VIRUS LIFE CYCLE

Plus sense ssRNA Viruses

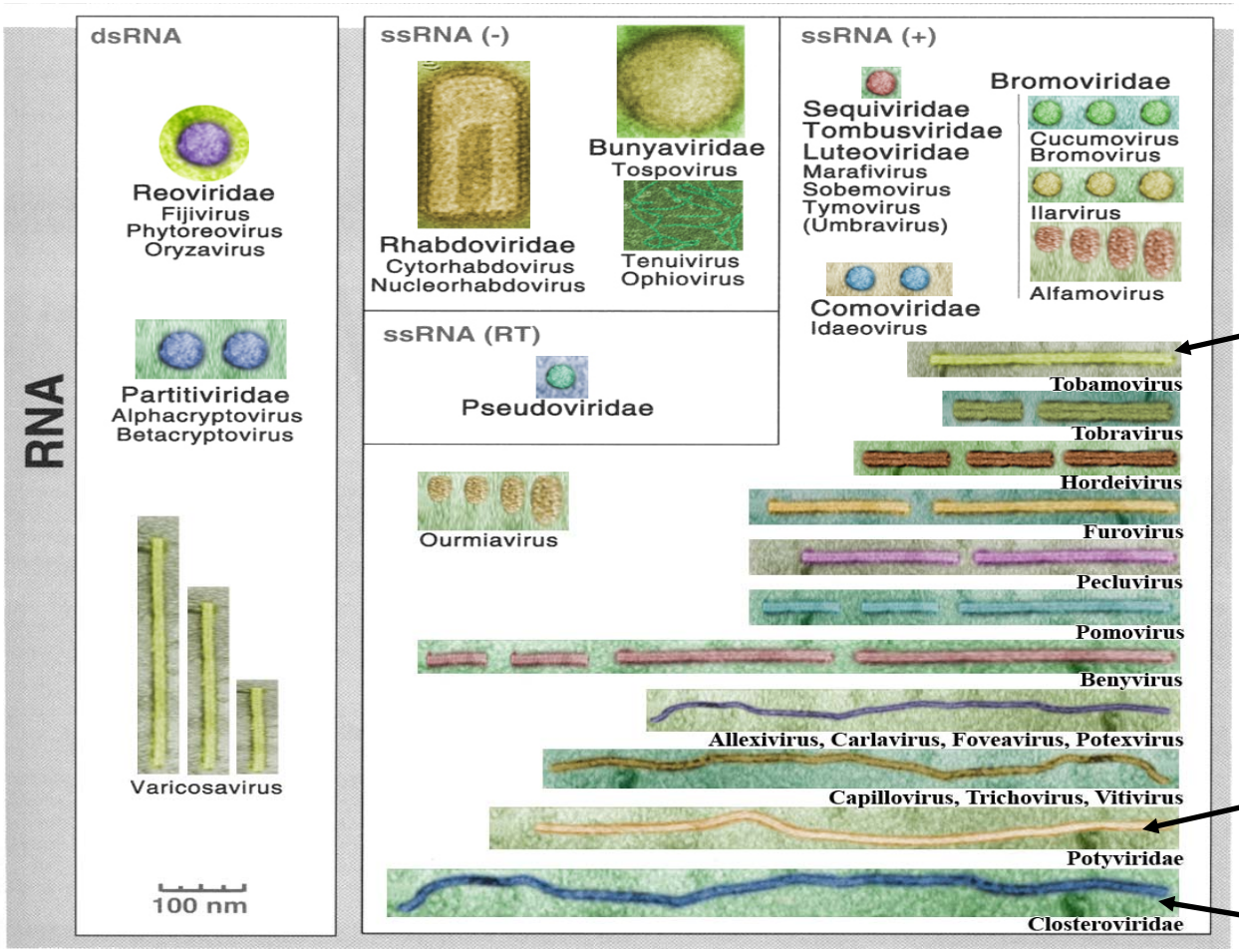
Topics:

1. Replication of species in the genus *Tobamovirus*

2. Replication of species in the genus *Potyvirus*

3. Replication of species in the family *Closteroviridae*

RNA Virus Families



Genus: *Tobamovirus*

Genus: *Potyvirus*

Family: *Closteroviridae*

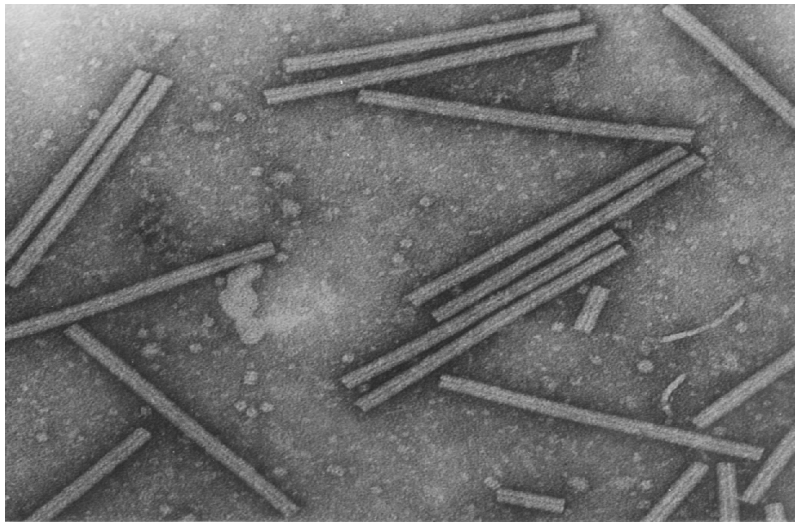
General Steps in the Replication Cycle of (+) Sense RNA Viruses

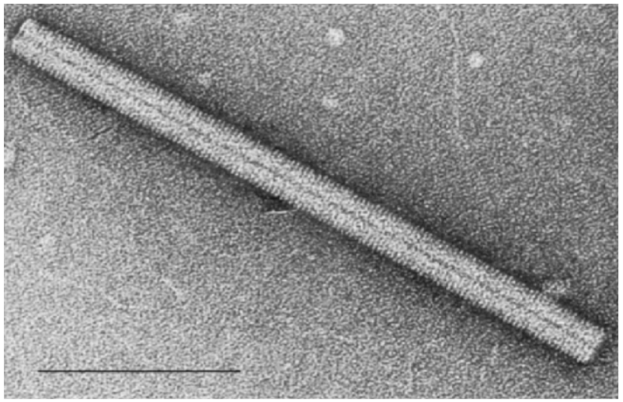
1. Disassembly
2. Primary Translation – usually it's the genes required for replication
3. Transcription – production of (-) sense RNA
4. Replication – production of (+) sense RNA (RNA genomes)
5. Secondary Translation – coat protein and movement protein synthesis
6. Encapsidation
7. Translocation

