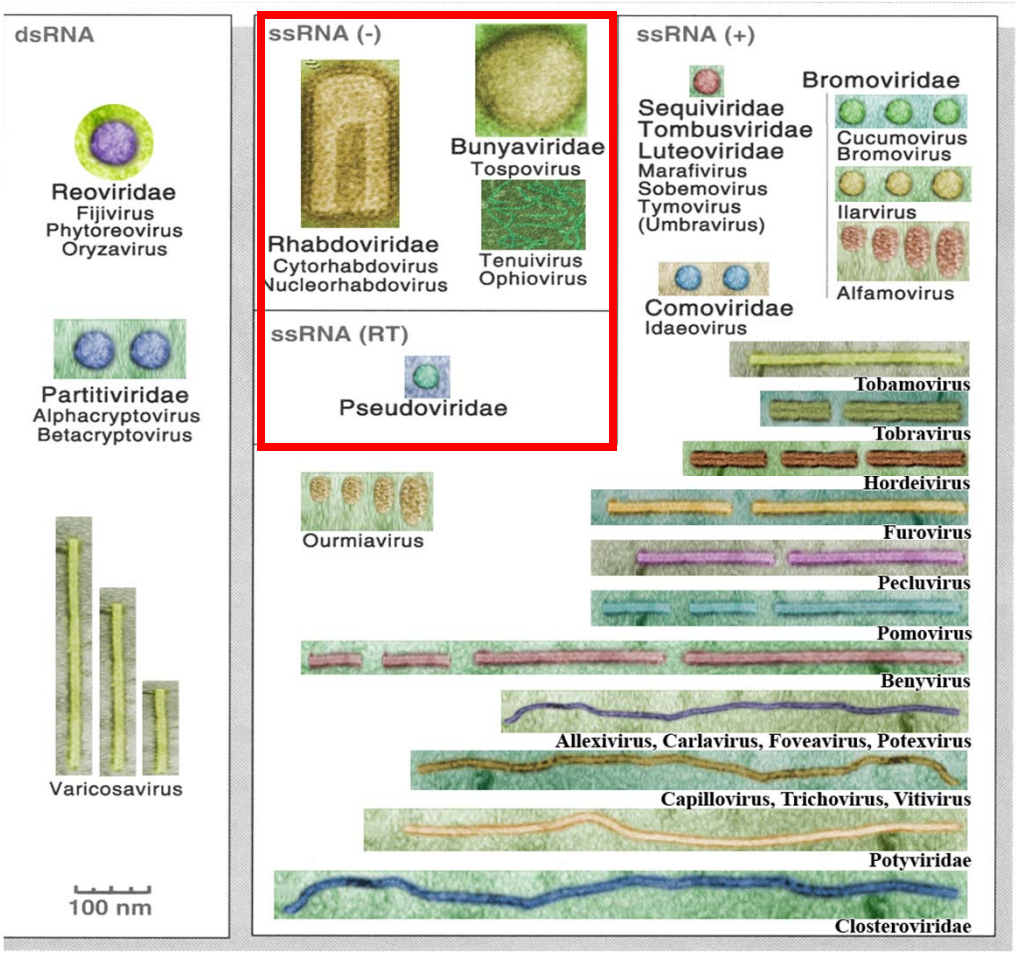
Negative Sense ssRNA Viruses

Families and Genera of Plant Viruses – RNA Plant Viruses



Life Cycles of (-) sense ssRNA viruses

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

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

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

FAMILIES of NEGATIVE STRAND VIRUSES

Non-segmented (-) Strand Viruses

RHABDOVIRIDAE **- Rabies, VSV, & Plant viruses**

FILOVIRIDAE **- Marburg & Ebola viruses**

PARAMYXOVIRIDAE **- Measles, Mumps, RSV, & Distemper**

BORNAVIRIDAE **– Neurological diseases of humans, animals**

Segmented (-) Strand Viruses

ORTHOMYXOVIRIDAE **- Influenza virus**

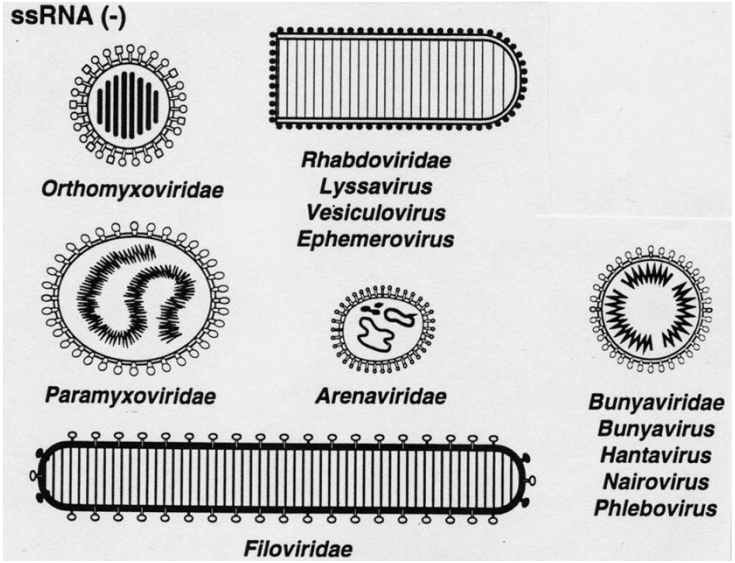
Segmented Ambisense Viruses

BUNYAVIRIDAE **- Hantavirus, plant *Tospovirus* and *Tenuivirus***

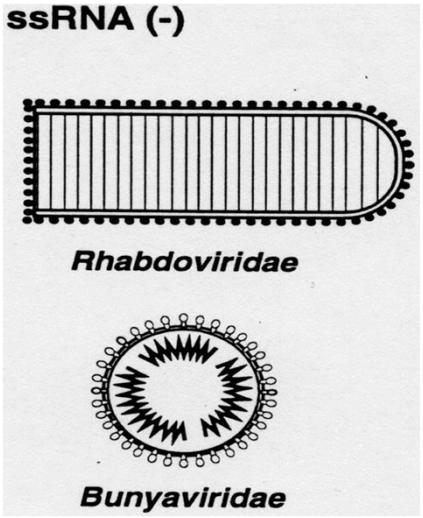
ARENAVIRIDAE **- Lassa fever**

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

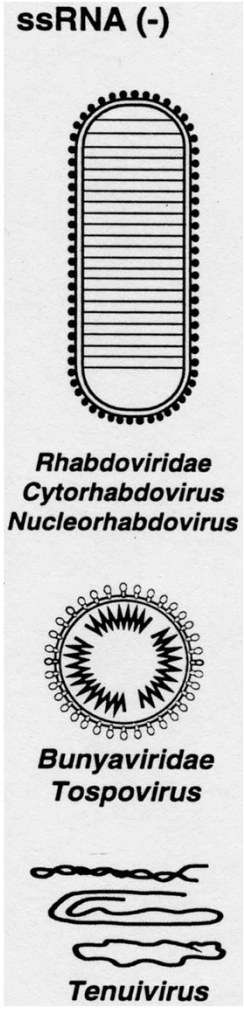
Vertebrate



Invertebrate



Plant



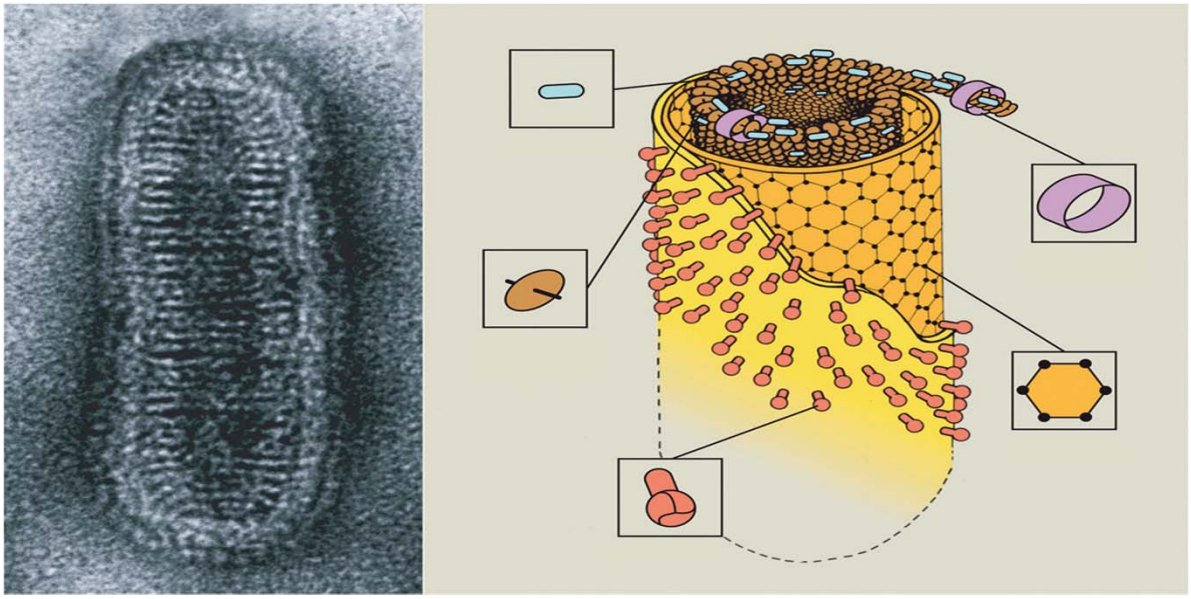
Plant-Infecting ss (-) sense Viruses:

Ex. *Rhabdoviridae* and *Bunyaviridae*

- Most ss (-) sense plant viruses are enveloped
- 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*

2 genera that infect plants:

Genus *Cytorhabdovirus*,

type species *Lettuce necrotic yellows virus*.

