of (-) sense RNA (progeny genomes)
5. Encapsidation
6. Translocation

Cytorhabdovirus and *Nucleorhabdovirus*

Expression Strategies Used:

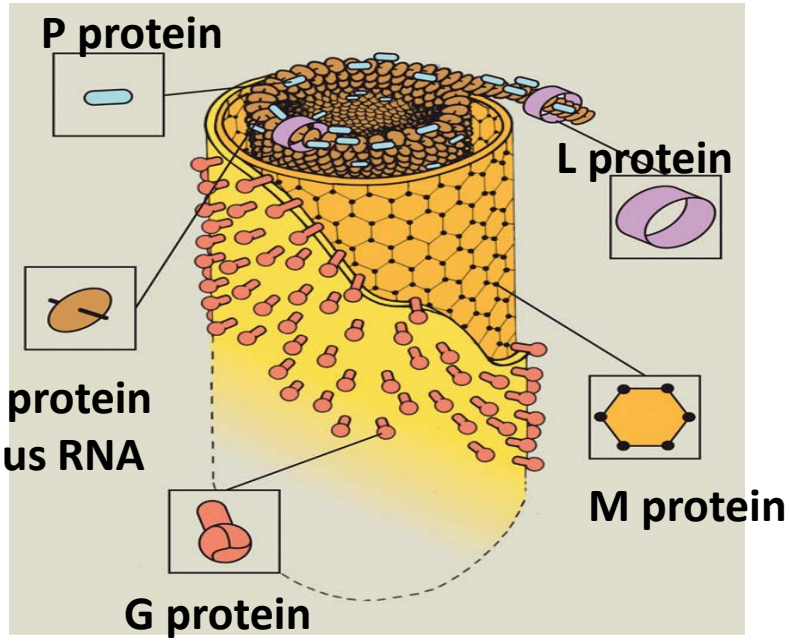
- √ - subgenomic RNAs
- - multipartite genomes
- - polyprotein (proteolytic processing)
- - translational read-through
- - translational frame-shift
- - ambisense RNAs
- - cap snatching

Family *Rhabdoviridae*

Nucleorhabdovirus Genome:

- a single, negative sense ssRNA
- 13,500 nts
- 6 genes
- 5’ cap
- 3’ end - no poly A or t-RNA structure

Structure of Rhabdoviruses



The nucleocapsid core is composed of:

- minus-sense genomic RNA
- the nucleocapsidprotein (N)
- the phosphoprotein(P)
- the polymerase protein (L)

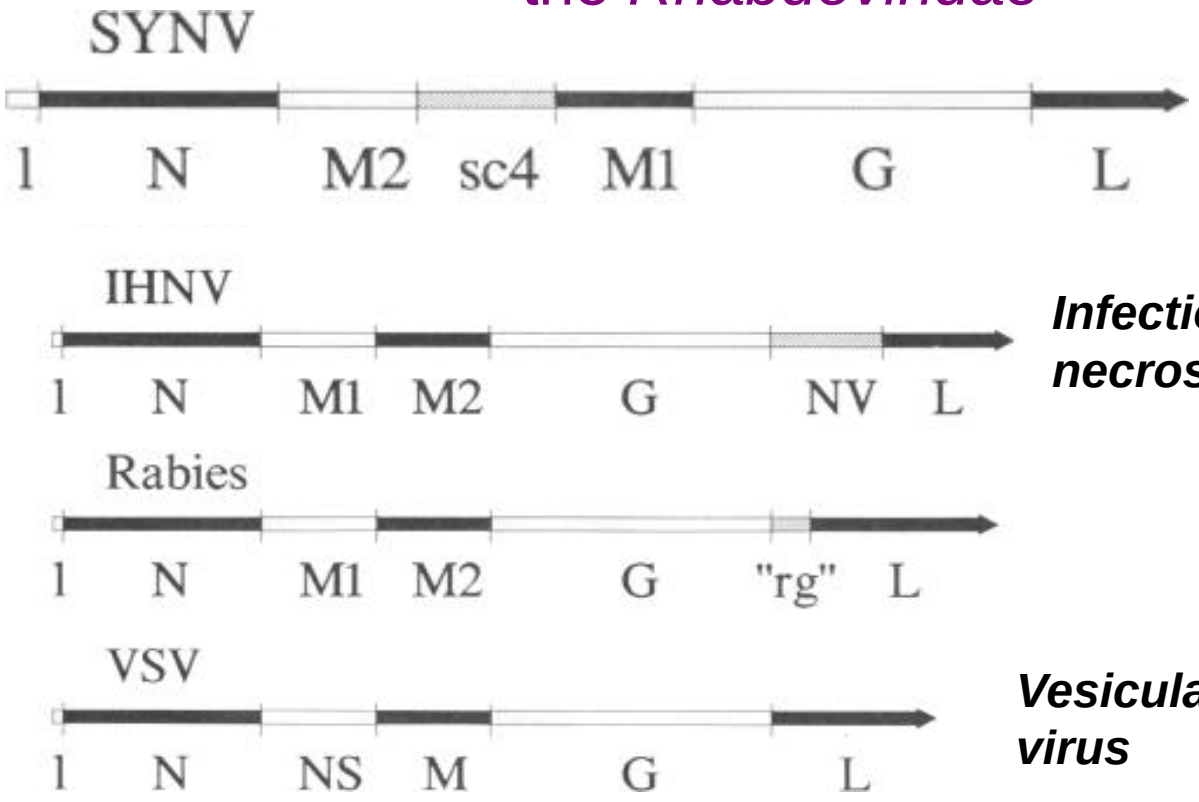
Most of the proteins encoded by rhabdovirus genomes are part of the virion

Genome Organization of Plant Rhabdoviruses:

/ represents the leader RNA
t represents the trailer sequence
N - nucleocapsidprotein
P – phosphoprotein
L - polymerase protein
M - Matrix protein involved in coiling the nucleocapsid, attachment of the nucleocapsid to the envelope, and associations with the transmembrane glycoprotein
G – Transmembrane glycoprotein
X denotes putative movement protein gene
Y shows the location of open reading frames of unknown function in the genomes of several plant and animal rhabdoviruses

Annual Review of Phytopathology, Volume 43

Arrangement of viral proteins is conserved among genera in the *Rhabdoviridae*

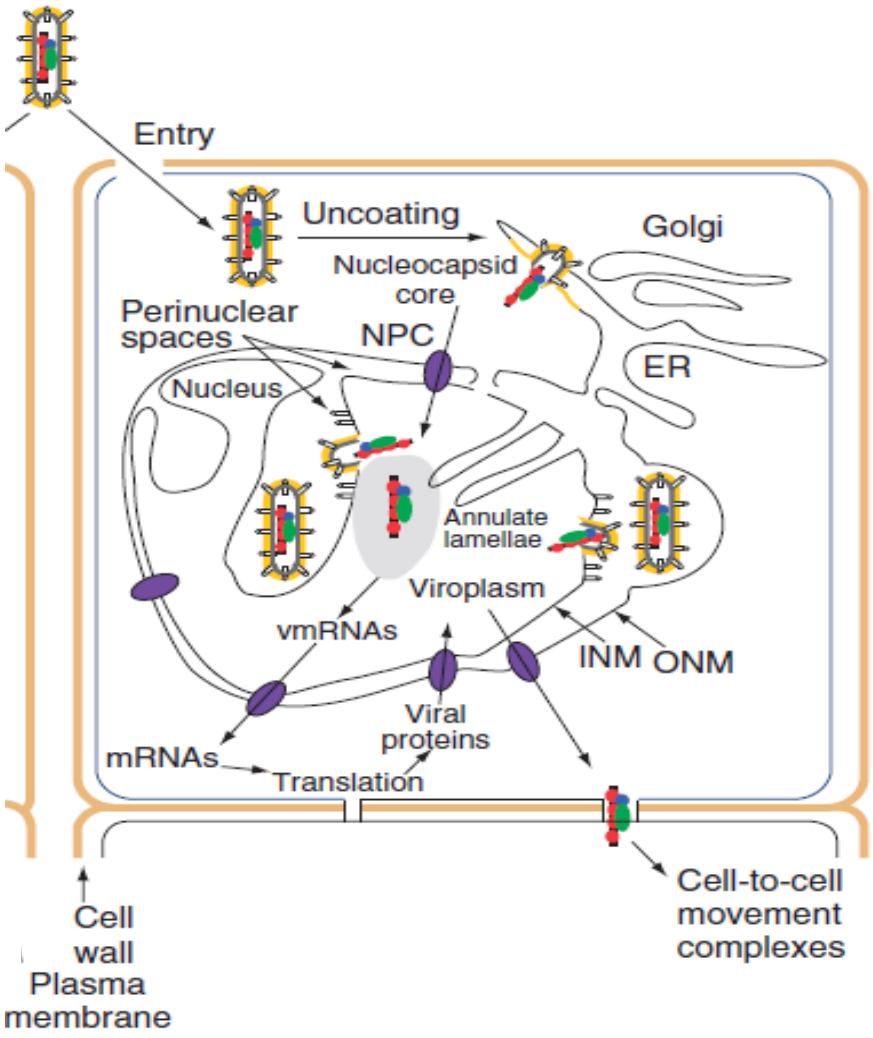


Infectious hematopoietic necrosis virus

Vesicular stomatitis virus

Replication of Nucleorhabdoviruses:

- Studied in more detail than cytorhabdoviruses
- Shows many similarities to vertebrate rhabdoviruses
- Replication occurs in the nucleus
- Replication of cytorhabdoviruses is similar but occurs in the cytoplasm



Nucleorhabdovirus: Interaction and effects on cell membranes

Whole cell (Protoplast)
Green – ER tubules

Nucleus
(Green – nuclear membranes)