Family: *Virgaviridae*

Genus: *Tobamovirus*
ssRNA(+), rod-shaped virions,
no known vector – mechanically transmitted

Type species - *Tobacco mosaic virus*





Species in the Genus *Tobamovirus*

Tobacco mosaic virus **, - U1, vulgare type species**

Tobacco mild green mosaic virus **– (formerly TMV U2 strain)**

Plus 23 more species

Criteria demarcating different species:

- **Sequence similarity (less than 90% similarity)**
- **Host range**
- **Antigenic relationships among the coat proteins**

Genomic organization and expression of Tobamoviruses

Tobacco mosaic virus,* type member** of the genus ***Tobamovirus

Rod-shaped virions (18 nm x 300 nm) and are very stable.

Genome: **(+) ssRNA (ie. messenger sense),**
Monopartite, 6,395 nt
Contains at least four genes
Capped 5' end, t-RNA at 3' end

Tobacco mosaic virus:

- Important disease of tobacco, tomato & other plants.
- Easily mechanically transmitted (only means of transmission).
- Very high concentration in plants.
- First plant virus disease characterized (1898).
- First virus strains demonstrated;
- first cross protection shown.
- First virus crystallized (1946 Stanley was awarded the Nobel prize).
- First demonstration of infectious RNA (1950s).
- First virus to be shown to consist of RNA and protein.
- First virus characterized by X-ray crystallography.
- First plant virus genome to be completely sequenced.
- First virus used for coat protein mediated protection.
- First virus to have a resistance gene characterized.

www.rci.rutgers.edu/~bhillman/comparative_virology/

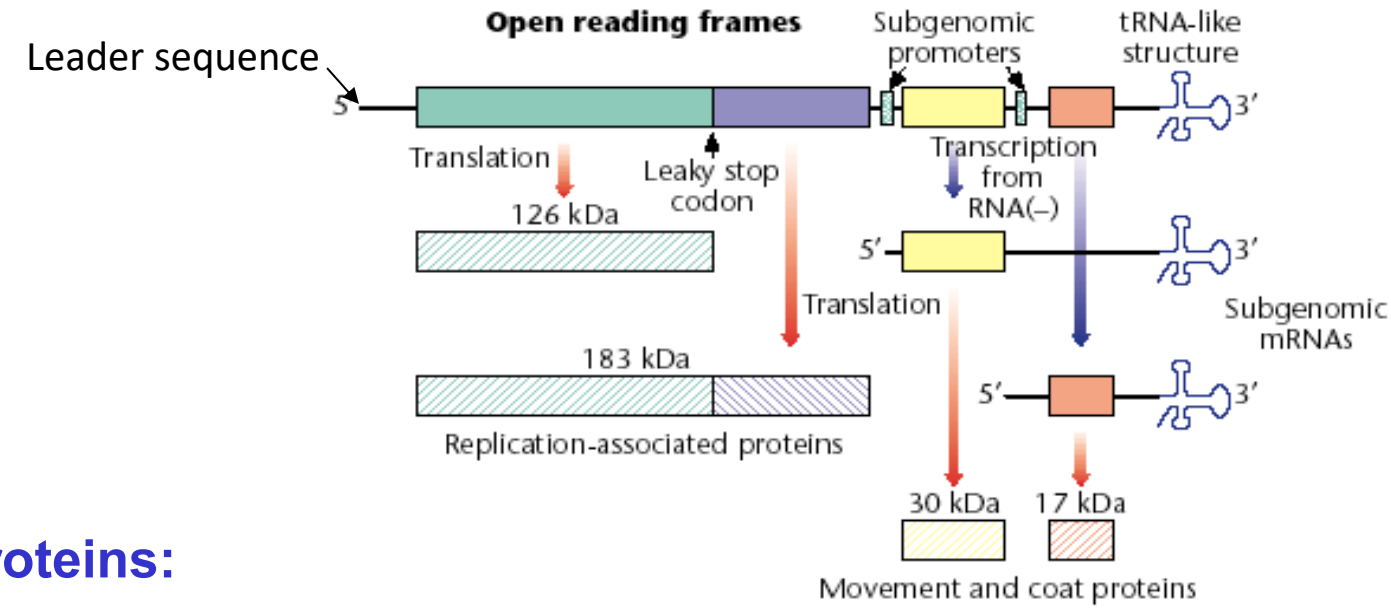
Replication: Various strategies are used by viruses with different types of genomes

- subgenomic RNAs
- multipartite genomes
- polyprotein
- translational read-through
- translational frame-shift
- ambisense RNAs

Every virus does not use all these strategies but uses different combinations of the above strategies

Tobamovirus Replication

- subgenomic RNAs
- multipartite genomes
- polyprotein
- translational read-through
- translational frame-shift
- ambisense RNAs



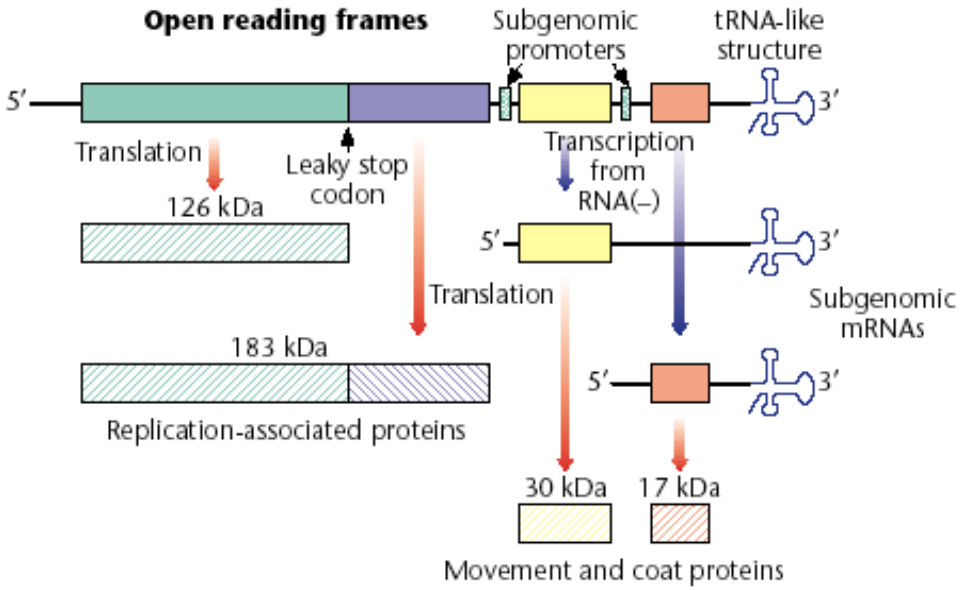
TMV Proteins:

126 kd
183 kd

} These plus host proteins form the RNA dependent RNA polymerase (RdRp) and replicase

30 kd movement protein
17 kd coat protein

TMV Replication: Subgenomics

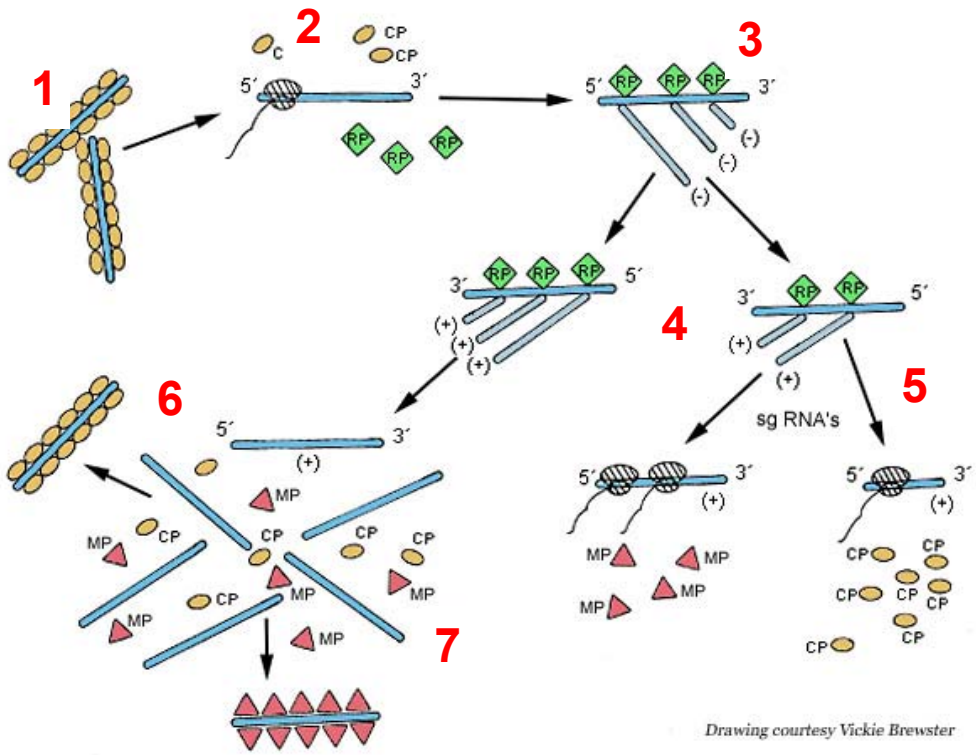


- Subgenomic mRNAs are formed by binding of RdRp to internal (subgenomic) promoters.
- The 2 promoters are located so that the subgenomic mRNAs are in different reading frames, so only one protein is produced per subgenomic mRNA.

"Life cycle" of *Tobacco mosaic virus* (TMV)

[1] TMV enters a wounded plant cell to begin the replication cycle.

[2] Host ribosomes bind to the RNA genome at the 5' end; coat protein (CP) molecules are pushed out of the way from the RNA; host ribosomes begin to translate the two replicase-associated proteins.

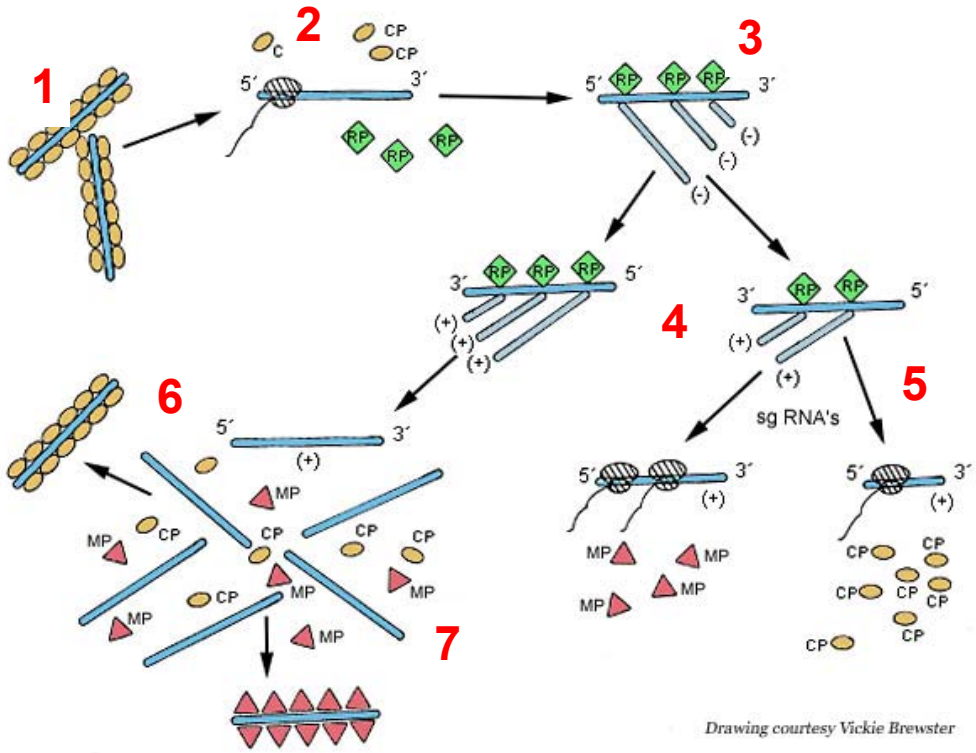


[3] The replicase proteins (RP) are used to generate a negative-sense (- sense) RNA template from the virus RNA.