8 member species

aphid or leafhopper vectors

Genus *Nucleorhabdovirus*,

type species *Potato yellow dwarf virus*

7 member species

aphid or leafhopper vectors

More than 48 species unassigned to Genus

Vectors - aphids, leafhoppers, planthoppers, mites, lacebugs

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

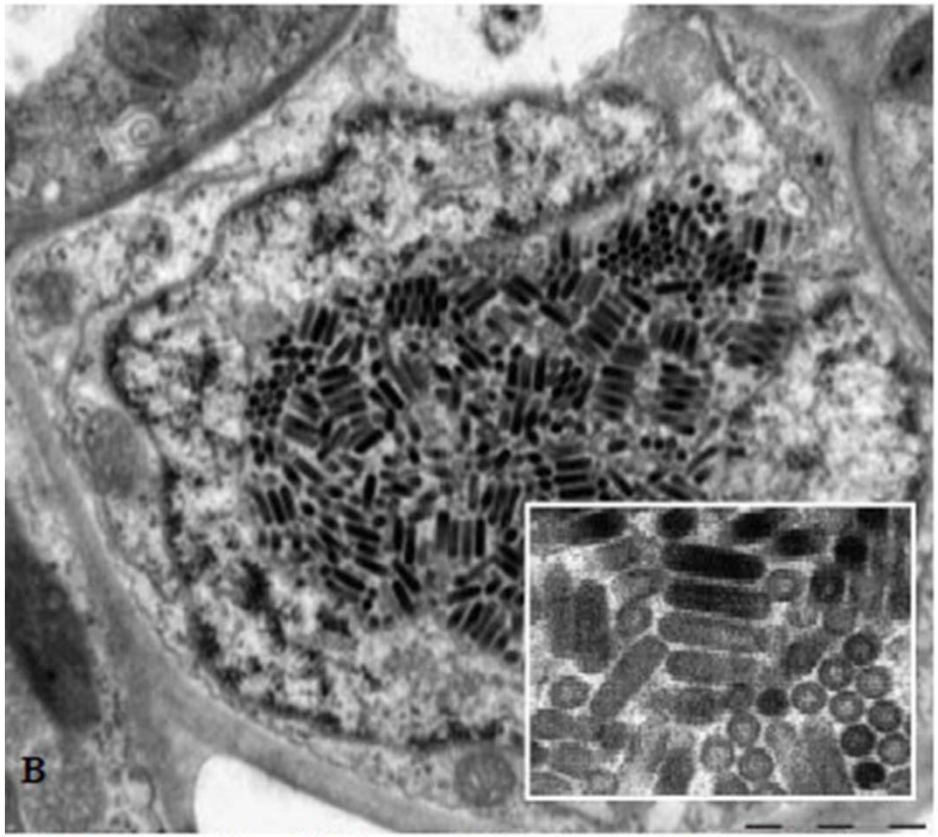
Sonchus oleraceus - vein clearing, yellow patches between veins

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)

Rhabdovirus particles

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 (RNA genomes)
5. Encapsidation
6. Translocation

Cytorhabdovirus and *Nucleorhabdovirus*

Expression Strategies Used:

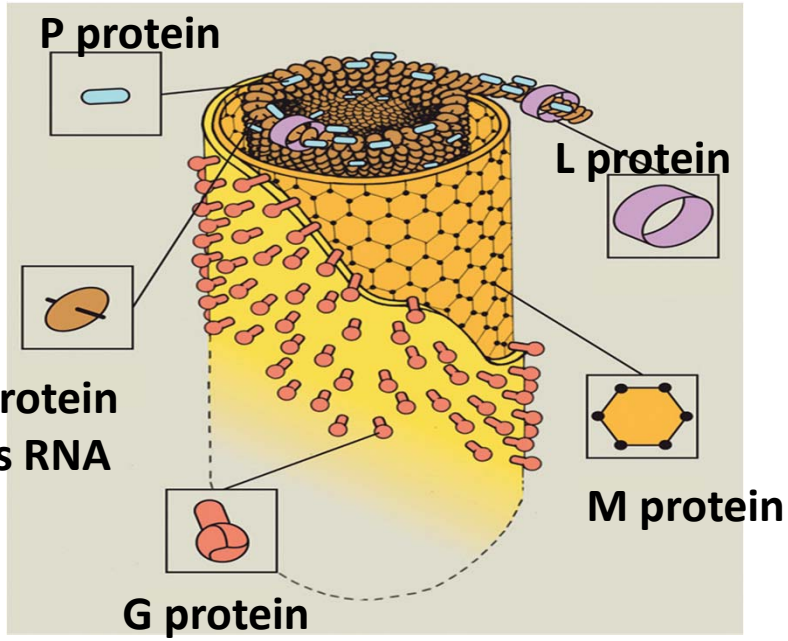
- √ - subgenomic RNAs
- - multipartite genomes
- - polyprotein (proteolytic processing)
- - translational read-through
- - translational frame-shift
- - ambisense RNAs
- - cap snatching

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)

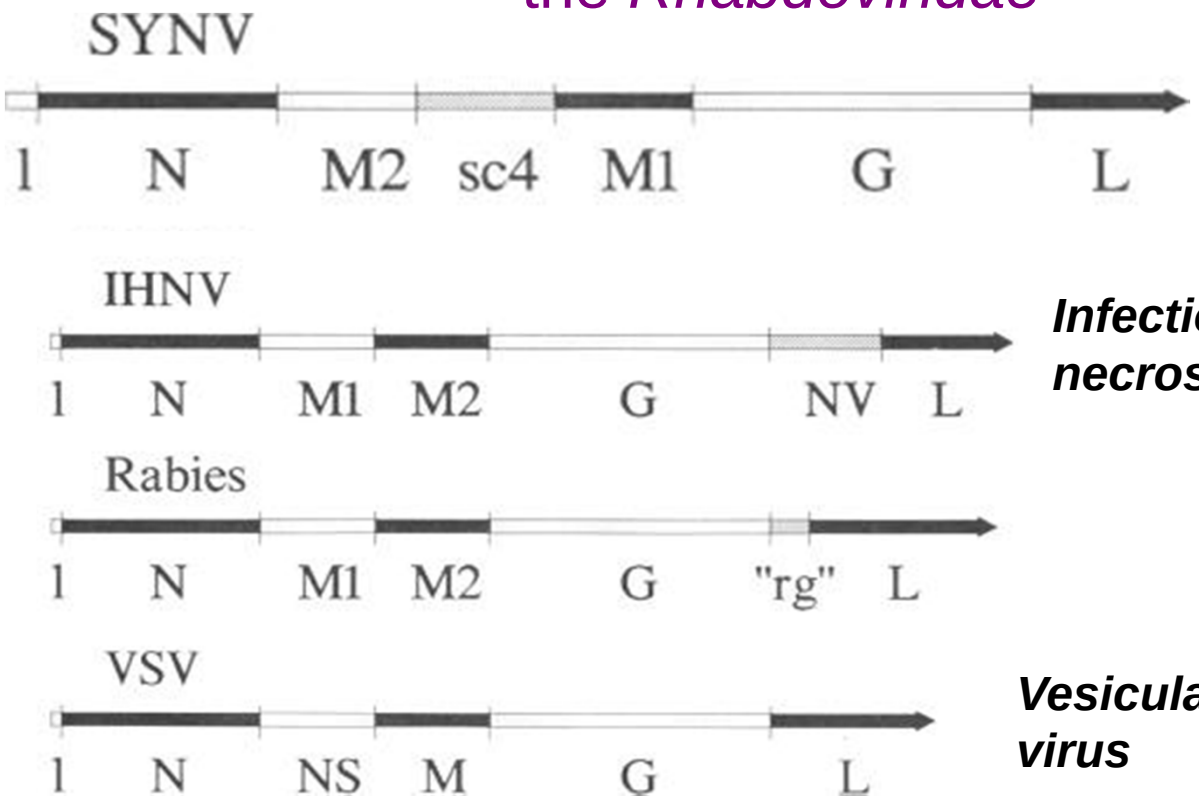
Most of the proteins encoded by rhabdovirus genomes are part of the virion

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*

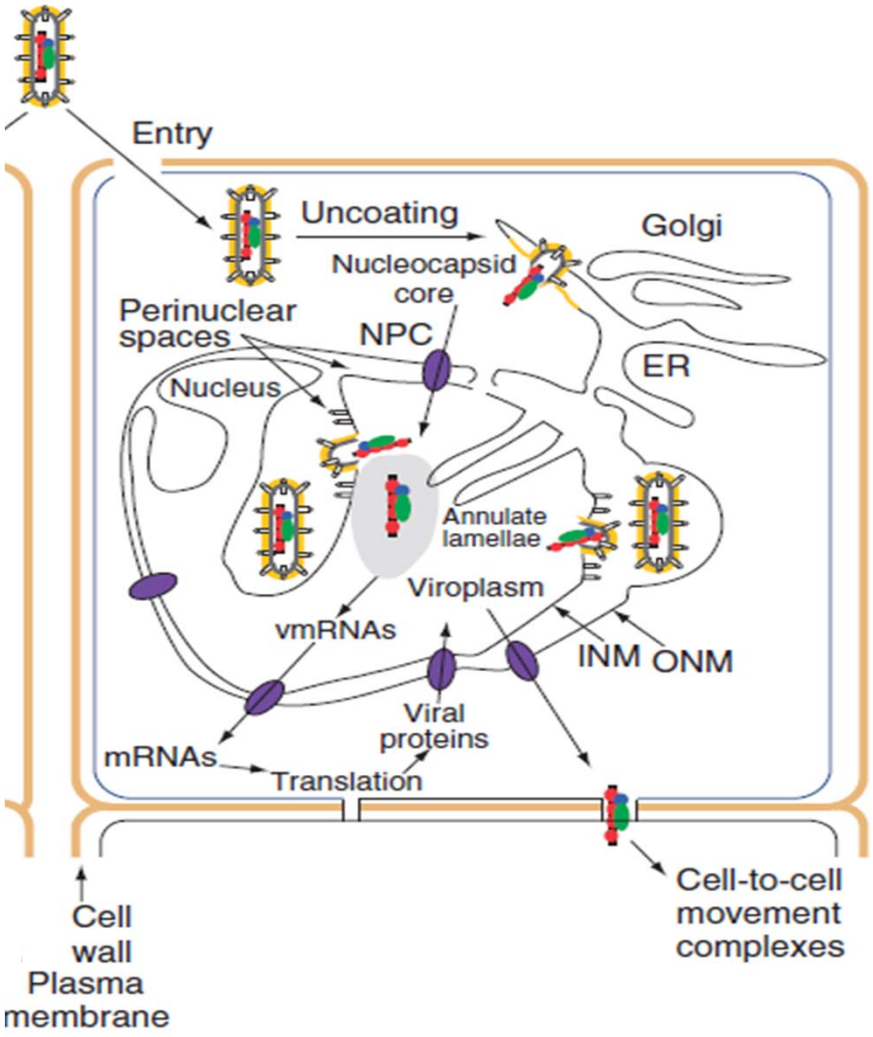


Infectious hematopoietic necrosis virus

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 *Cytorhabdovirus* is similar but occurs in the cytoplasm