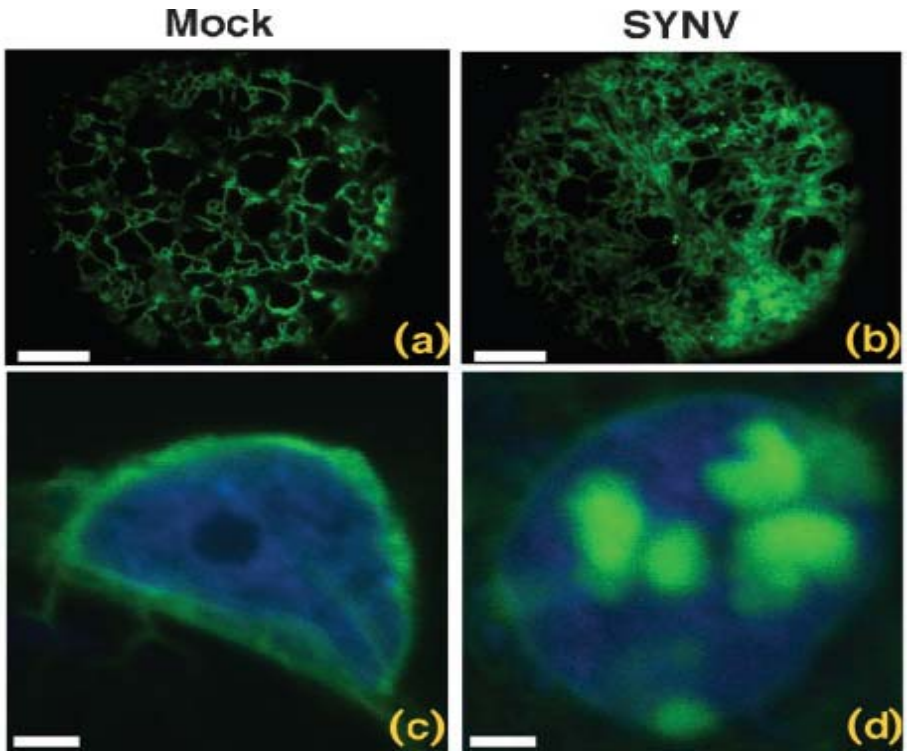
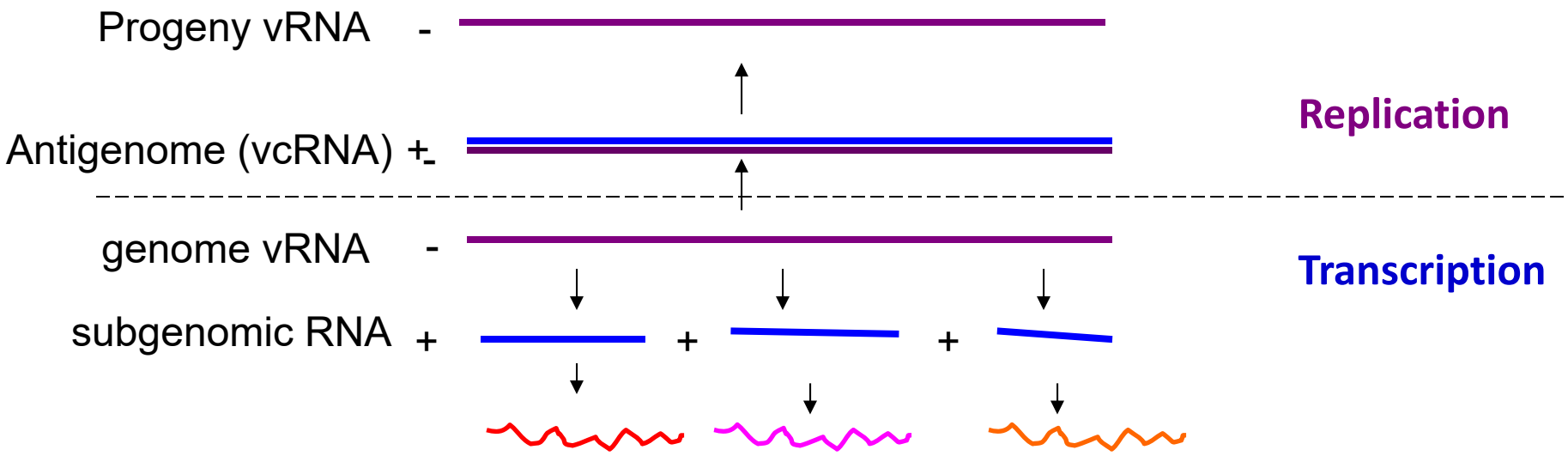


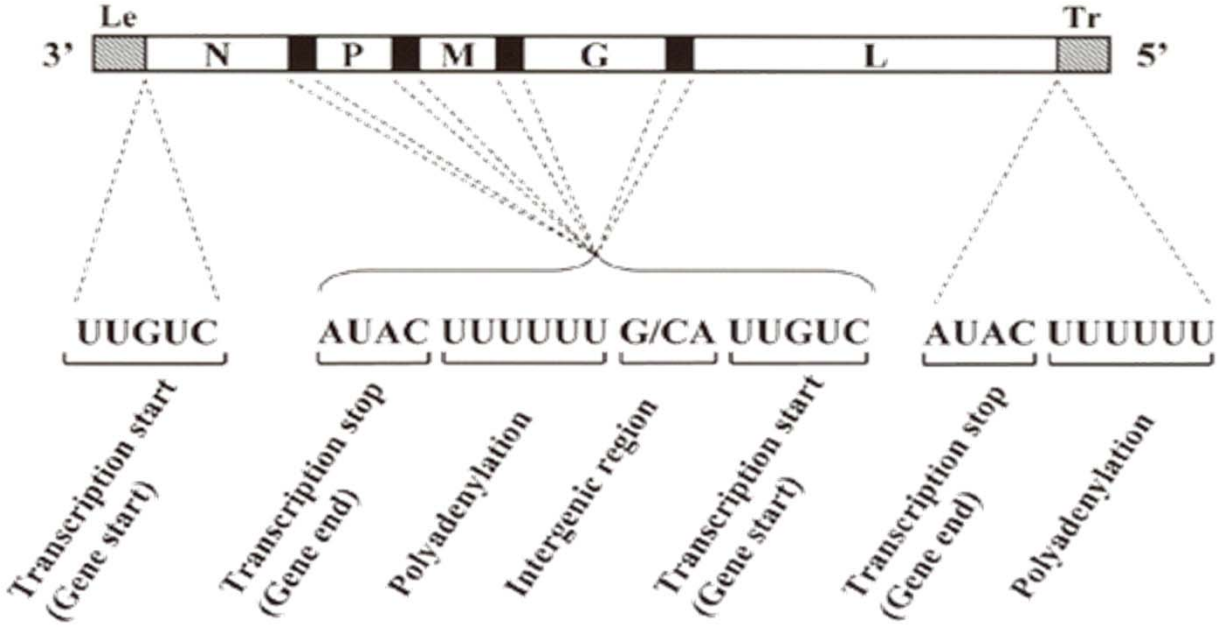
Fig. 1. (a, b) Micrographs showing the distribution of ER tubules in protoplasts derived from leaves of mock-inoculated (a) or SYNV-infected (b) mGFP5-ER *N. benthamiana* plants. Bars, 10 mm.
(c, d) Confocal micrographs showing nuclear membranes (green) of mock-inoculated (c) or SYNV-infected (d) leaf epidermal cells. Nuclei were counterstained with the DNA-selective dye 4,6-diamidino-2-phenylindole (DAPI; blue). Bars, 2 mm. From: Goodin et al J. of General Virology (2007), 88, 1810–1820

“Streamlined” Diagram: Replication Cycle of Rhabdoviruses:

Transcription occurs first, the Replication



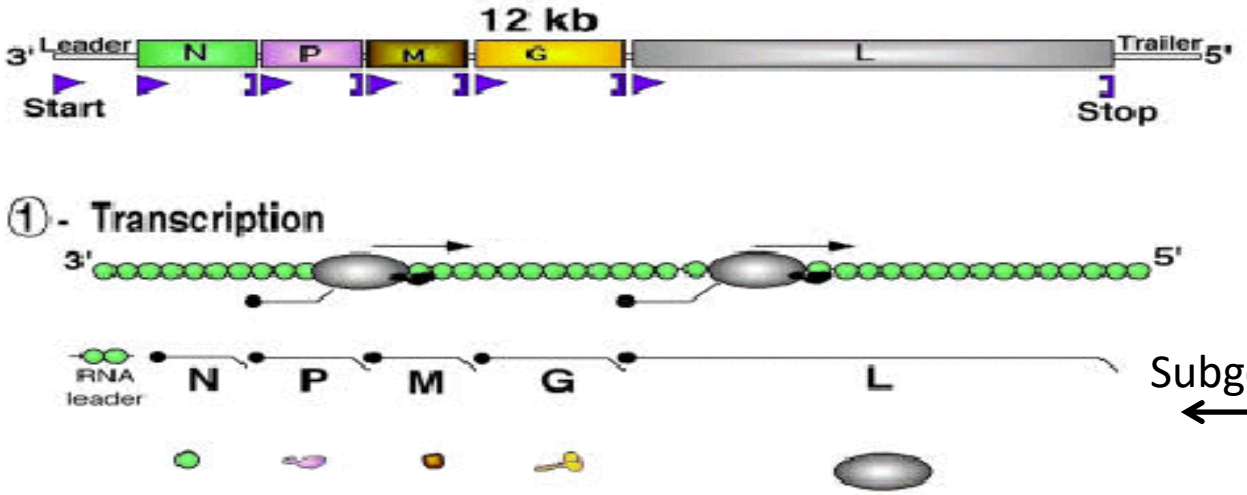
Regulatory sequences in VSV control transcription of the subgenomic RNAs