"Life cycle" of *Tobacco mosaic virus* (TMV)

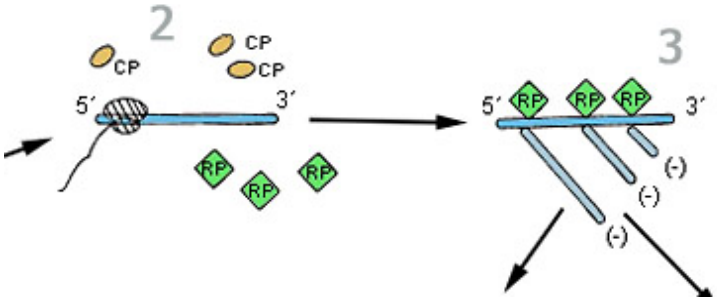
[4] This (- sense) RNA is, in turn, used to generate both full-length positive-sense (+ sense) TMV RNA and the + sense subgenomic RNAs (sgRNAs)

[5] Subgenomic RNAs are used to express the movement protein (MP) and CP.



The + sense TMV RNA is either encapsidated by the CP to form new TMV particles [6] or wrapped with MP [7] to allow it to move to an adjacent cell for another round of replication.

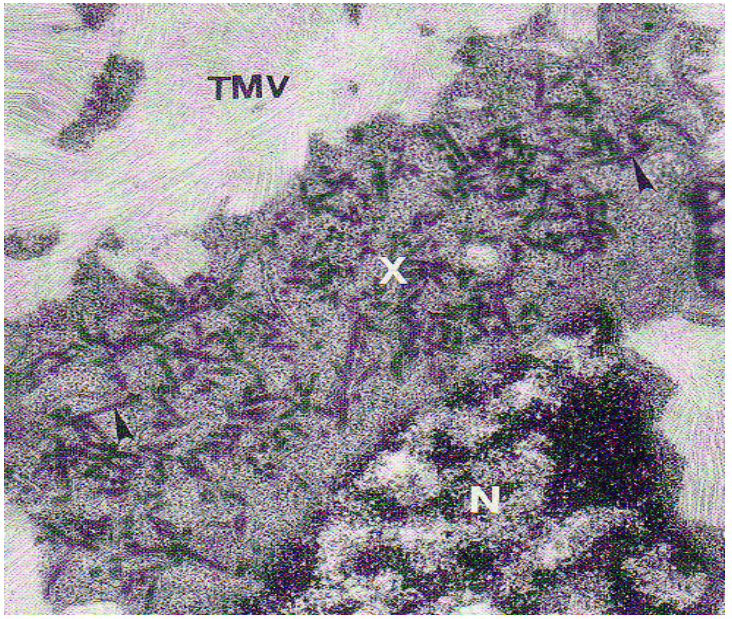
1. Uncoating (Disassembly):



- After virion entry through a wound in the host cell, the virus particle must disassemble. pH of the microenvironment may play a role in the destabilization of the protein subunits of the virion capsid which result in the exposure of the 5' end of the RNA to ribosomes and the initiation of translation.
- Host ribosomes attach to the 5' leader sequence and move down the RNA, displacing coat-protein subunits as it moves (known as Co-translational disassembly). The 5‘ end leader sequence interacts weakly with coat protein because it contains no G residues

2. **Primary Translation**

- Production of the viral replicase assoc. proteins 126 kd + 183 kd
- Viral RNA(s) are translated by host ribosomes to form protein(s) directly or indirectly involved in viral RNA replication.
- The 123 kDa and 183 kDa proteins aggregate with host components (membrane) to form the replication complex also known as the **viroplasm, or inclusion bodies or X-bodies**.

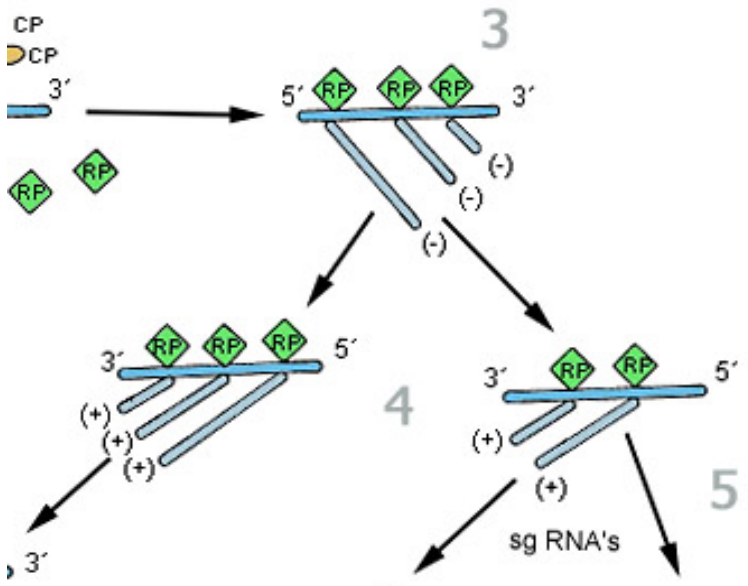


3. **Transcription**

An RNA-dependent, viral-specified polymerase generates (-) sense RNA strands from the genomic RNA [(+) RNA]