Nucleorhabdovirus: Interaction and effects on cell membranes

Whole cell (Protoplast)
Green – ER tubules

Nucleus
(Green – nuclear membranes)

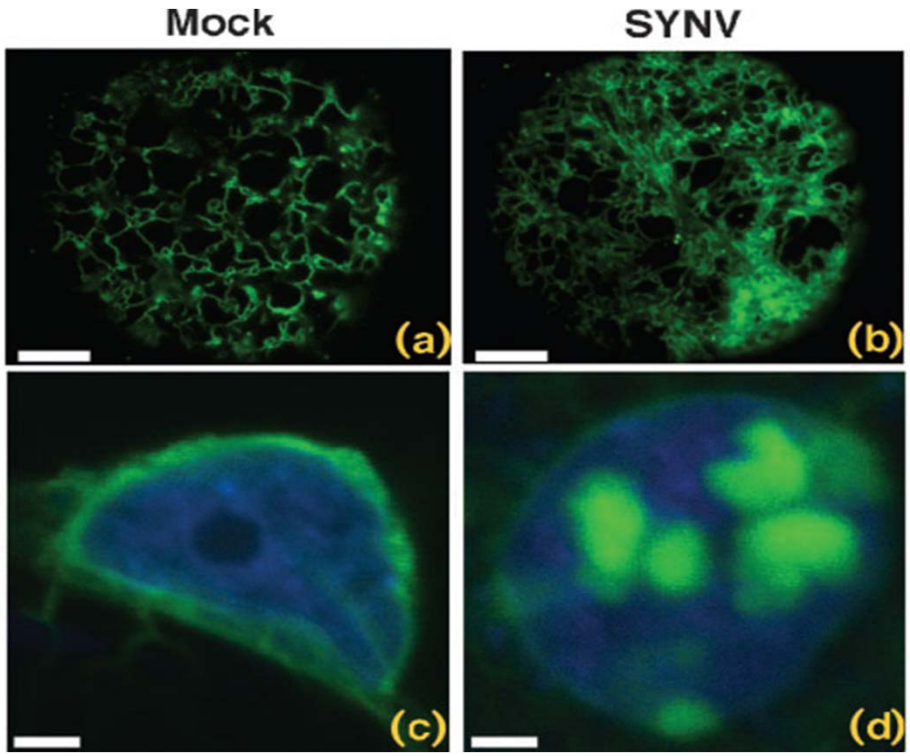
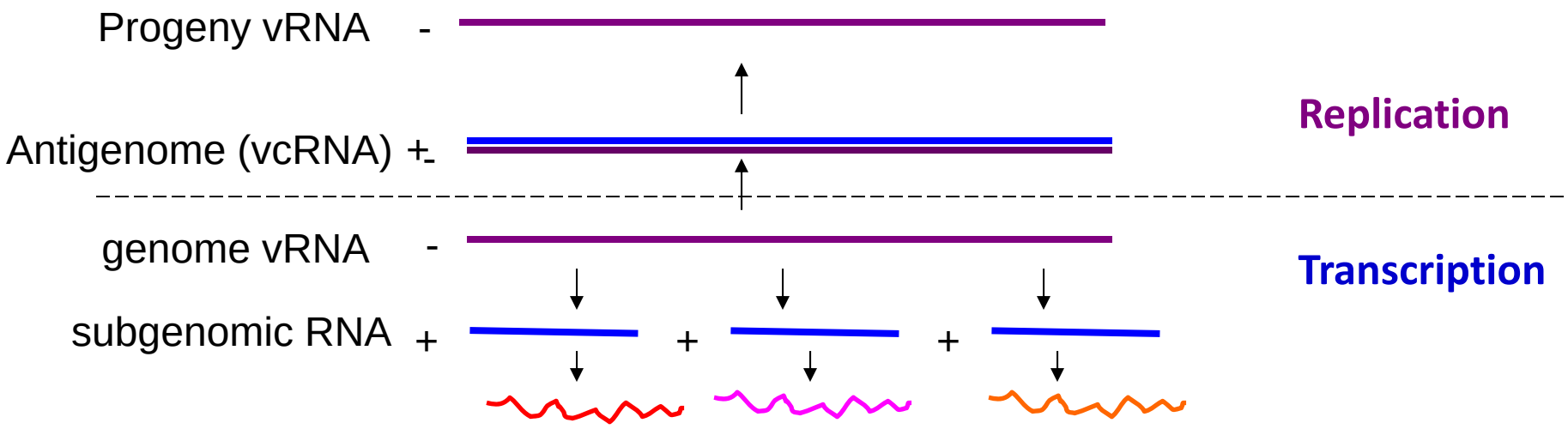


Fig. 1. (a, b) Micrographs showing the distribution of ER tubules in protoplasts derived from leaves of mock-inoculated (a) or SYNV-infected (b) mGFP5-ER *N. benthamiana* plants. Bars, 10 mm.
(c, d) Confocal micrographs showing nuclear membranes (green) of mock-inoculated (c) or SYNV-infected (d) leaf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

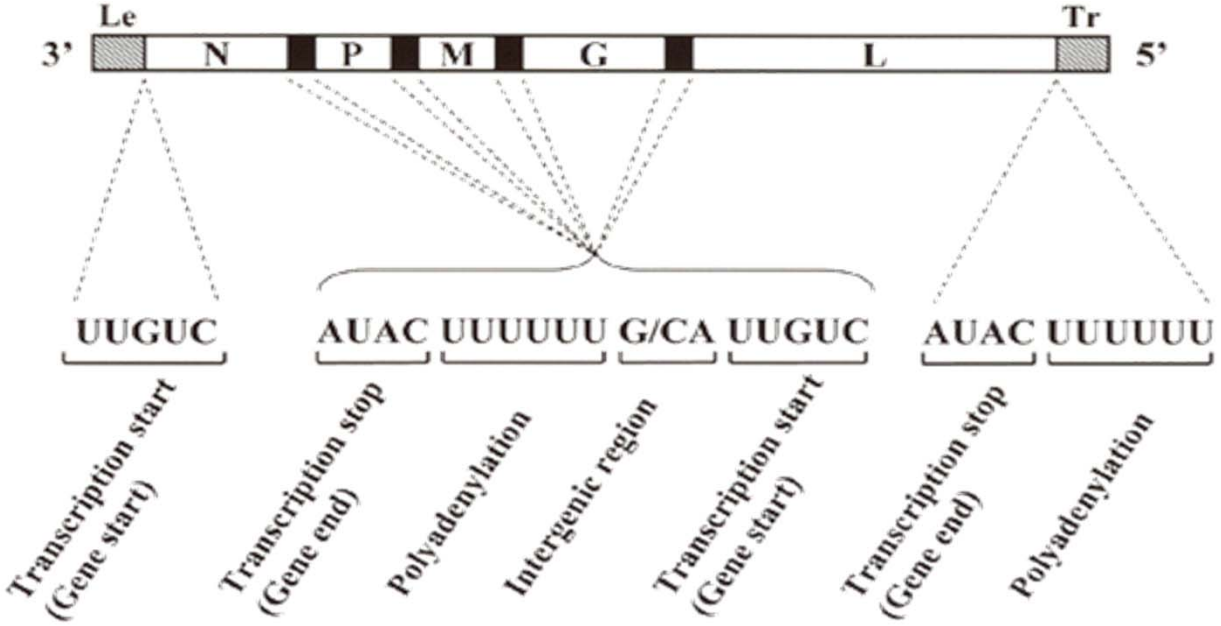
From: Goodin et al J. of General Virology (2007), 88, 1810–1820

“Streamlined” Diagram: Replication Cycle of Rhabdoviruses:

Transcription occurs first, the Replication



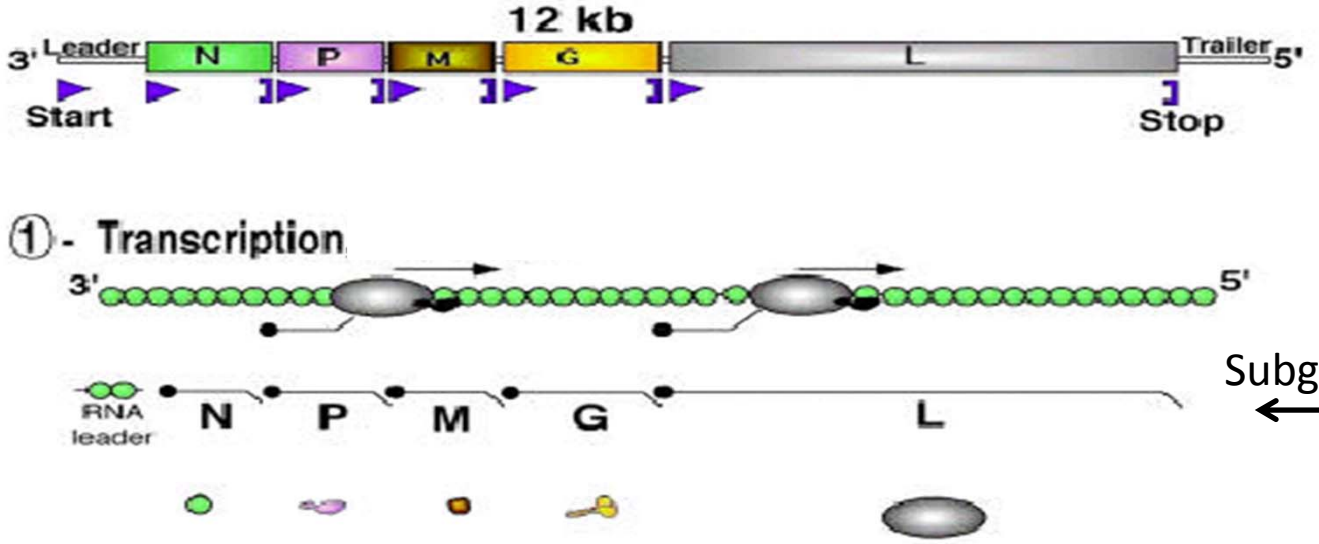
Regulatory sequences in VSV control transcription of the subgenomic RNAs



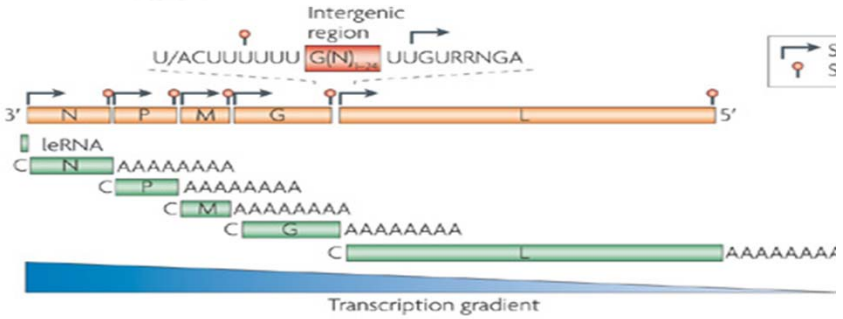
(N) nucleoprotein
(P) phosphoprotein
(M) matrix protein
(G) glycoprotein
(L) polymerase protein

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

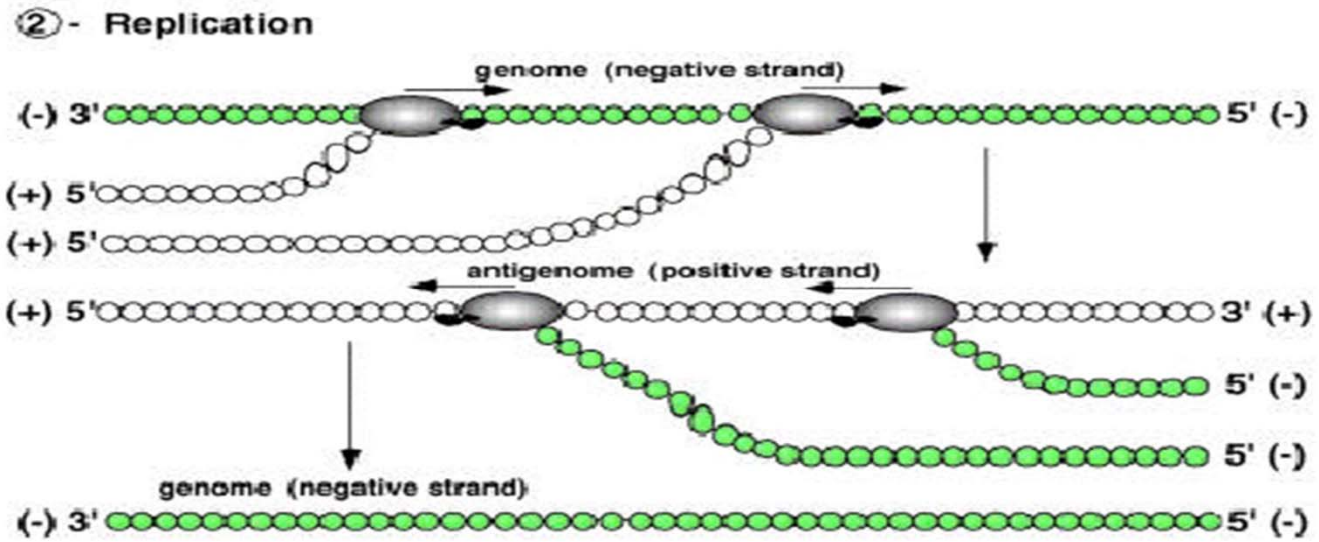
Rhabdovirus (VSV) Transcription and Replication



- Rhabdovirus genes are transcribed sequentiall 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



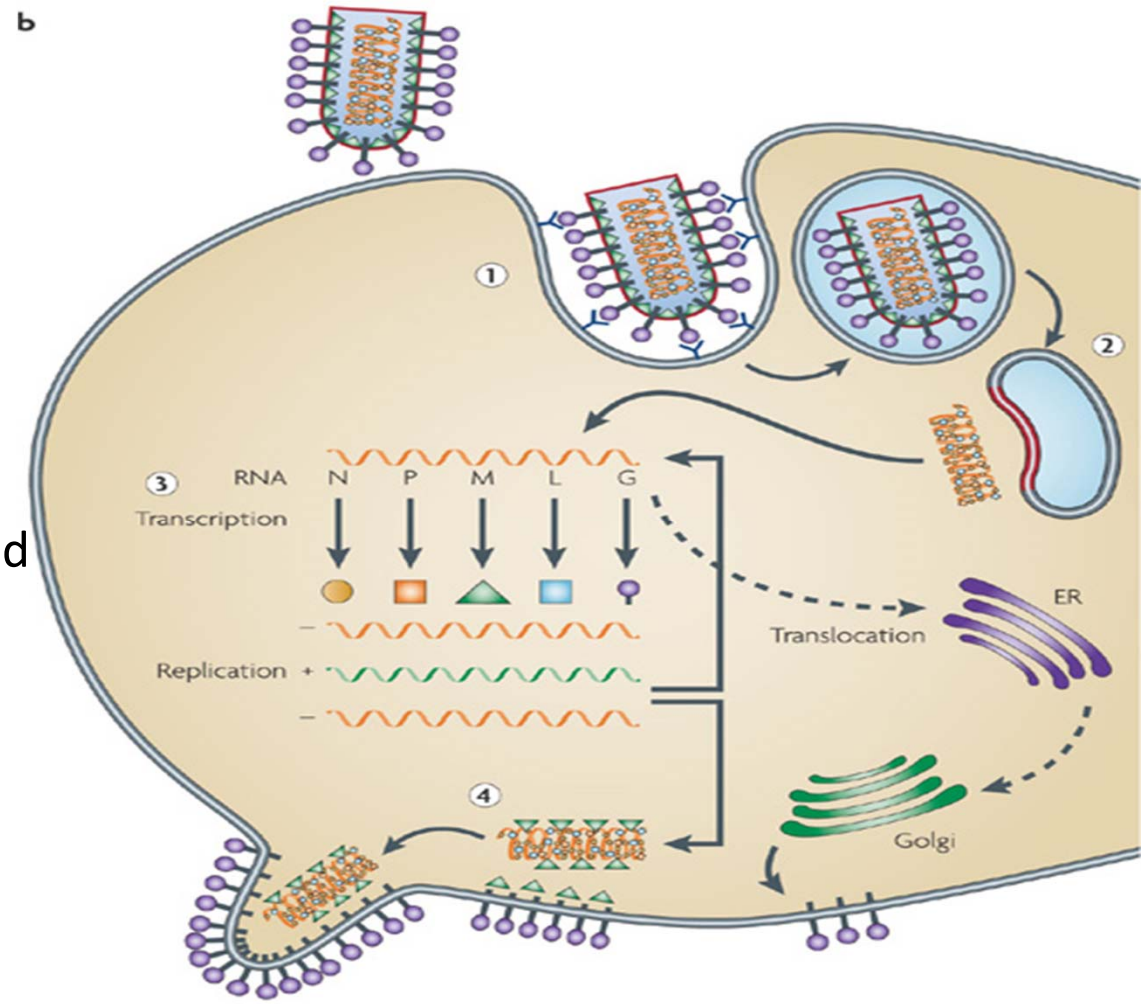
Rhabdovirus (VSV) Transcription and Replication



- 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: b

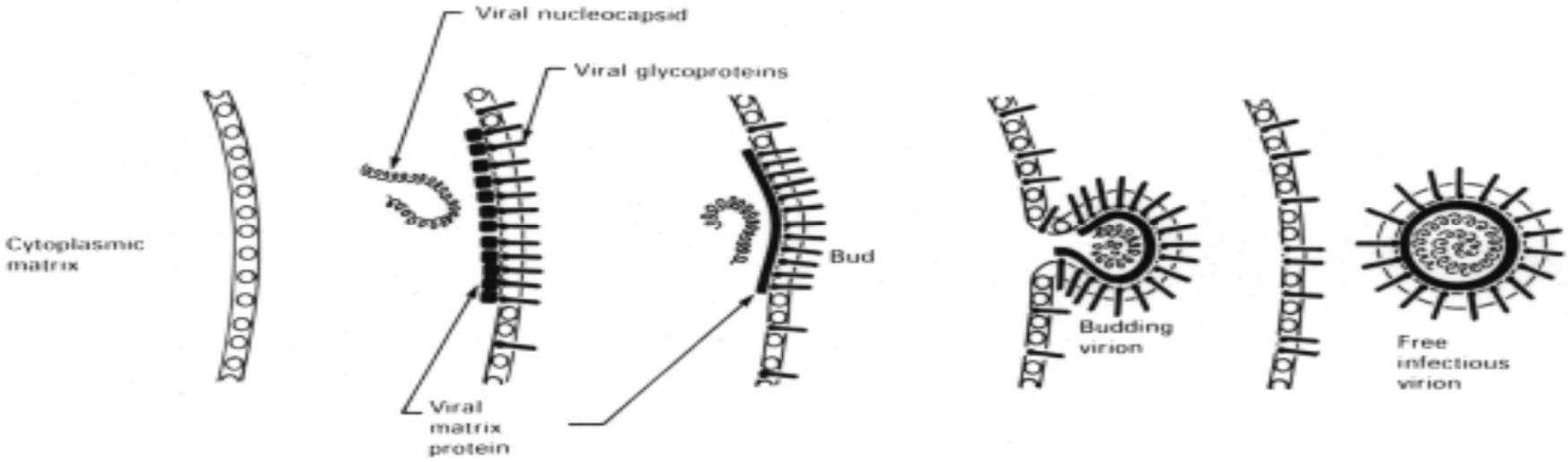
1) Binding and entry into the host cell by endocytosis (animal cell)
2) Fusion of the viral membrane and endosome membrane to release the viral genome (uncoating).
3) Virion components are produced (transcription, replication, protein synthesis;
4) Assembly of the viral components, budding and release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



Nature Reviews | Microbiology

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.