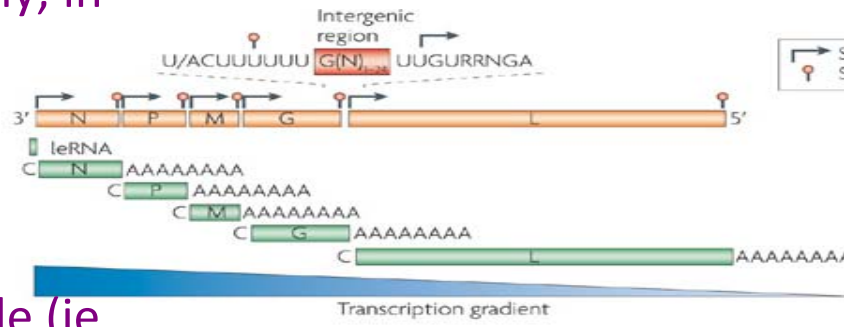
(N) nucleoprotein
(P) phosphoprotein
(M) matrix protein
(G) glycoprotein
(L) polymerase protein

Coding regions are separated by regulatory sequences that contain a transcription stop (gene end) signal, a polyadenylation signal, a nontranscribed intergenic region and a transcription start (gene start) signal. Transcription units are flanked by a leader (Le) and trailer (Tr) region that contain the genomic and antigenomic viral promoters, respectively. Note that the genome sense strand is shown, so by convention, the 3'-end is on the left. Neumann et al, J. Gen Virol DOI 10.1099/vir.0.18400-0

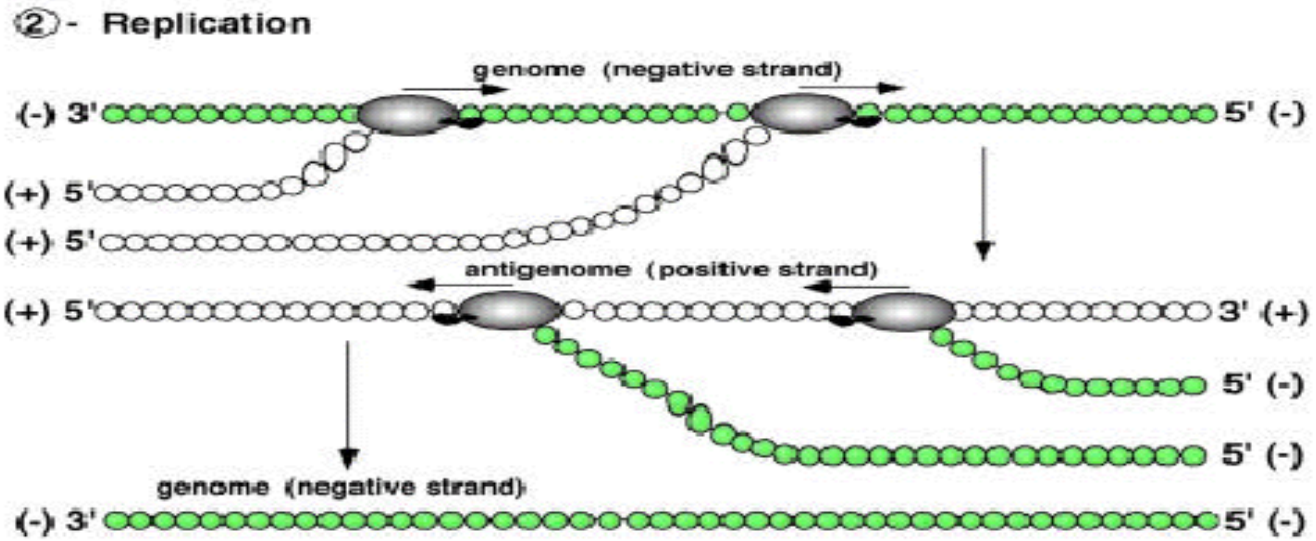
Rhabdovirus (VSV) Transcription and Replication



- Rhabdovirus genes are transcribed sequentially, in order of greatest requirement and timing of requirement
- Regulatory leader sequence is transcribed first and binds N protein early and often, resulting in maintenance of transcription mode (ie production of subgenomic RNAs)



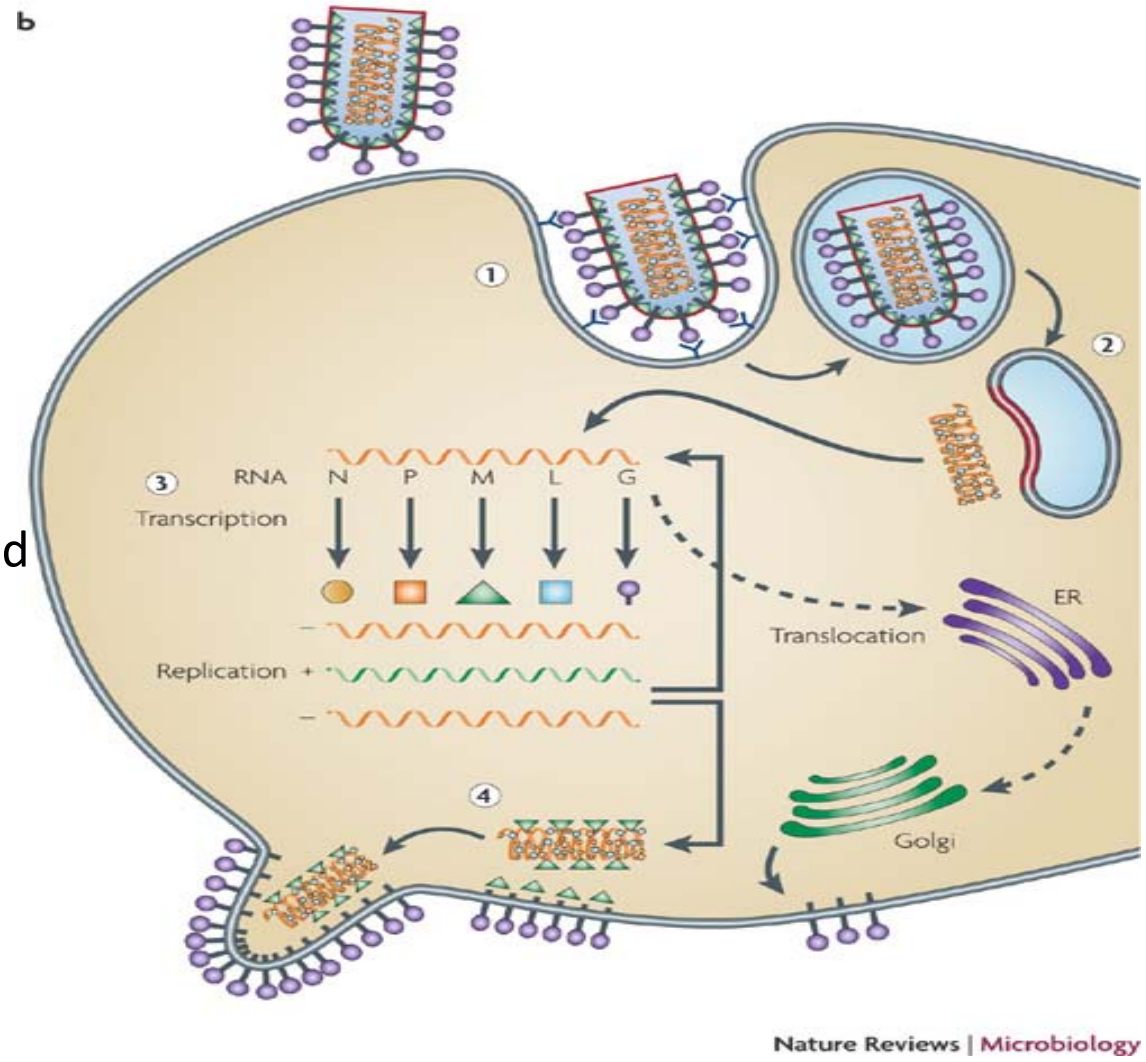
Rhabdovirus (VSV) Transcription and Replication



- Sufficient N-protein accumulation and phosphorylation state of P protein results in switch from transcription to replication
- Full-length antigenome (+ sense RNA) is template for - sense genome replication

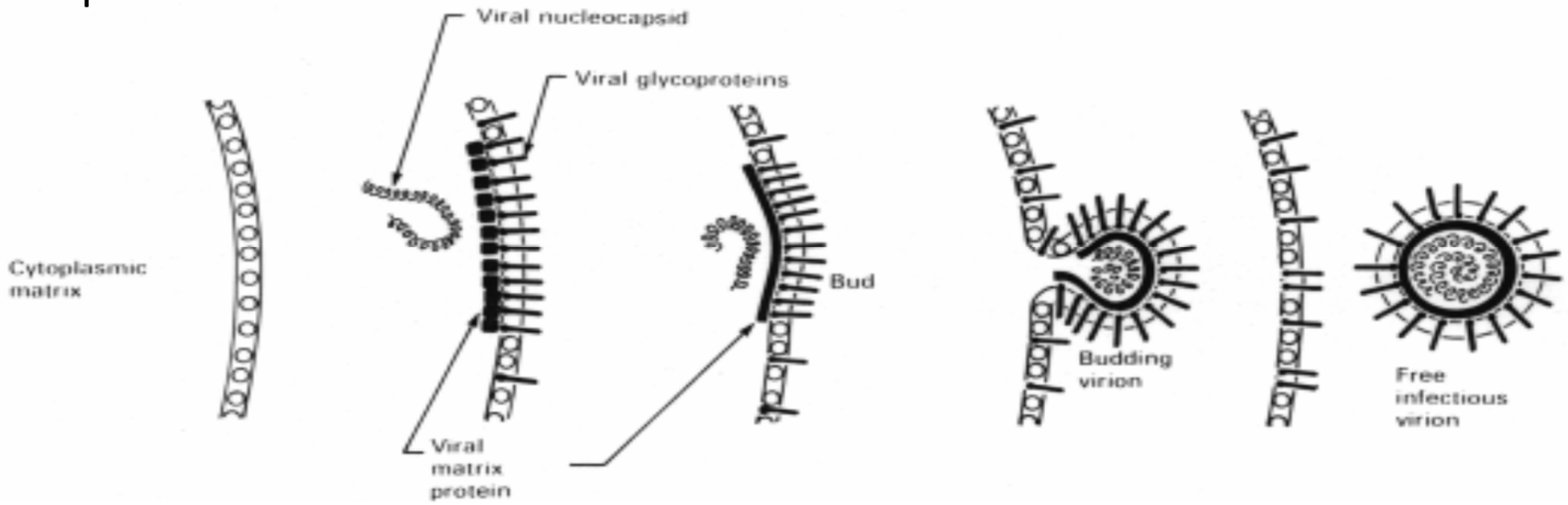
A simplified rabies virus life cycle:

1) Binding and entry into the host cell by endocytosis (animal cell)
2) Fusion of the viral membrane and endosome membrane to release the viral genome (uncoating).
3) Virion components are produced (transcription, replication, protein synthesis;
4) Assembly of the viral components, budding and release of the rabies virus virions.