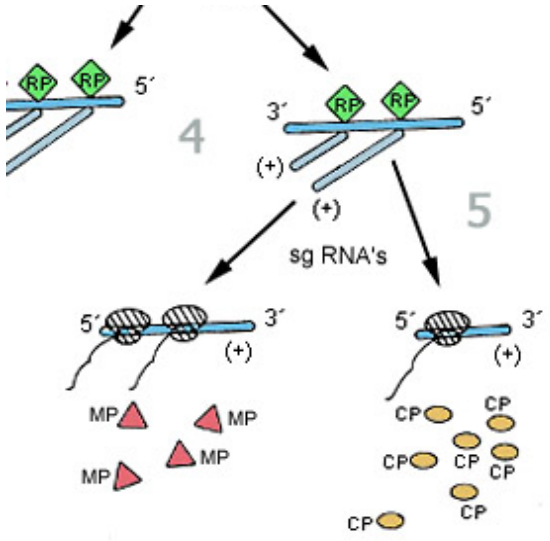
4. **Replication**

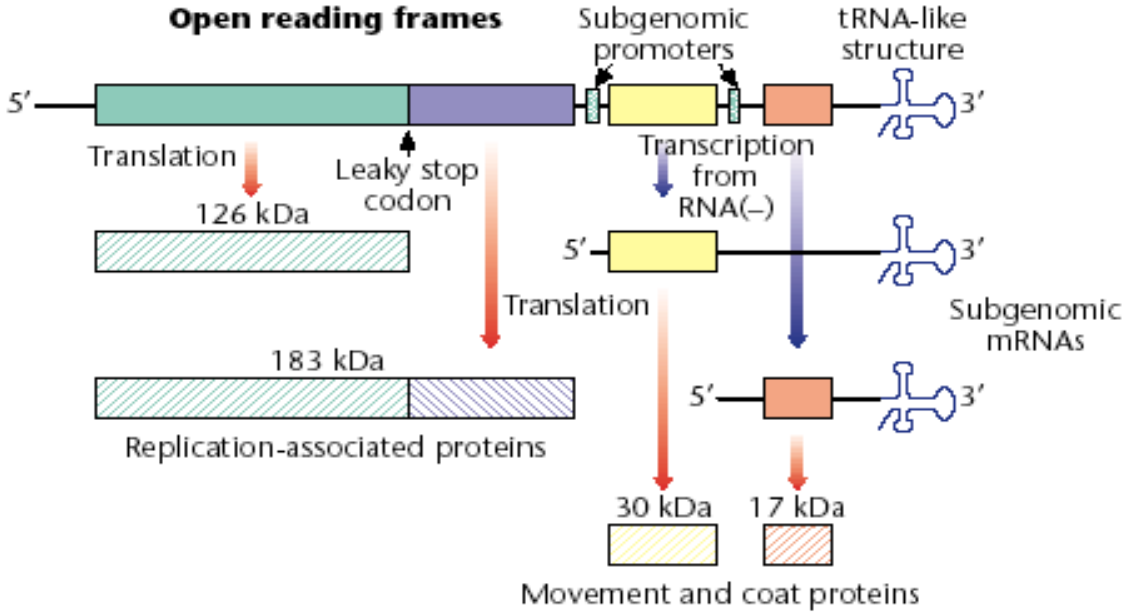
Progeny virus RNA strands are then synthesized on the negative-sense template(s), yielding genomic RNA(s) and in some cases, subgenomic RNA(s). More (+) strands are produced than (-) strands.



5. Secondary Translation - Coat protein and movement protein synthesis.

The genes downstream from the 5' end gene are not available for translation from the full-length genomic RNA. These 3' end genes are expressed via subgenomic RNAs.

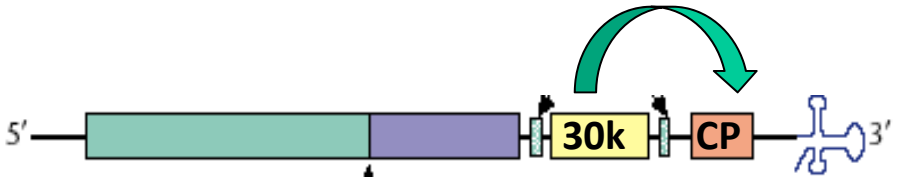




5. Secondary Translation – Synthesis of coat and movement proteins Con't.

For TMV the two internal genes for the 30 kDa movement protein and the coat protein are regulated differently, both temporally and quantitatively.

The promoter/leader sequences in front of the ORFs of TMV determine the timing of gene expression.

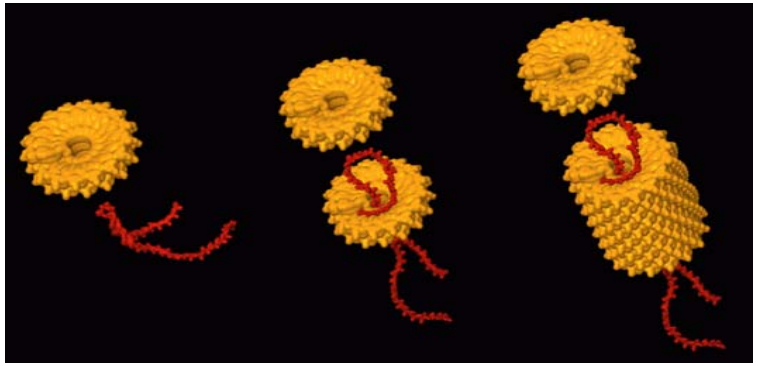


Positioning the 30 kDa gene mRNA in approximately the same location as the coat protein resulted in significant increase in 30kDa protein synthesis

(Culver et al., 1993 Proc. Natl. Acad. Sci. USA 90, 2055-2059).

6. **Encapsidation.**

Some of the plus-sense RNAs are encapsidated (self-assembly) by the coat protein subunits to form mature virions. The origin of assembly is the region of the movement protein gene (900-1300 nt from the 3' end).



7. **Translocation**.

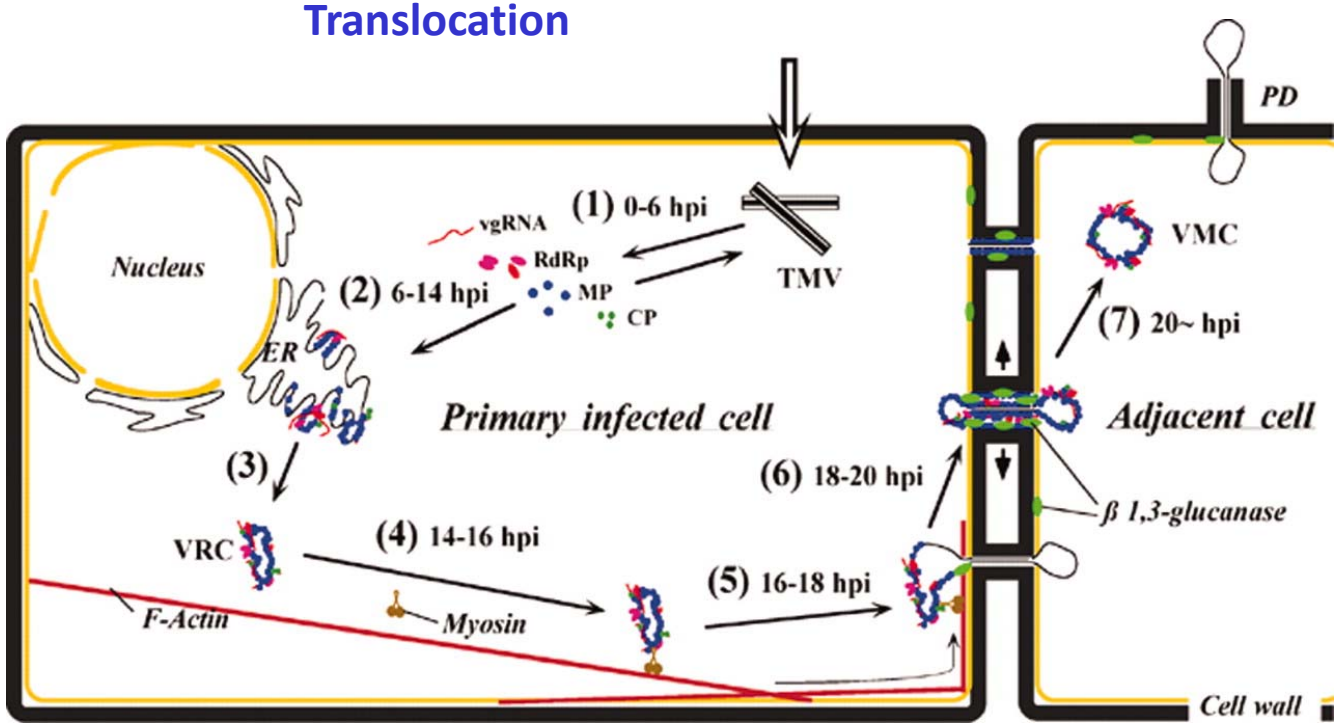
Is by cell to cell movement through plasmodesmata and later by transportation in the phloem to other locations within the plant.