Summary of Nucleorhabdovirus Replication:

Entry Mechanism ……mechanical, insect vectors
Site of Transcription…….nucleus
Site of Genome Replication….nucleus
Replicase……..virus coded L protein
Replication Intermediate……..dsRNA
Site of Virion Assembly……..nuclear membrane
Egress mechanism…….plasmodesmata, insect vectors

Summary of Cytorhabdovirus Replication:

Entry Mechanism ……mechanical, insect vectors
Site of Transcription…….cytoplasm
Site of Genome Replication…cytoplasm
Replicase……..virus coded L protein
Replication Intermediate……..dsRNA
Site of Virion Assembly……..viroplasm in cytoplasm
Egress mechanism…….plasmodesmata, insect vectors

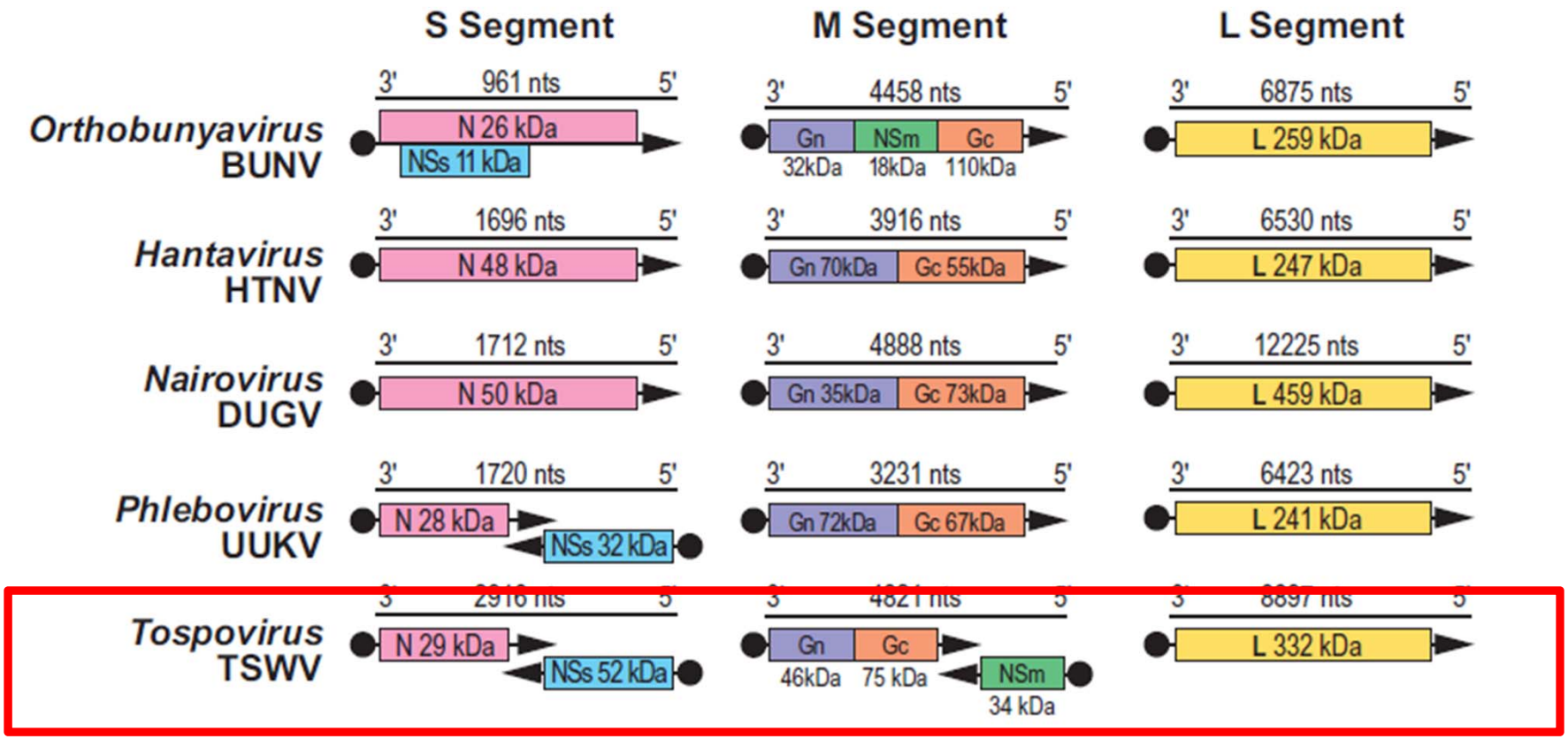
Family: *Bunyaviridae*

- Largest family of RNA viruses, more than 200 species
- All members replicate in invertebrates
- Maintained in insects and wild animals in nature; rarely transmitted to humans and domestic animals (which are considered as dead end hosts)

Five Genera in *Bunyaviridae*

- Genus *Bunyavirus*
  - Prototype *Bunyawera virus*; mosquito vectored
- Genus *Hantavirus*
  - Rodent alternate hosts; occasionally major outbreaks
- Genus *Nairovirus*
  - Cause hemorrhagic fevers; tick-borne
- Genus *Phlebovirus*
  - Cause Rift Valley Fever and similar disease; sandfly vectored
- **Genus *Tospovirus***
  - **Important plant pathogen with the widest host range of any plant virus; replication in thrips and plants**

Coding strategies of the genome segments of the 5 genera in the *Bunyaviridae*



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

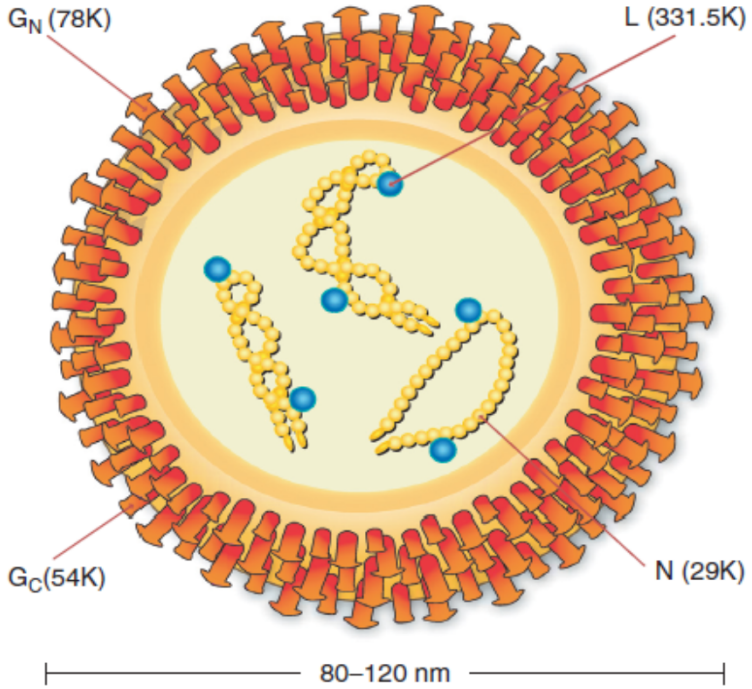
Tospoviruses:

Expression Strategies Used:

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

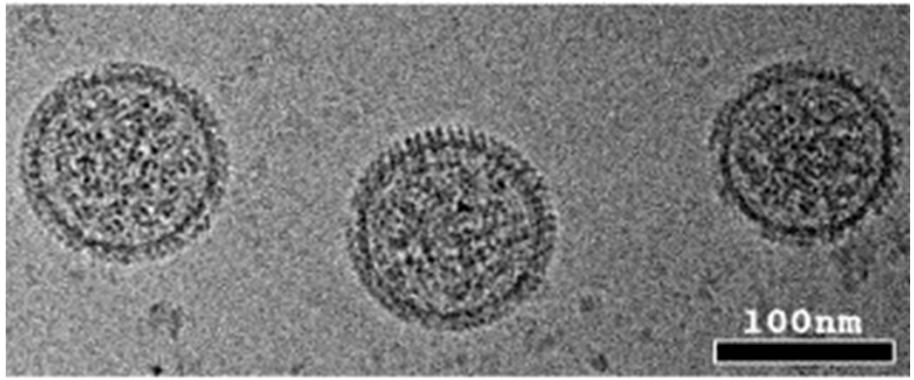
Structure of Tospoviruses

- 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"