How a vertebrate Rhabdovirus acquires its envelope

After assembly of the viral genomes with the coat protein (nucleocapsid).
the viruses acquire lipid membranes imbedded with viral-encoded glycoproteins.
Virus-specified envelop proteins (glycoproteins) go directly to the appropriate membrane (nuclear membrane or endoplasmic reticulum, or the Golgi apparatus) and displace host proteins. The glycoproteins determine where virion maturation takes place.



Bunyavirales

- Family *Fimoviridae*
  - Genus *Emaravirus*
- Family *Phenuiviridae*
  - Genus *Tenuivirus*
- Family *Peribunyaviridae*
  - Genus *Tospovirus*
  - TSWV: Important plant pathogen with the widest host range of any plant virus; replication in thrips and plants

Fimoviridae

Tenuivirus

Tospoviridae

Order: Bunyavirales

Fimoviridae
- *Emaravirus*

Hantaviridae
- *Orthohantavirus*

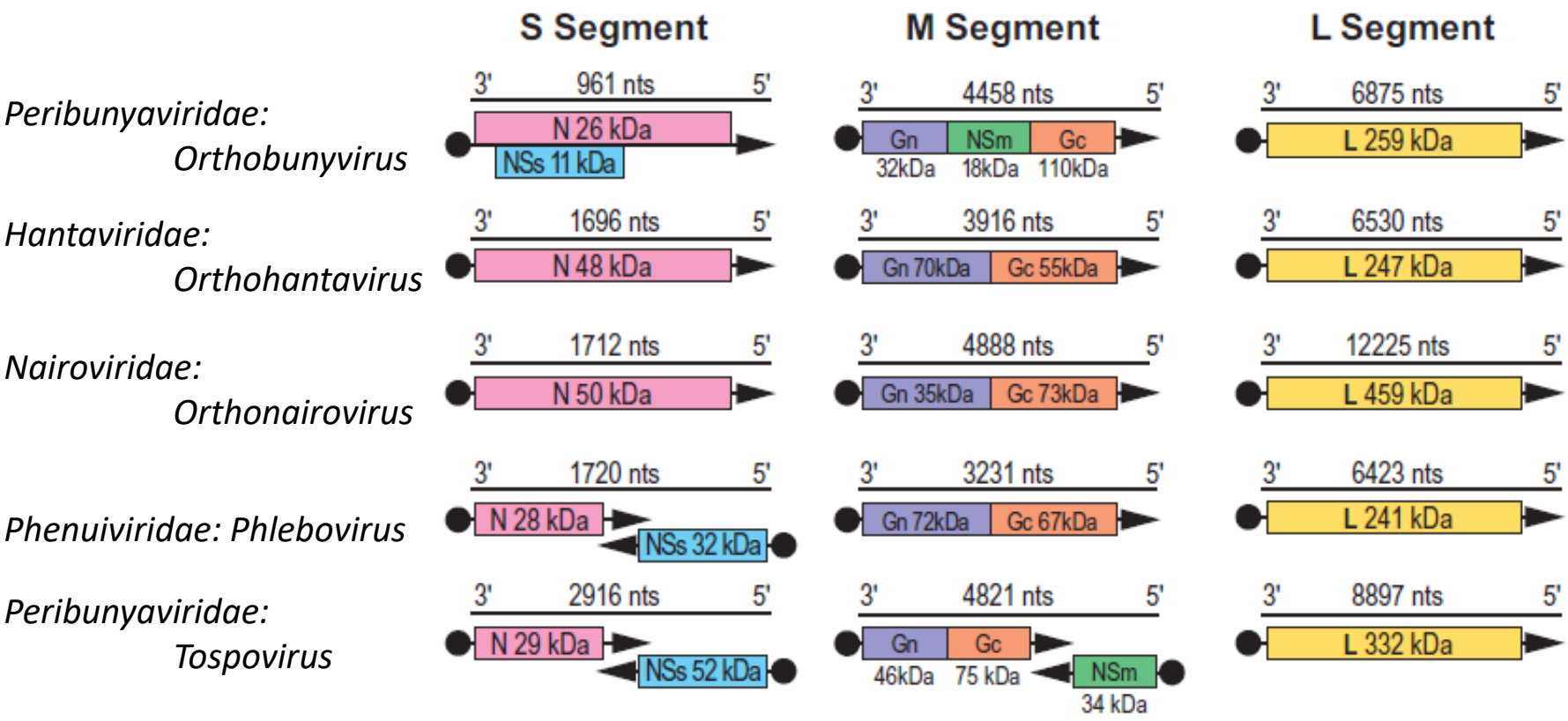
Nairoviridae
- *Orthonairovirus*

Phasmaviridae
- *Jonvirus* Moved 2018
- *Feravirus* Moved 2018
- *Orthophasmavirus*

Phenuiviridae
- *Goukovirus*
- *Phasivirus*
- *Phlebovirus*
- *Tenuivirus*

Peribunyaviridae
- *Herbevirus*
- *Orthobunyavirus*
- *Tospovirus*

Coding strategies of the genome segments of the 5 genera in the Order *Bunyavirales*

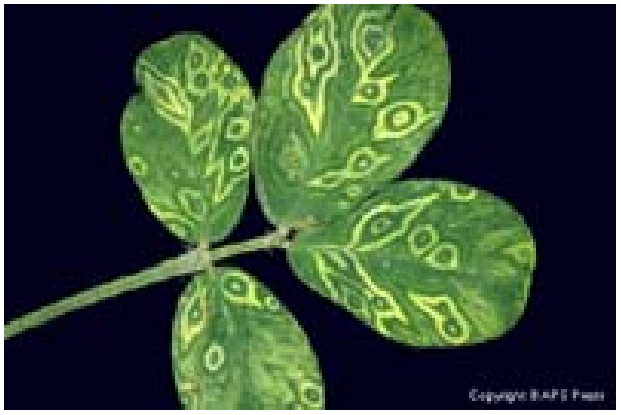


genus ***Tospovirus,*** Type member:

Tomato spotted wilt virus (TSWV)





TSWV

Hosts: 800+ known host species (80+ plant families); monocots and dicots
Many horticultural and agronomic crops susceptible to TSWV infection:
ex. tomato, pepper, potato, lettuce, papaya, peanut, tobacco chrysanthemum
Symptoms tend to be unusual and severe: necrosis, ringspots, wilting,

Distribution: Temperate, subtropical and tropical regions of the world

Vectors: several species of thrips (*Thysanoptera, Thripidae*)

Economic Significance: Incidences of 50 to 90% lead to major losses in crops
TSWV is one of the 10 most economically destructive plant viruses: worldwide losses exceed **one billion dollars** annually.

General Steps in the Replication Cycle of (-) Sense RNA Viruses

1. Disassembly
2. Transcription – production of (+) sense RNA
3. Translation of proteins
4. Replication – production of (-) sense RNA (RNA genomes)
5. Encapsidation
6. Translocation

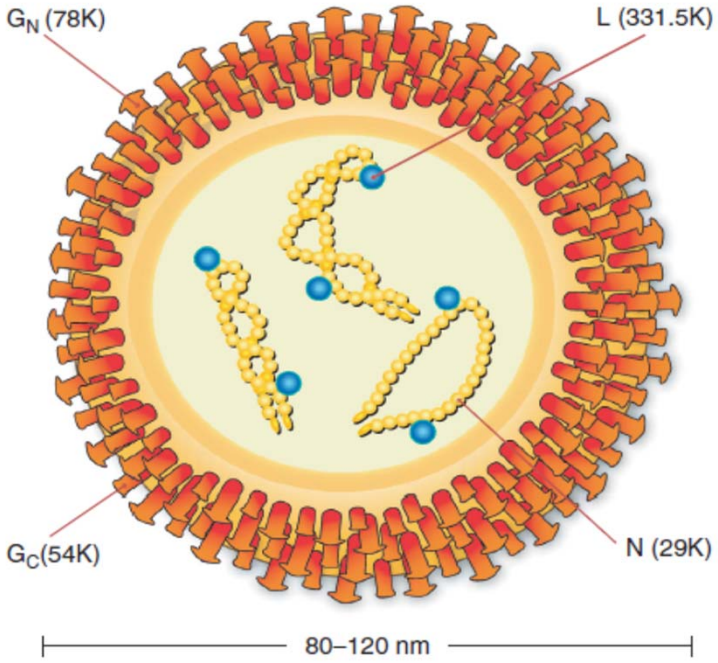
Orthotospoviruses:

Expression Strategies Used:

√ - subgenomic RNAs
√ - multipartite genomes
√ - polyprotein (proteolytic processing)
- translational read-through
- translational frame-shift
√ - ambisense RNAs
√ - cap snatching

Structure of Orthotospoviruses

- Negative sense ssRNA genome
- Monopartite virion, 80-120 nm
- Multipartite genome –
  3 RNA genome segments (S,M, L)
- Segments have partially complimentary terminal sequences that allow the RNAs to form into pseudocircles or "panhandles"