- TMV movement protein (MP) binds and elongates single-stranded nucleic acids (RNA, DNA).
- TMV MP increases plasmodesmatal size exclusion limit from 0.7-kDa to approx. 20-kDa.
- Also appears to make cell more susceptible to infection

Translocation

Step 1: from 0 to 6 hpi, TMV infection yields viral replicase (RdRp), MP, and CP, as well as viral RNA sequences.

Step 2: from 6 to 14 hpi, viral RNA and viral proteins are produced. MP (movement protein) is phosphorylated, and MP is localized on perinuclear and cytoplasmic ER to form viral replication complexes (VRCs) composed of MP, RdRp and viral RNA.



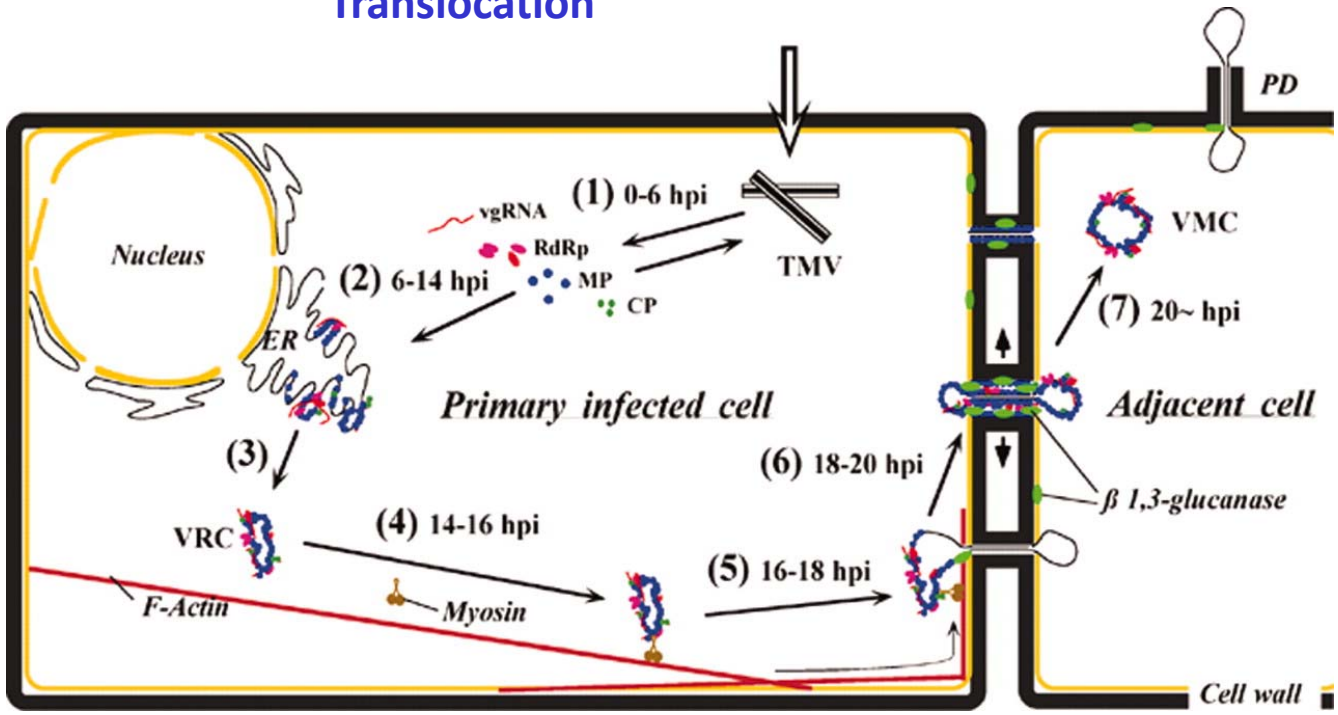
Steps 3 and 4: from 14 to 16 hpi, VRCs increase in size on cytoplasmic ER and in association with protein cytoskeleton, in particular with actin and myosin filaments. VRCs exhibit rapid intracellular movement in cytosol of infected cells.

Step 5: 16 to 18 hpi: movement of VRCs is stopped and some of the VRCs lodge adjacent to plasmodesmata (PD).

Step 6: 18 to 20 hpi: plasmodesmata are modified and opened by action of the MP or VRCs, perhaps involving β-1,3 glucanase of the host.

Step 7: viral movement complexes (VMCs) are spread from primary infected cells to adjacent cells, and replication is initiated in secondarily infected cells.