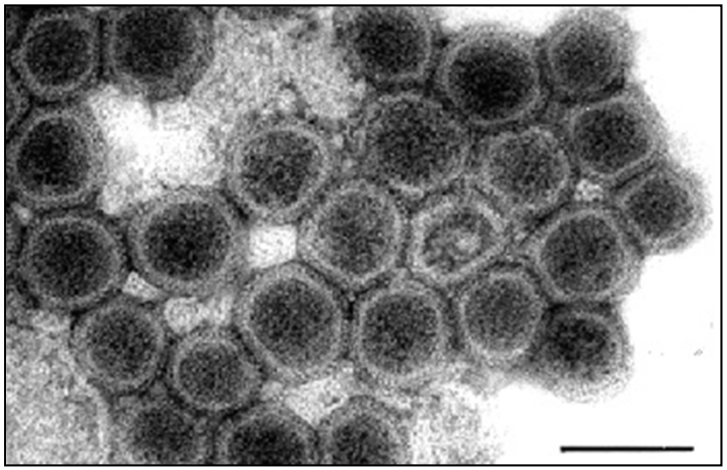


Virion Structure

Pleomorphic virions



Cryo-EM micrograph of Hantaan virus particles

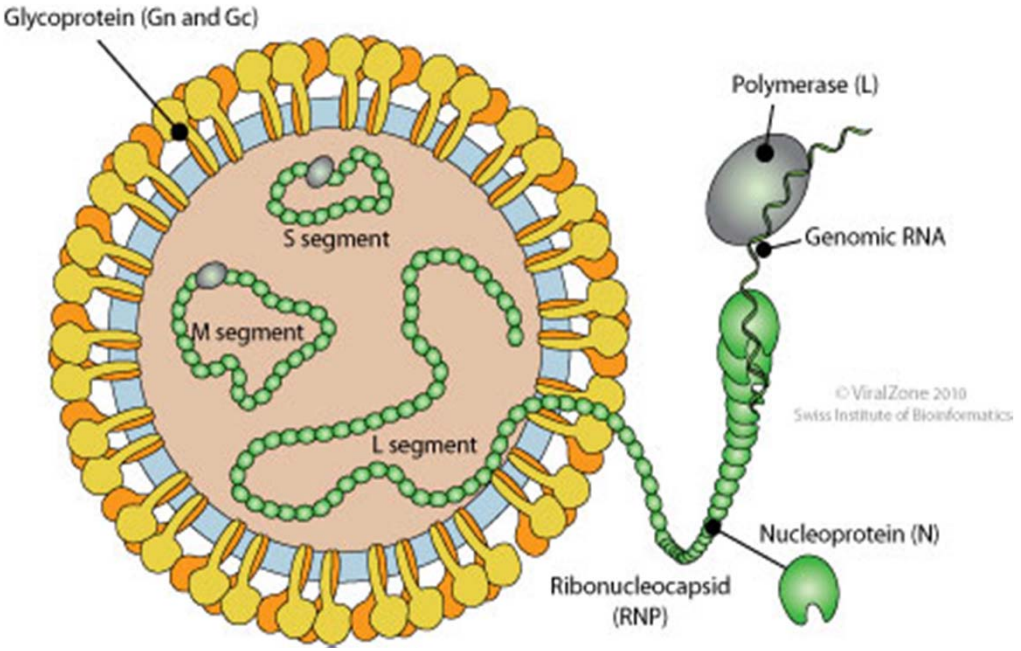


EM of negatively stained particles of TSWV (bar represents 100 nm

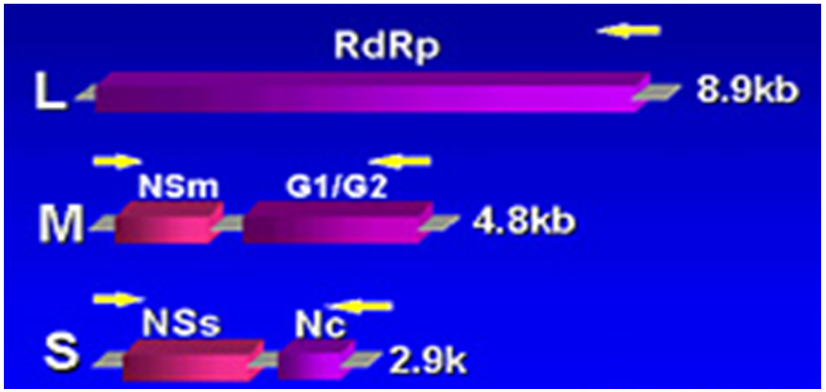
Tospovirus Particle Structure

Nucleocapsids are enclosed in host-derived membrane bi-layer 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**)



Tospovirus ORFs :



L RNA

RdRp – replicase 331 kDa
[produced from one long mRNA)

M RNA

NSm – Non-structural protein 33.6 kDa, movement protein in plants [in viral sense]

GN, GC – glycoproteins (58 and 78 kDa) in the bilayer membrane
[viral complimentary sense] function in assembly of virions

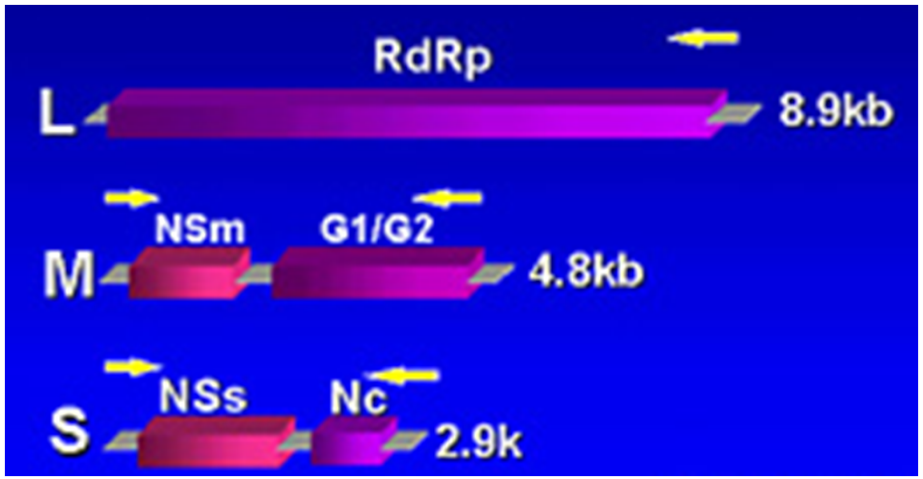
S RNA

NSs – Non-structural protein (52.4 kDa) [viral sense, subgenomic]

N (or Nc) – nucleocapsid protein (29 kDa) binds to RNA and forms the nucleocapsid [compli. sense, subgenomic]

Replication of Tospoviruses

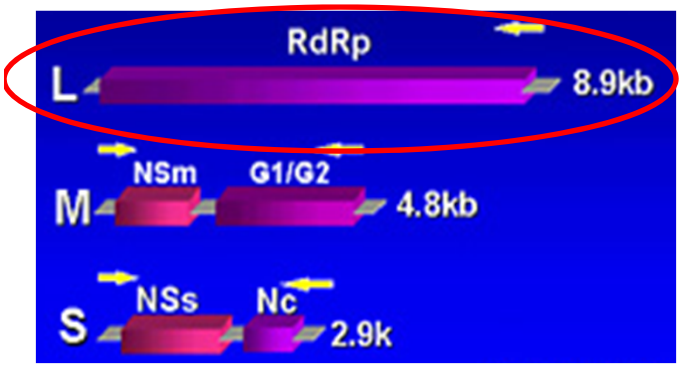
- Proteins on the L, M and S RNAs are not expressed in the same way