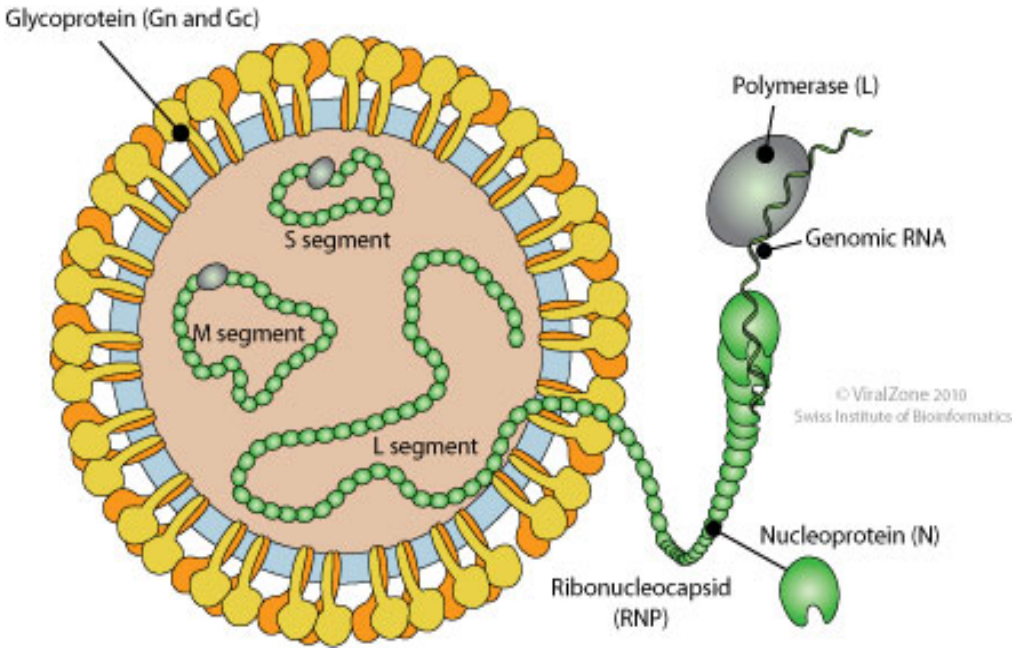


- Nucleocapsids are enclosed by a host-derived bi-layer (double) membrane

Orthotospovirus Particle Structure

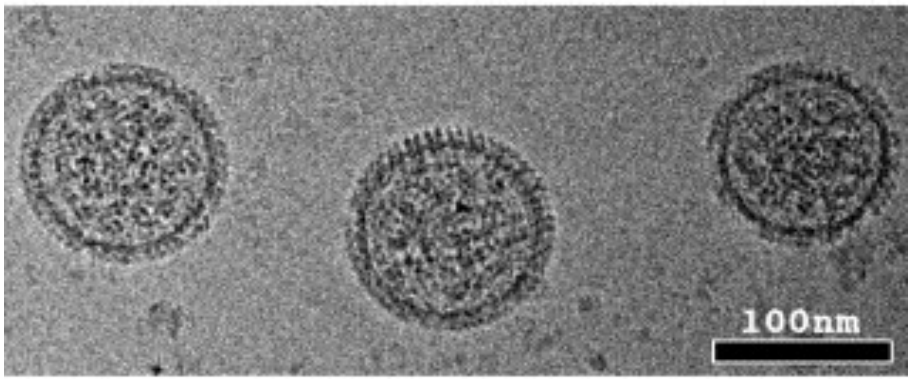
Nucleocapsids are enclosed in host-derived membrane with the RdRp (the L protein)

- Membrane contains two viral coded glycoproteins (GN, GC)
- Each RNA is encapsidated by the viral nucleocapsid protein (N protein) and thus forms a ribonucleoprotein structure (**nucleocapsid**)

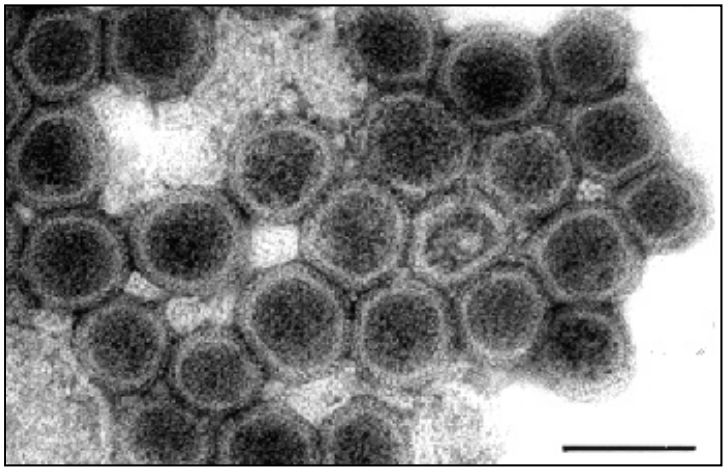


Virion Structure

Pleomorphic virions

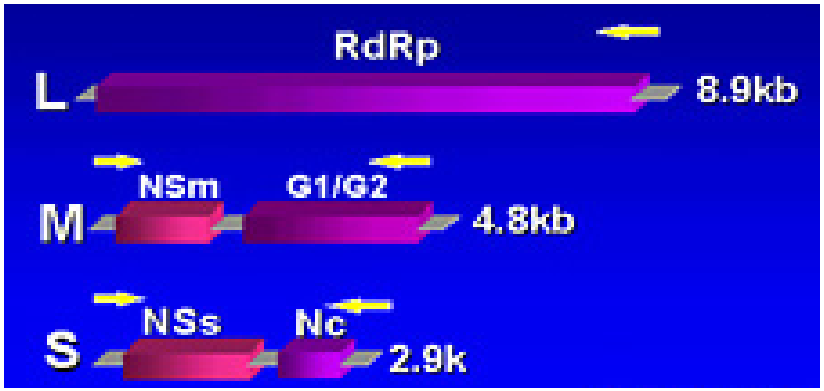


Cryo-EM micrograph of Hantaan virus particles



EM of negatively stained particles of TSWV (bar represents 100 nm

Tospovirus ORFs :



L RNA

RdRp – replicase 331 kDa
[vc sense, from one long mRNA)

M RNA

NSm (viral sense)– Non-structural protein 33.6 kDa, important in tubule formation, important for cell-to-cell and systemic movement in plants, trigger of HR (programmed cell death) in resistant plants (SW5, *Tsw*),

GN, GC (vc sense) – glycoproteins (58 and 78 kDa) in the bilayer membrane function in assembly of virions, role in infection of thrips cells

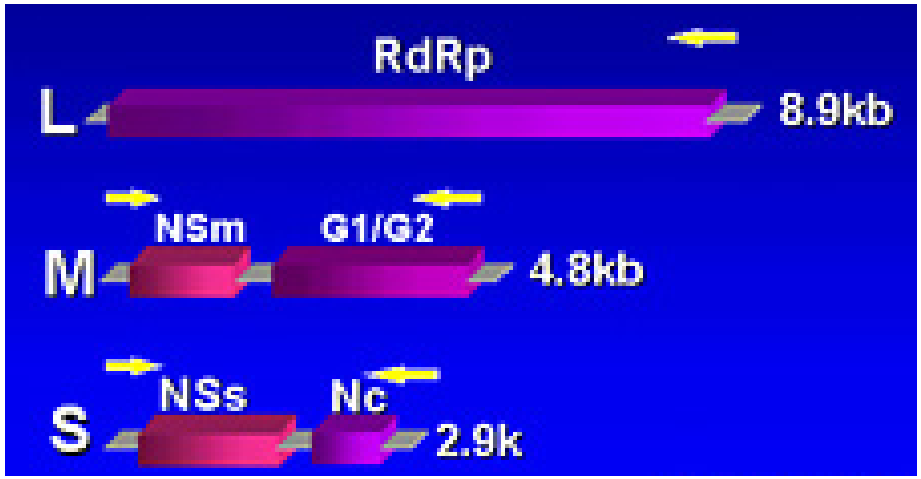
S RNA

NSs (viral sense, sub genomic)– Non-structural protein, silencing suppressor

N (or Nc) (vc sense, sub genomic)– nucleocapsid protein (29 kDa), binds to RNA and forms the nucleocapsid, plays a role in HR (programmed cell death)

Replication of Orthtospoviruses

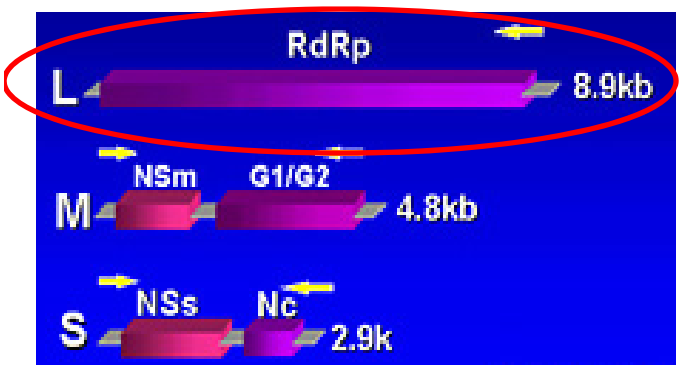
- Proteins on the L, M and S RNAs are not expressed in the same way