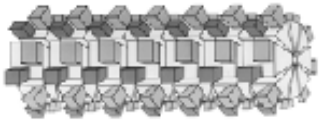
Translocation

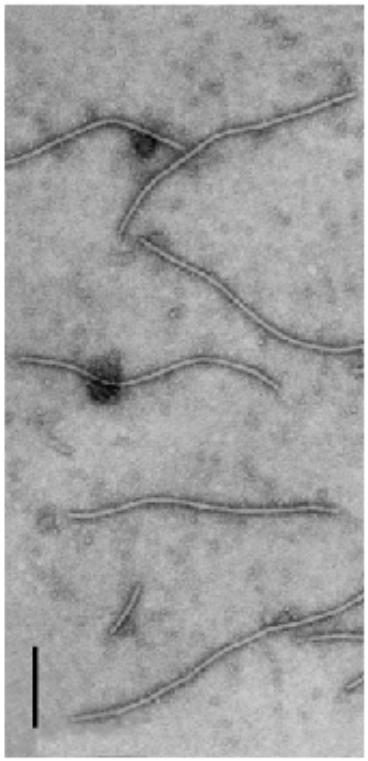


Summary:
At 17 hpi, 90% of the infection sites included single cells; at 20 hpi, 51% of the sites included two or more cells; and by 24 hpi, 64% of sites were multicellular.

Family *Potyviridae*

***Watermelon mosaic virus* in pumpkin**





***Potato virus Y* in Pepper**

Family *Potyviridae*

8 Genera:

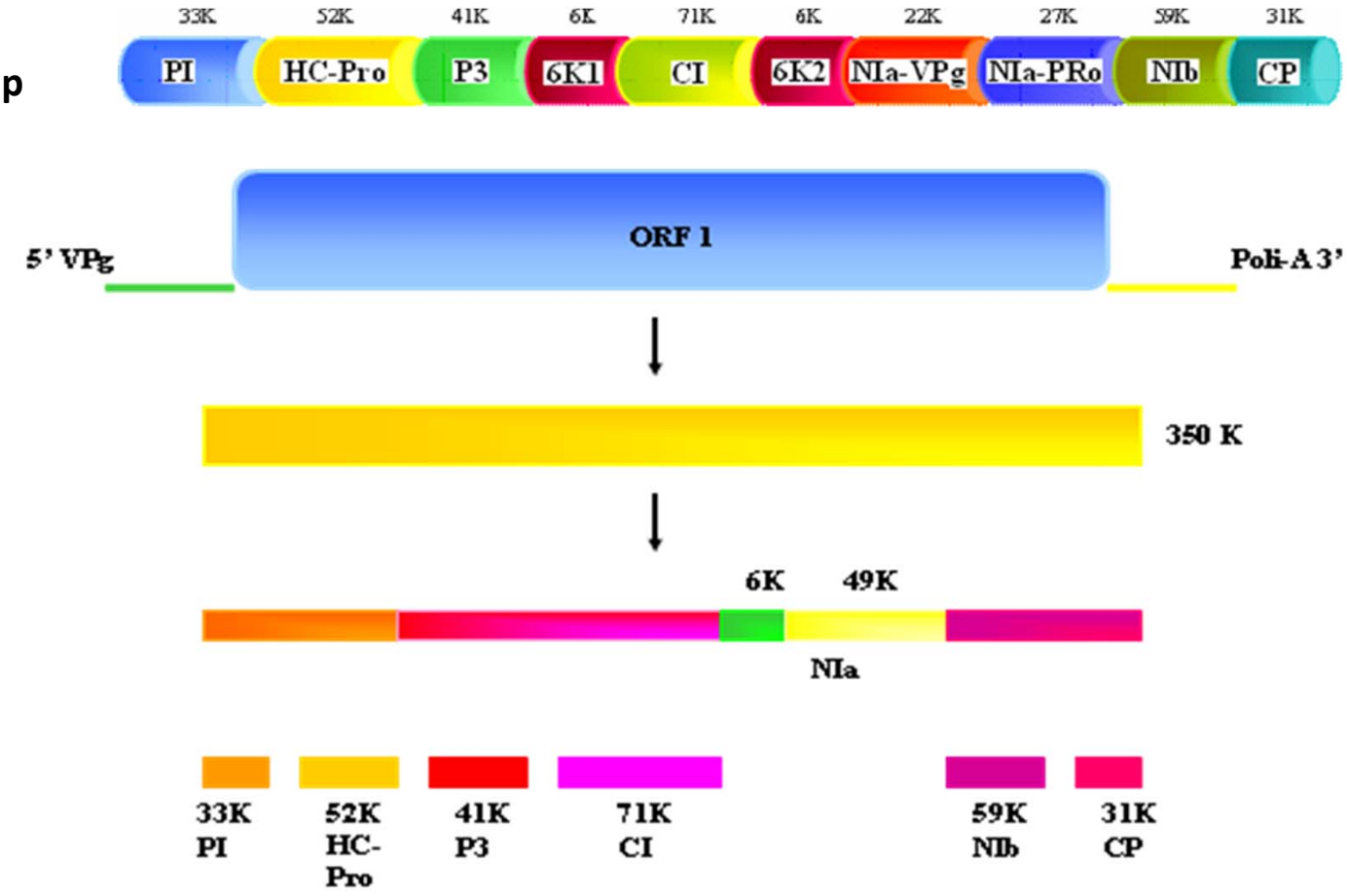
Genus *Brambyvirus*…*Blackberry virus Y,* vector unknown
Genus *Bymovirus*……*Barley yellow mosaic virus*, bipartite, fungal vector
Genus *Ipomovirus….* *Sweet potato mild mosaic virus*, whitefly vector
Genus *Macluravirus* *Maclura mosaic virus,* aphid vectors
Genus *Poacevirus…..* *Triticum mosaic virus,* mite vector
Genus *Potyvirus …….*Potato virus Y*, aphid vectors
Genus *Rymovirus* …..*Ryegrass mosaic virus*, mite vector
Genus *Tritimovirus* ..*Wheat streak mosaic virus,* mite vector

Monopartite virions: 680-900 x 12 nm,
Bipartite virions: 275 nm and 550 nm x 12 nm

General Steps in the Replication Cycle – Genus Potyvirus of (+) Sense RNA Viruses

1. Disassembly
2. Primary Translation – polyprotein of the entire genome
3. Transcription – production of (-) sense RNA
4. Replication – production of (+) sense RNA (RNA genomes)
5. Secondary Translation – does not occur
6. Encapsidation
7. Translocation

Genomic Map


33K
52K
41K
6K
71K
6K
22K
27K
59K
31K
PI
HC-Pro
P3
6K1
CI
6K2
NIa-VPg
NIa-PRo
NIb
CP
5' VPg
ORF 1
Poli-A 3'
350 K
6K
49K
NIa
33K PI
52K HC-Pro
41K P3
71K CI
59K NIb
31K CP