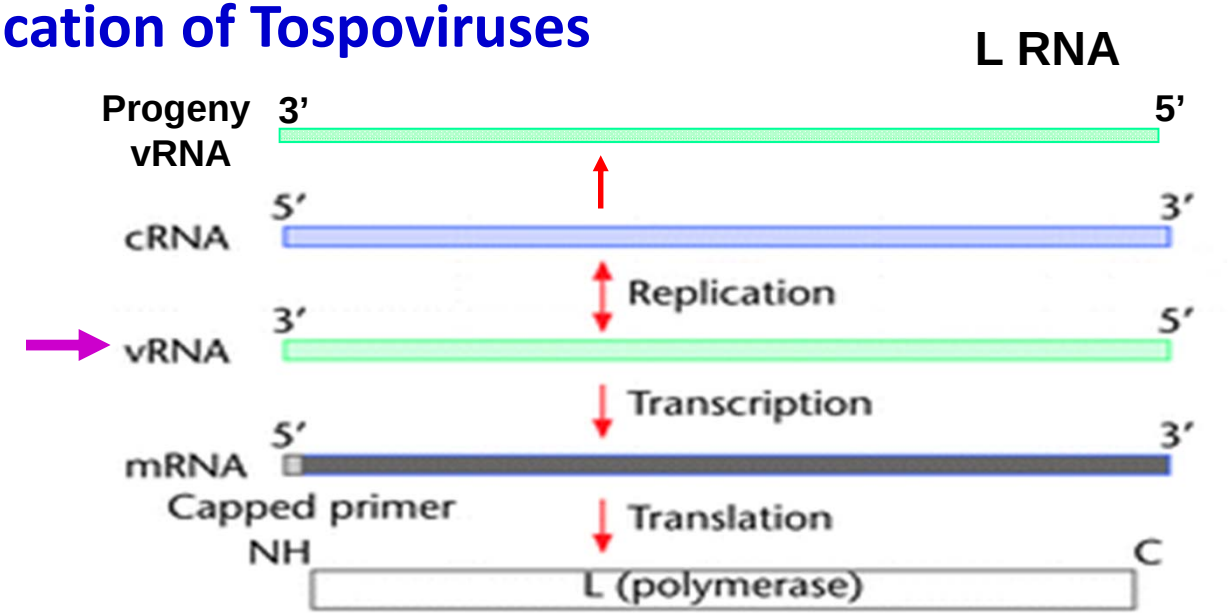


- L RNA – negative sense
- S RNA and M RNA - ambisense

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

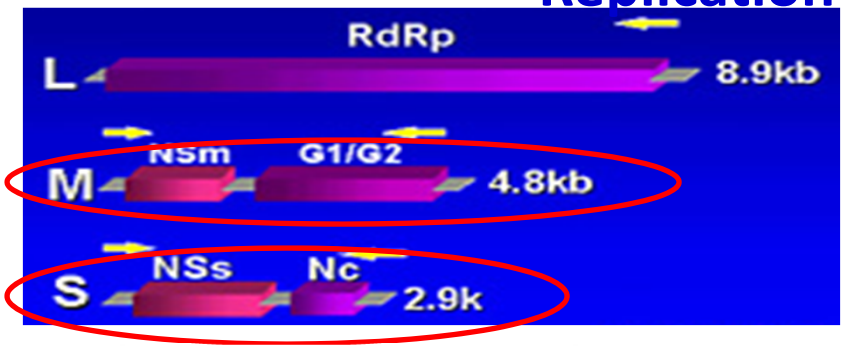
Replication of Tospoviruses



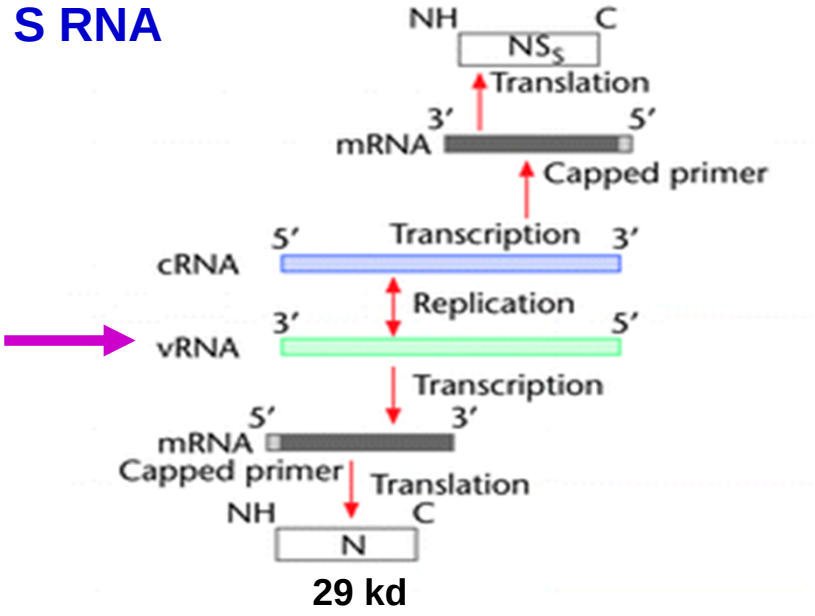


- vRNAs stay bound to the N protein. The polymerase can move down the RNAs without interference from the N protein.
- vRNAs can either be transcribed or replicated and both can occur off the same RNA. The concentration of N protein determines transcription or replication - at low N concentrations, the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