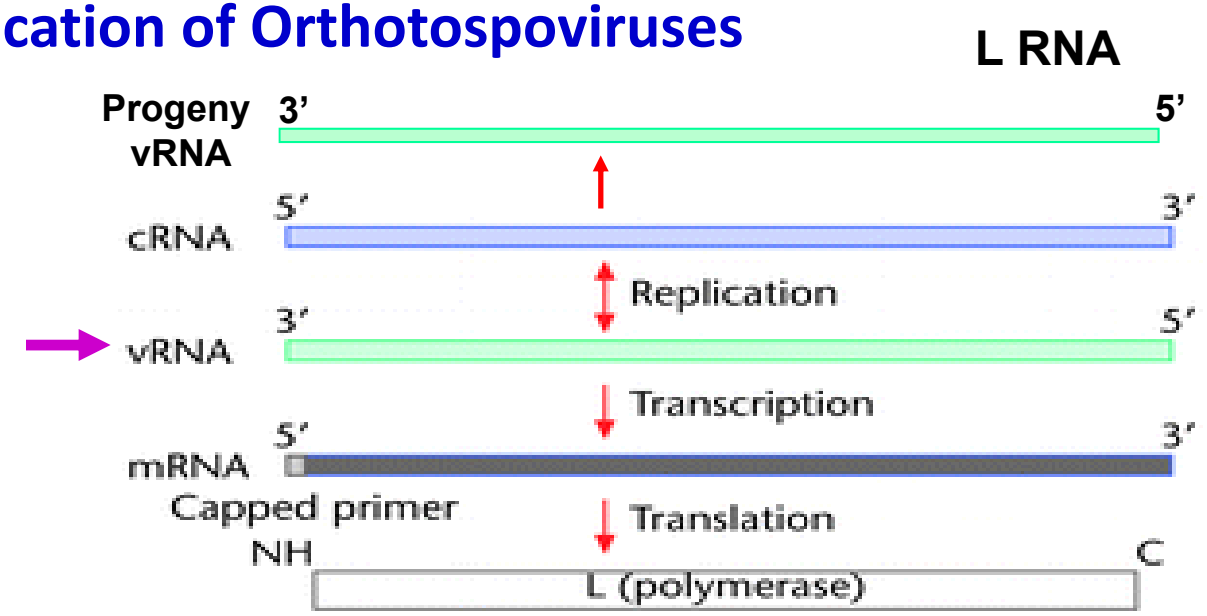


- L RNA – negative sense
- S RNA and M RNA - ambisense

Ambisense – a genome in which both nucleic acid strands encode for one or more proteins

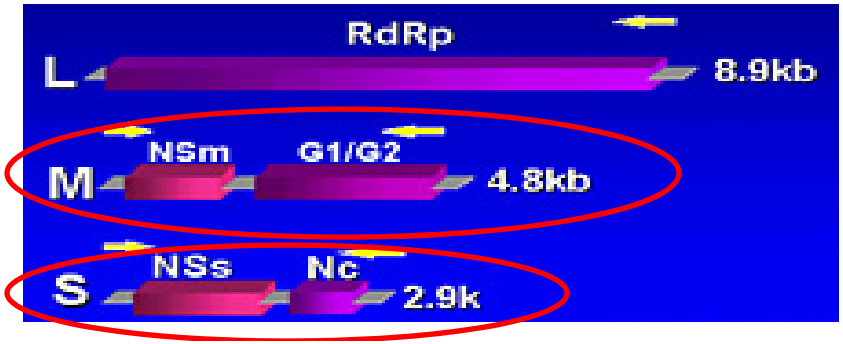
Replication of Orthotospoviruses



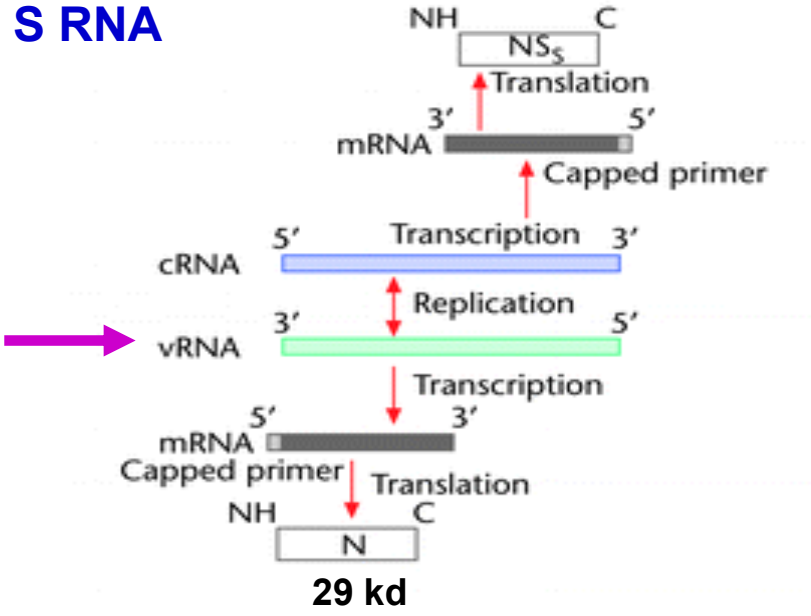


- vRNAs stay bound to the N protein. The polymerase can move down the RNAs without interference from the N protein.
- vRNAs can either be transcribed or replicated and both can occur off the same RNA. The concentration of N protein determines transcription or replication - at low N concentrations, the polymerase transcribes mRNAs that are translated into the viral proteins.

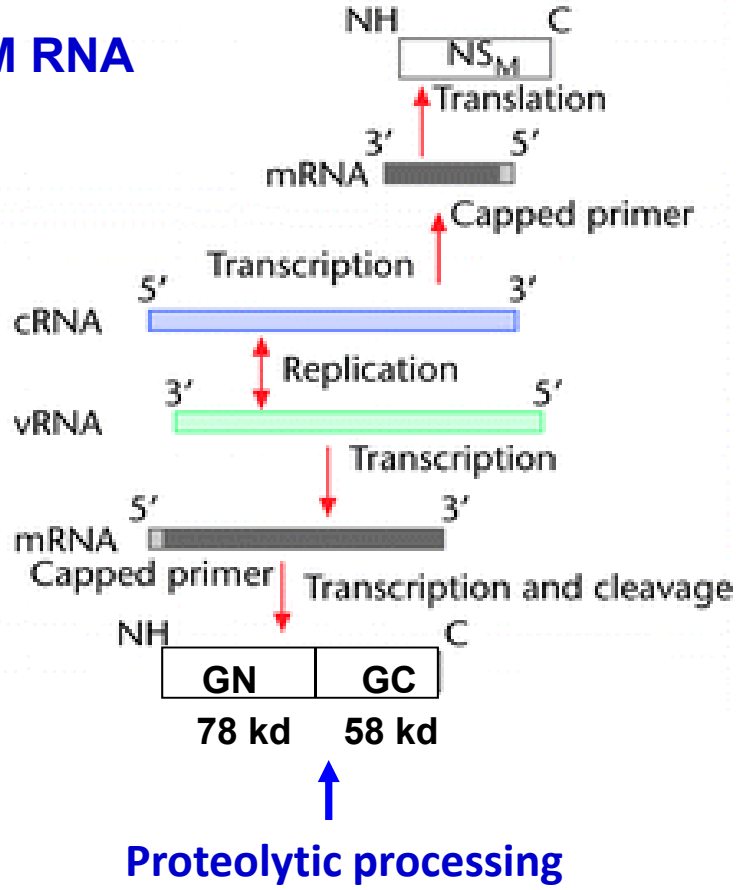
Replication of Orthotospoviruses



S RNA

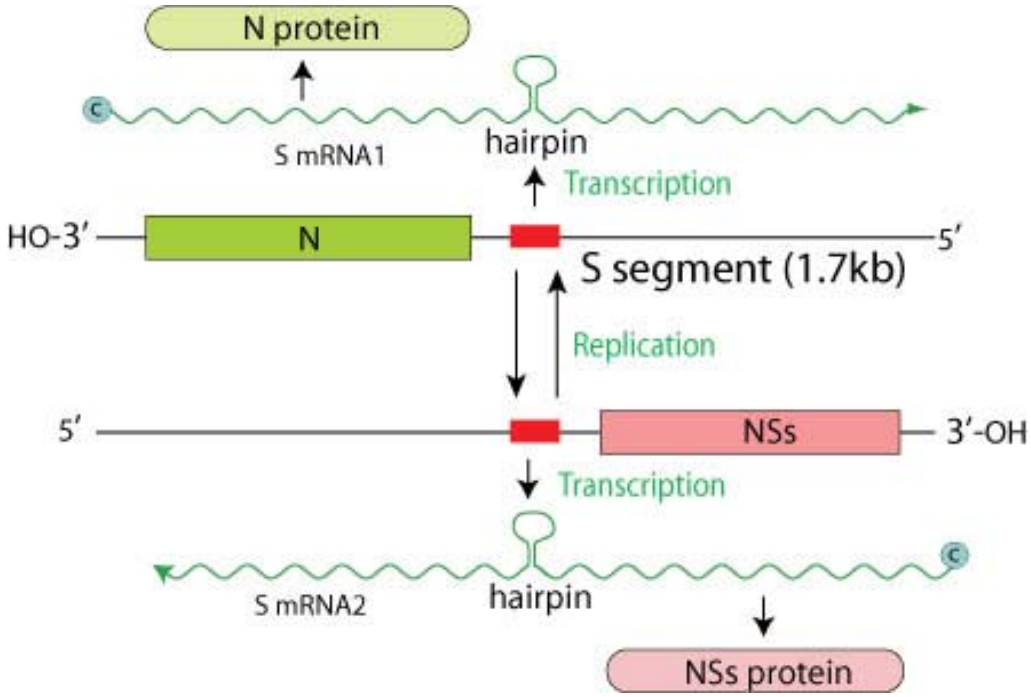


M RNA



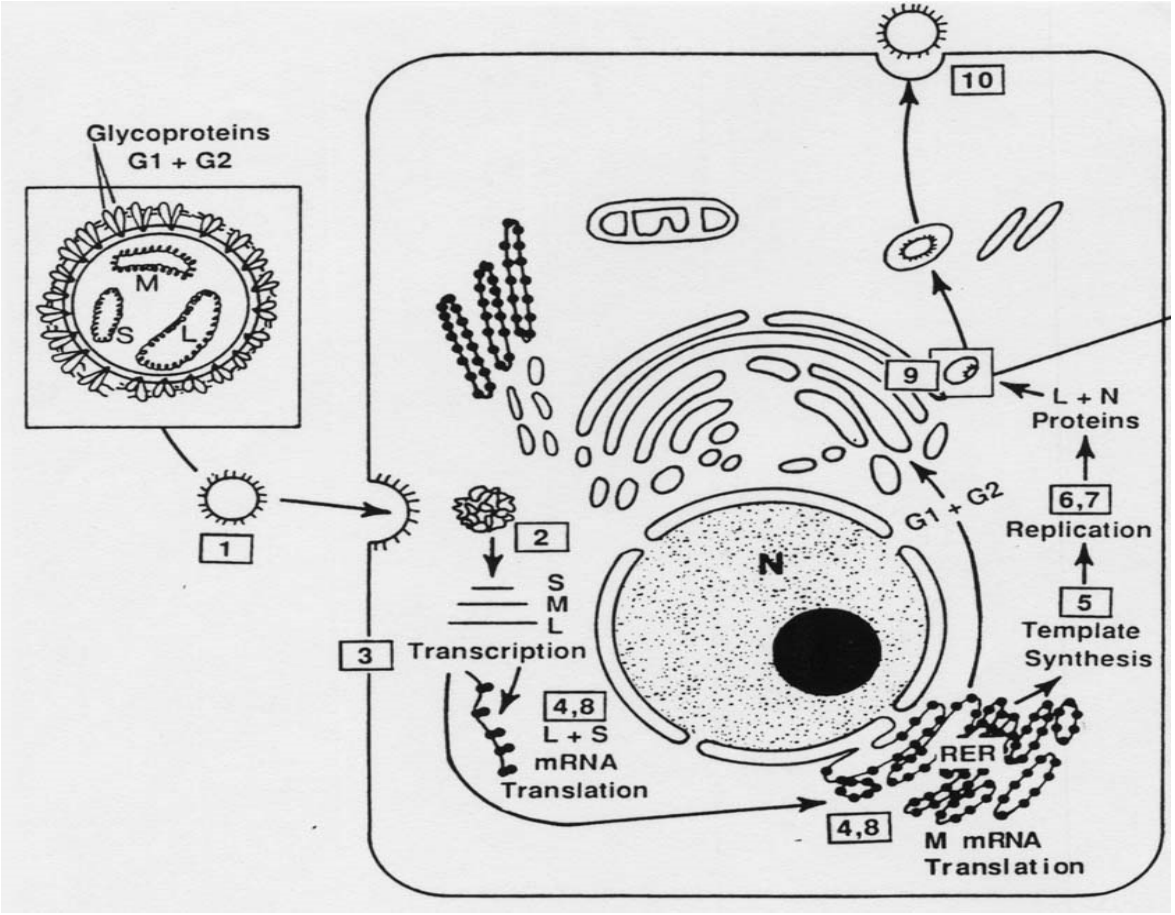
Proteolytic processing

Ambisense is possible because of a hairpin that strongly stops transcription



- The viral RNA dependent RNA polymerase (L) binds to a promoter on each encapsidated segment, and transcribes the mRNA. Transcription is terminated by a strong hairpin sequence at the end of each gene. mRNAs are capped with L protein during synthesis.

Replication

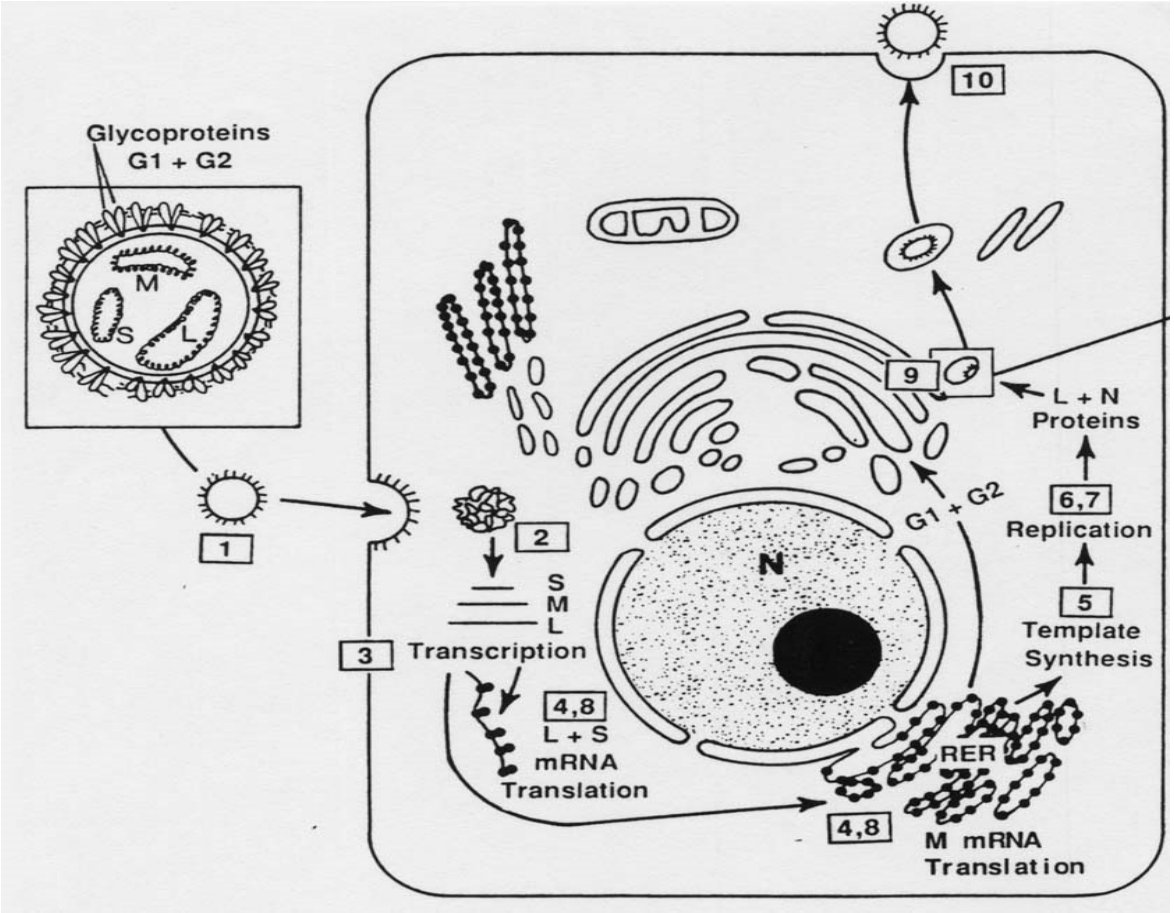


Replication of is cytoplasmic; there is no nuclear component.

1. Virus enters plant cell through wound (mechanical or through puncture by thrips)

2. In the thrips host, virus attaches to host receptors though Gn-Gc glycoprotein dimer and is endocytosed into vesicles into the host cell.

Replication

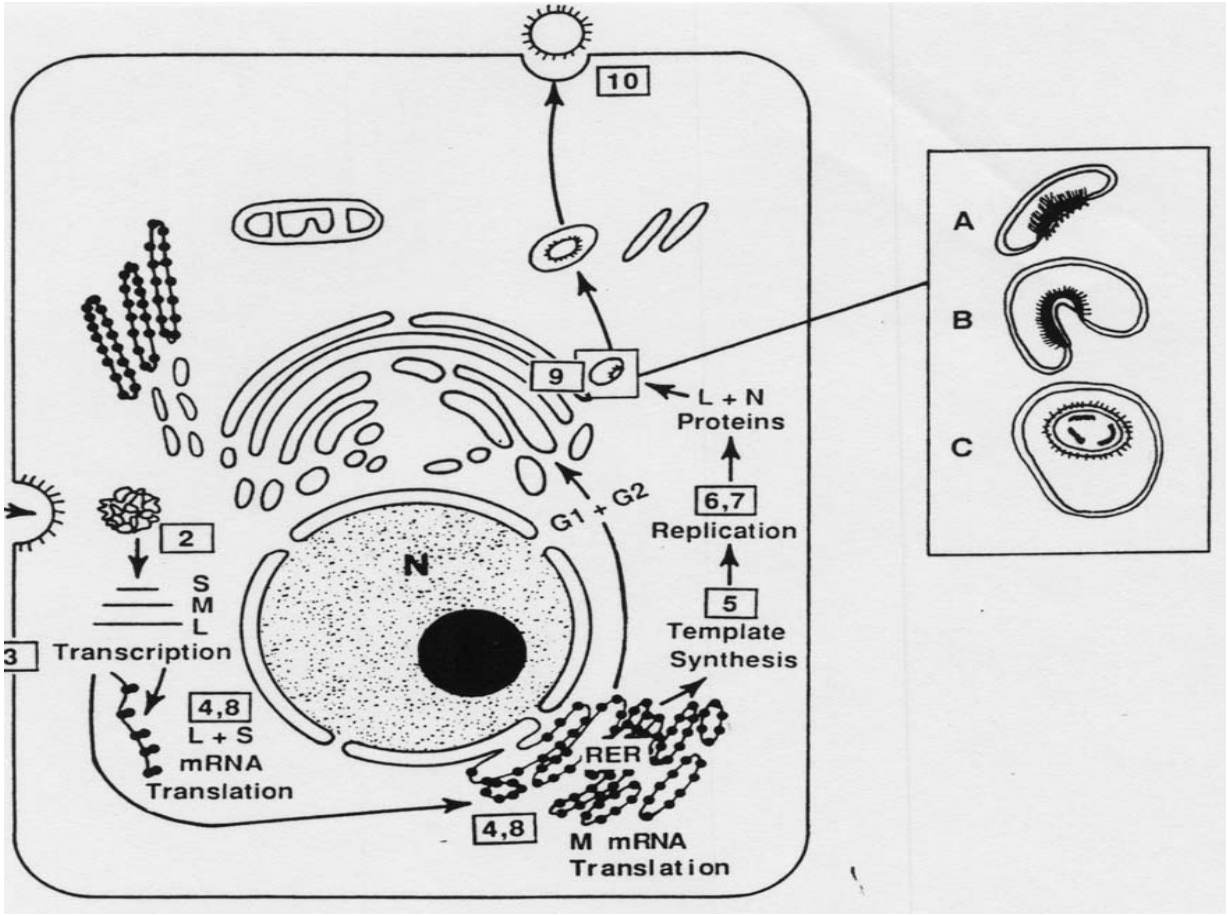


3. Virus membrane fuses with the vesicle membrane; ribonucleocapsid segments are released into the cytoplasm.

4. Transcription, viral mRNAs are capped in the cytoplasm.

5. Replication presumably starts when enough nucleoprotein is present to encapsidate newly-synthetized genomes.

Replication



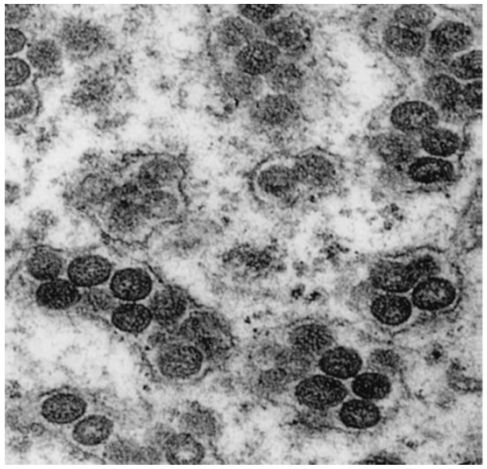
6. In non-plant hosts, the ribonucleocapsids migrate under the plasma membrane and then bud, releasing the virion.