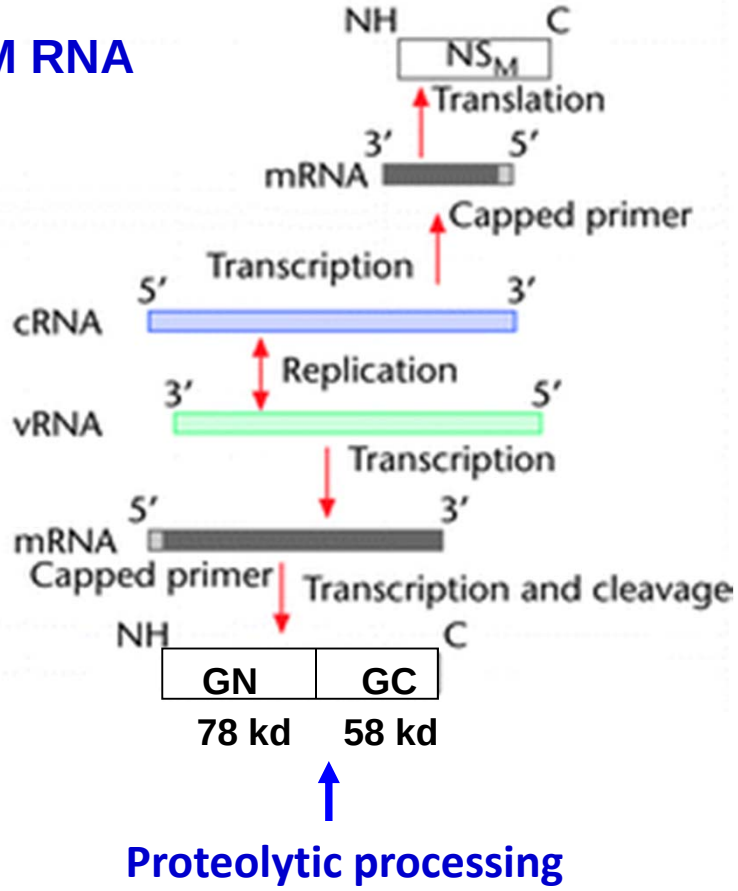
Replication of Tospoviruses



S RNA

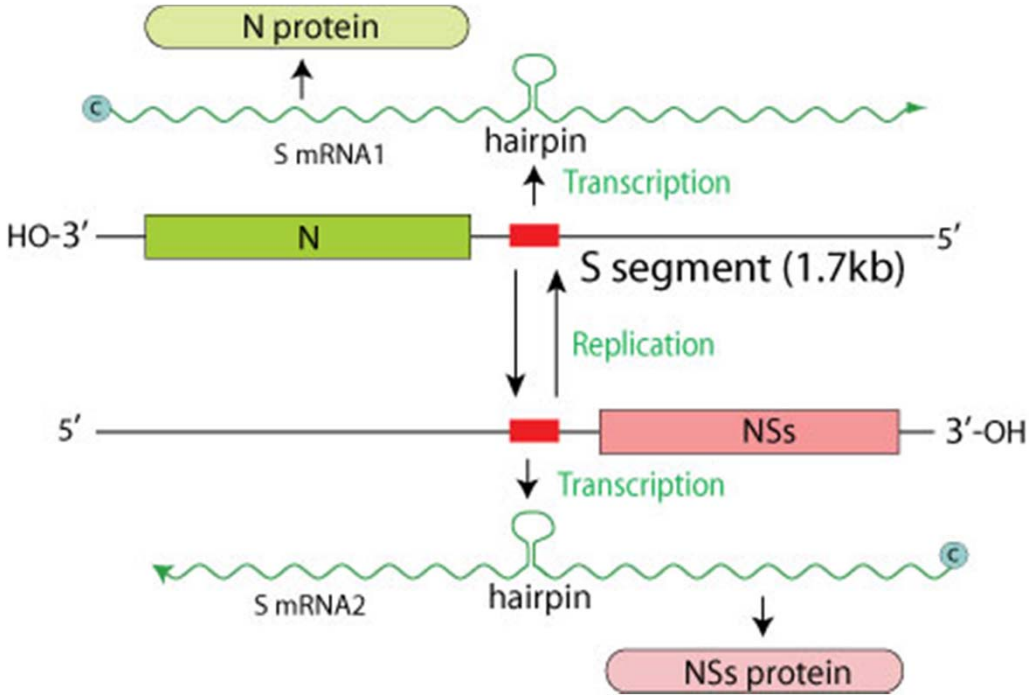


M RNA



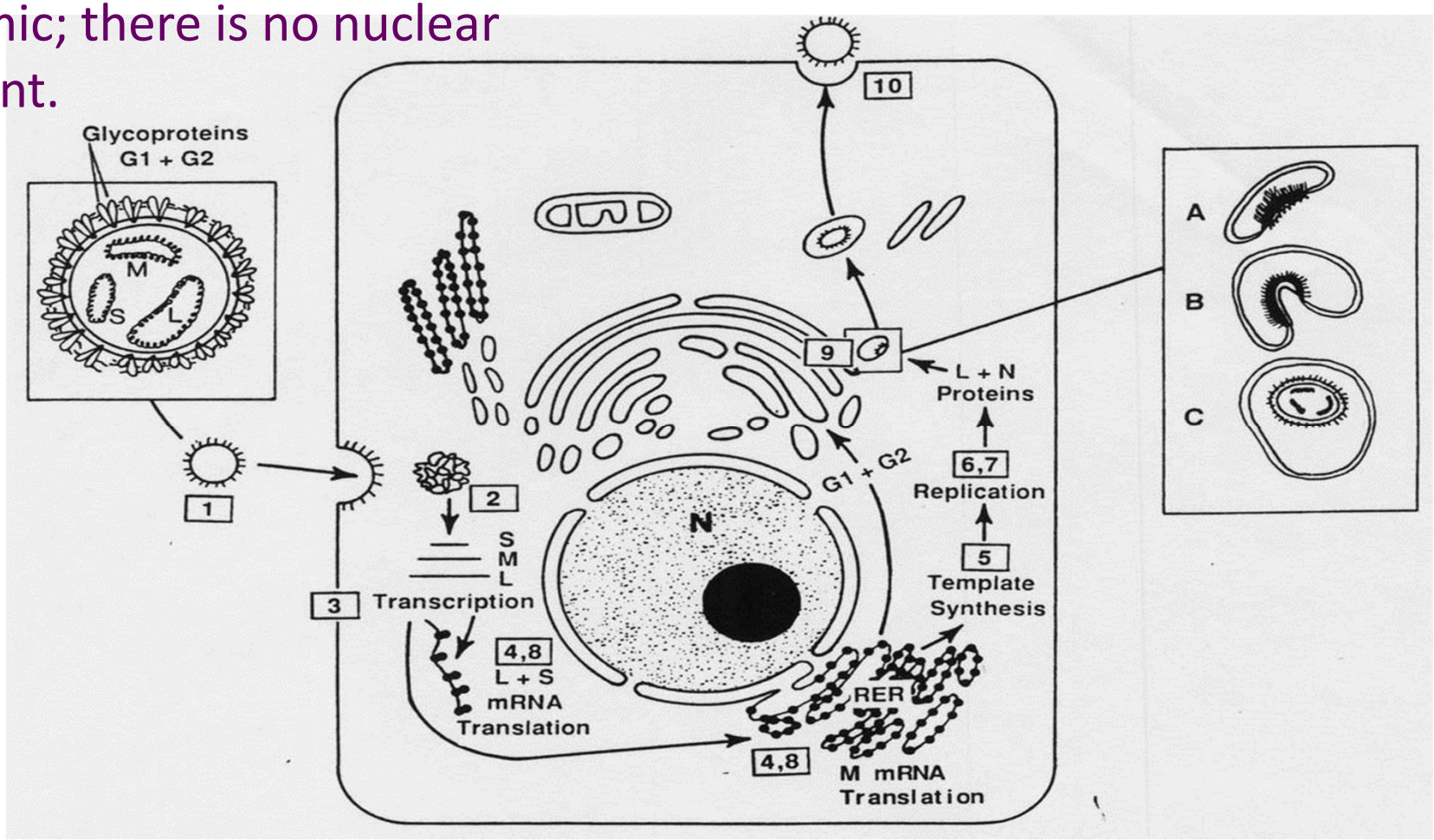
Proteolytic processing

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 of bunyaviruses is cytoplasmic; there is no nuclear component.



REPLICATION:

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

2. Virus attaches to host receptors though Gn-Gc glycoprotein dimer and is endocytosed into vesicles in the host cell (invertebrate host).

3. Fusion of virus membrane with the vesicle membrane; ribonucleocapsid segments are released in the cytoplasm.

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

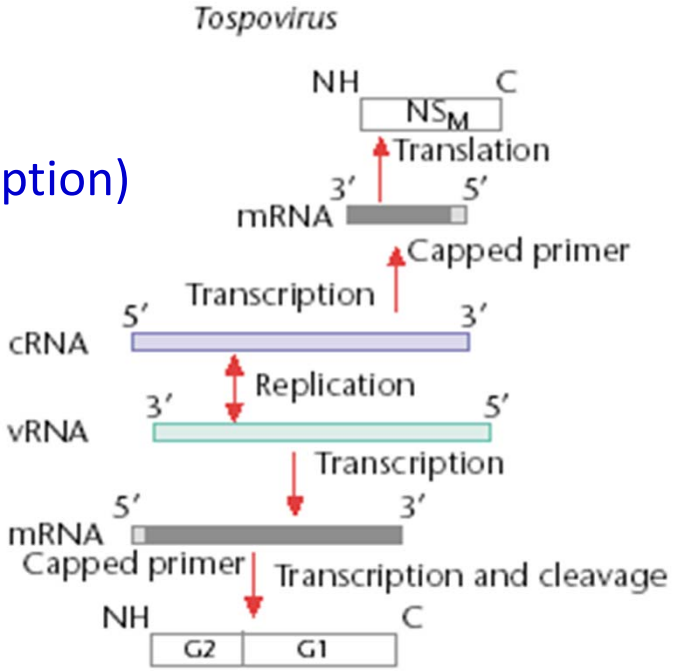
5. Replication presumably starts when enough nucleoprotein is present to encapsidate newly-synthetized anti-genomes and genomes.

6. The ribonucleocapsids migrate under the plasma membrane and buds, releasing the virion.

Cap-snatching:

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

Ex. Tospoviruses, Tenuiviruses

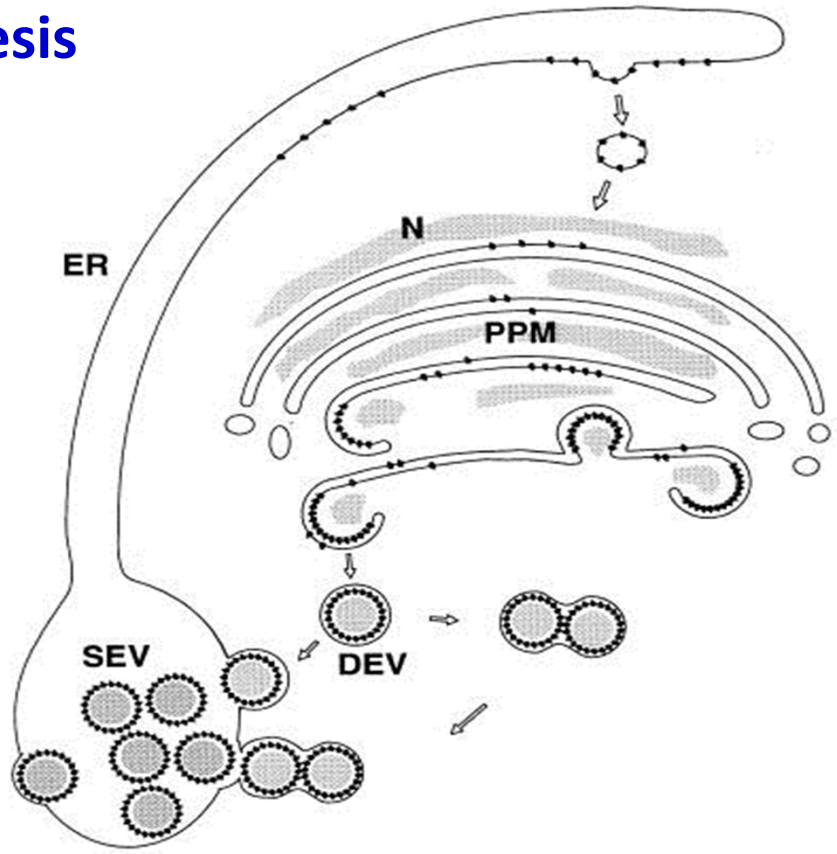


- Synthesis of the viral mRNAs is initiated by snatching the 5’ ends (caps) from host mRNAs (and other virus’s RNAs).

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

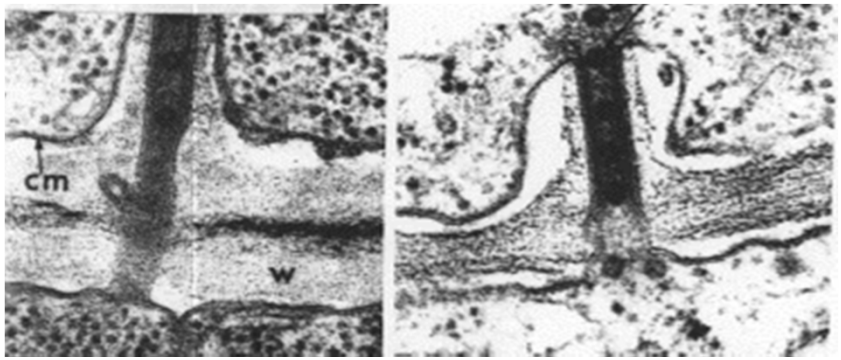
Model of TSWV particle morphogenesis

Doubly enveloped particles (DEV) are formed 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.

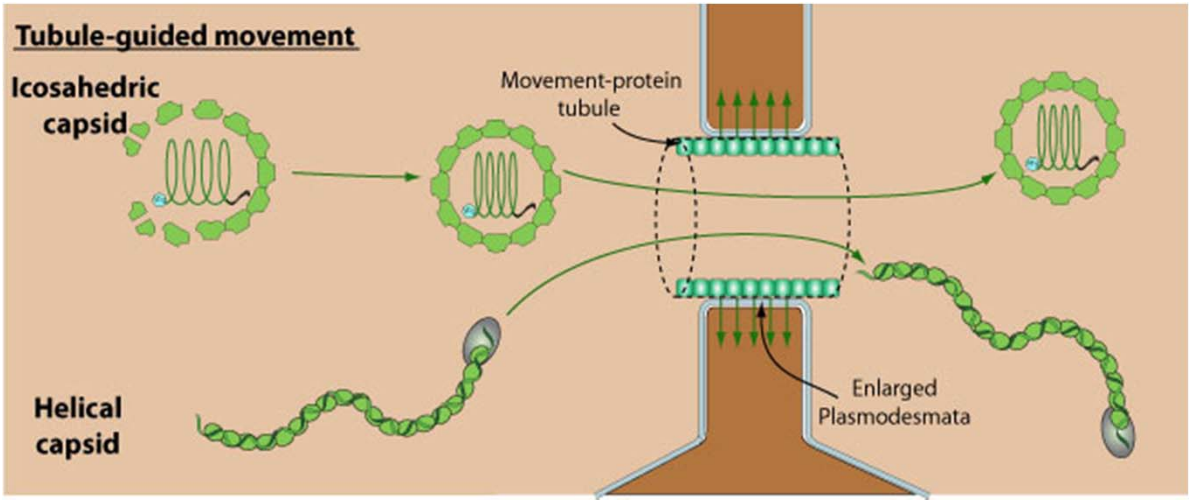


Marjolein Kikkert, Jan Van Lent, Marc Storms, Pentcho Bodegom, Richard Kormelink, and Rob Goldbach*
Tomato Spotted Wilt Virus Particle Morphogenesis in Plant Cells. *J Virol.* 1999 March; 73(3): 2288–2297.

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 that 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 envelope deficient

(they lose the M RNA sequences that code for GN and GC proteins)
and therefore lose the ability to be thrips transmitted.

Summary of Tospovirus Replication in Plants:

Entry Mechanism …….. thrips, mechanical (expt.)
Site of Transcription……cytoplasm
Site of Genome Replication…cytoplasm (Golgic stack membranes)
Replicase……..virus-encoded RdRp
Replication Intermediate……..dsRNA
Site of Virion Assembly…….cytoplasm - Golgi system (plants,thrips)
Egress mechanism……plasmodesmata, thrips