But this is not true for topsoviruses in plant cells.

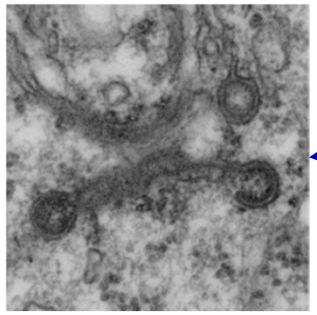
TSWV particle morphogenesis

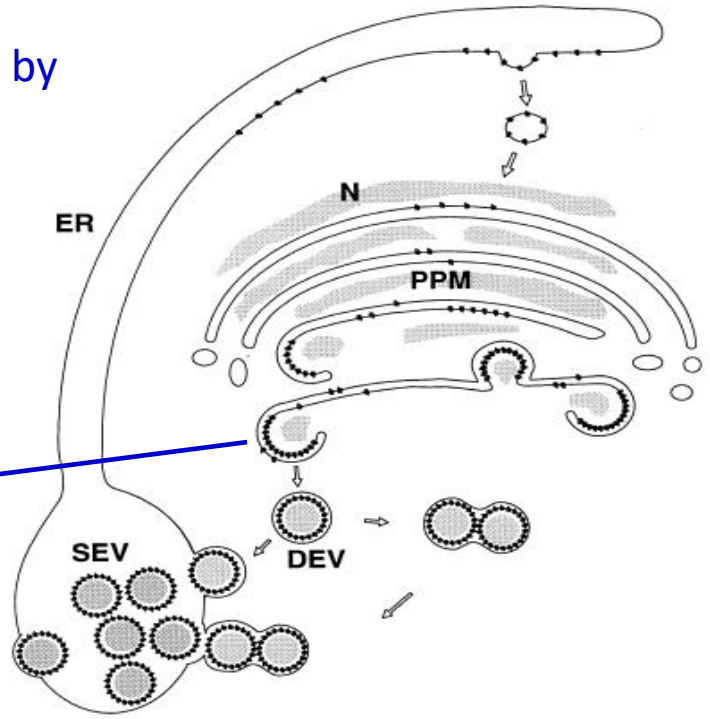
Viral glycoproteins (G1 and G2) accumulate in the Golgi. In a later stage of the maturation, these doubly enveloped particles fuse to each other and to the endoplasmic reticulum to form singly enveloped particles (SEV) clustered in membranes.

Doubly enveloped particles (DEV) are formed (within 18 hrs) by wrapping of modified Golgi membranes (PPM) around nucleocapsids in the cytoplasm, and subsequently singly enveloped particles are formed by fusion of DEV with each other or with ER membranes.



Clustered singly enveloped particles (SEV) inside smooth and rough ER membranes, 40 hours after inoc



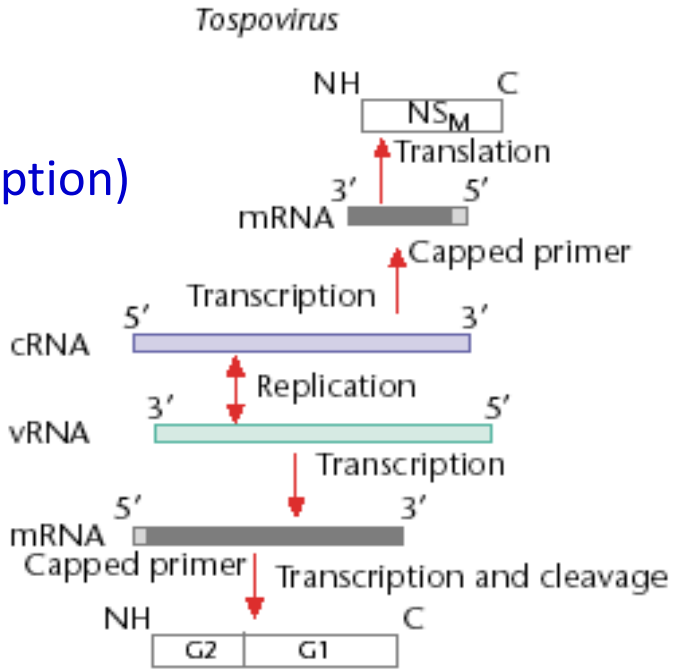


Kikkert et al 1999 *J Virol.* 73: 2288–2297.

Cap-snatching:

Cap (10 -20 nt) at 5’ end (functions as primer for transcription)
Occurs in viruses with (-) sense segmented genomes

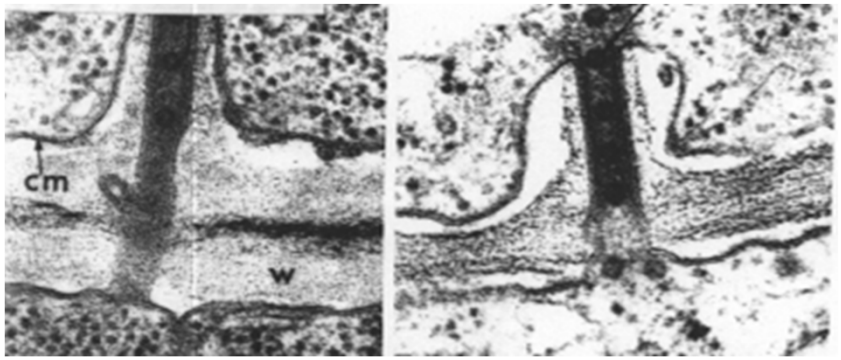
Ex. Tospoviruses (also Tenuiviruses)



- Synthesis of the viral mRNAs is initiated by snatching the 5’ ends (caps) from host mRNAs (and other virus’s RNAs).

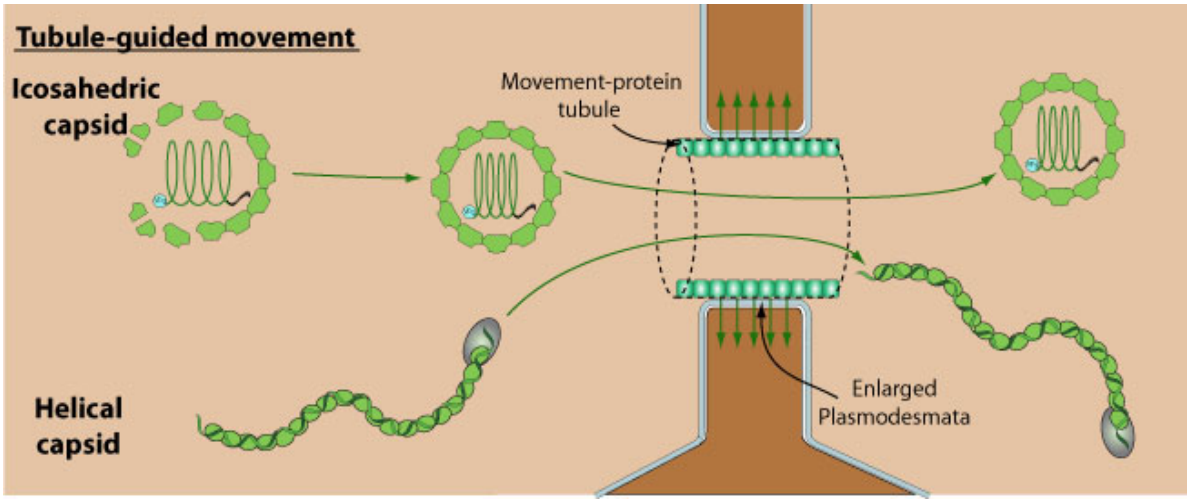
- This mechanism may enable viral RNAs to gain precedence over non-TSWV mRNAs

Cell to Cell Movement:



- **NSm – forms tubular structures** in the plasmodesmata of plant cells (and on the surface of thrips cells)
- these structures are assumed to alter the size exclusion limits of plasmodesmata.

- Virus moves as a nucleocapsid through the plasmodesmata from cytoplasm of one cell to the cytoplasm of an adjacent cell.



http://viralzone.expasy.org/all_by_protein/1018.html

TSWV isolates are highly divergent

1. In co-infections, TSWV isolates can exchange genome segments with each other (the S RNA is preferentially exchanged)

2. Mechanically-transmissible isolates are readily produced by serial mechanical transmission. (of 30 local lesions tested, only 3 could be transmitted by thrips)

(they lose the correct RNA sequences that code for GN and GC proteins) and therefore lose the ability to be thrips transmitted. These deletions, frameshift or nonsense mutations, may or may not affect virion formation, and have no impact on infection of plants, but are unable to be transmitted by thrips.

Sin et al 2005 PNAS 102: 5168–5173