Replication of Viruses in the Genus *Potyvirus*

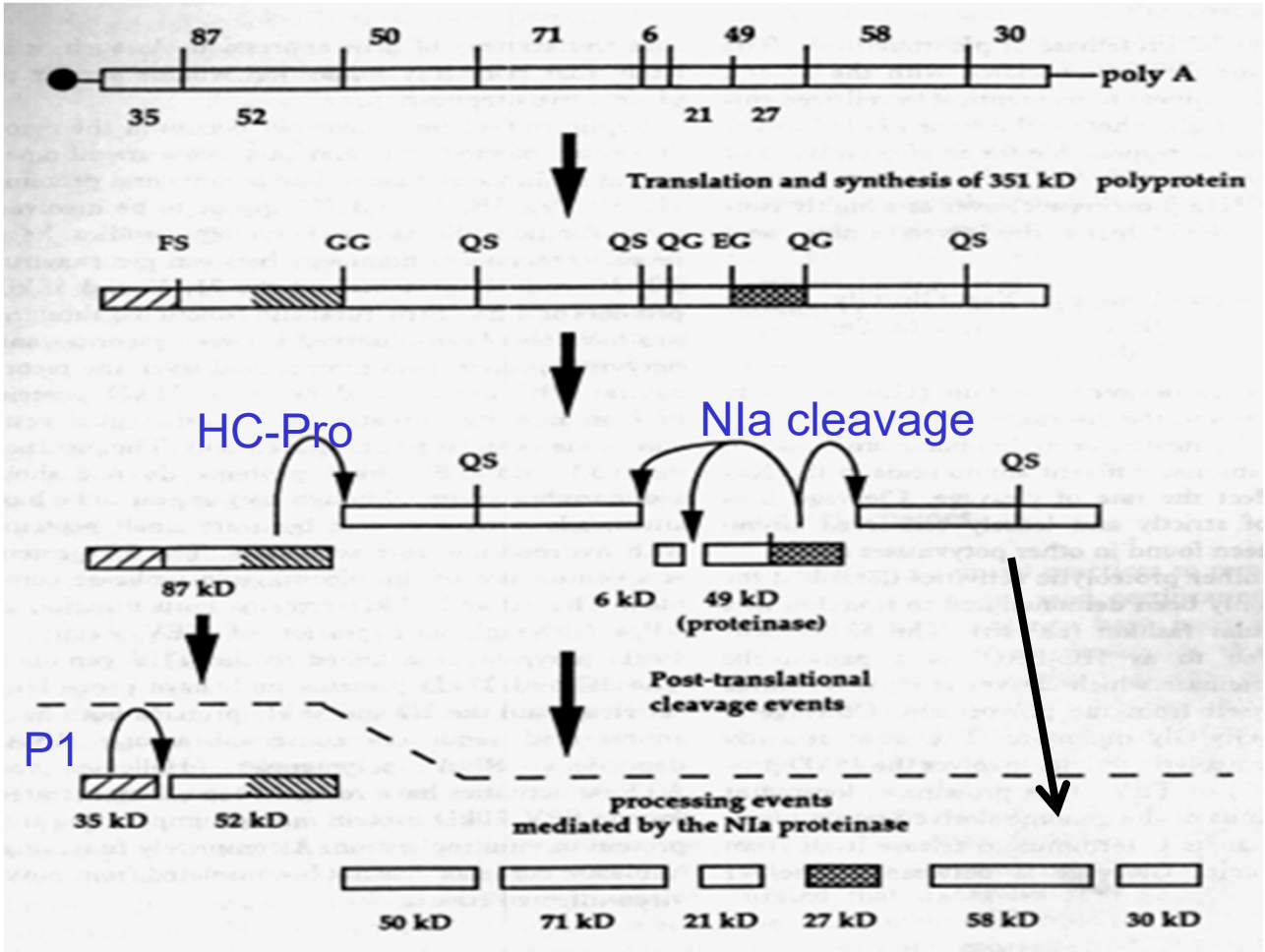
- subgenomic RNAs
- multipartite genomes
- polyprotein
- translational read-through
- translational frame-shift
- ambisense RNAs

Proteolytic processing scheme for the potyvirus *Tobacco etch virus*

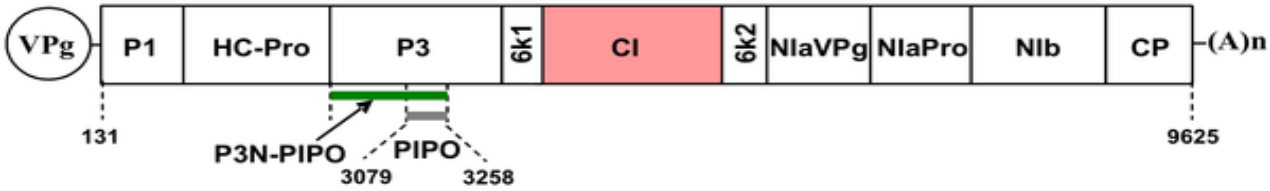
Genome is translated into one long polyprotein

Polyprotein is cleaved (self-cleaved) into smaller proteins

A total of 14 proteins produced

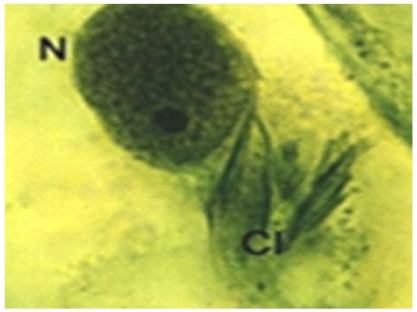


Genome Features:



5’ VPg
3’ poly A tail

RdRp =
CI + $6K_2$ +
NIa + NIb



Functions of Potyvirus Encoded Proteins:

HC-PRO – helper protein (vector trans.), host defense suppressor
P3 - ? - +2 frameshift -> P3N-PIPO: cell to cell movement
$6K_1$ - ?
CI – helicase, RNA binding, cylindrical inclusion, cell to cell movement
$6K_2$ – ER assoc., role in replication
NIa – major proteinase, nuclear inclusion
VPg – 5’ Cap and proteinase, primer for RNA synthesis, binds to many viral and host proteins
Pro - protease
NIb – RdRp, nuclear localization signal, nuclear inclusion
CP (Coat protein) – capsid, vector transmission (1700 – 2000 subunits per virion), cell to cell movement

Polyprotein Processing Strategy

Advantages:

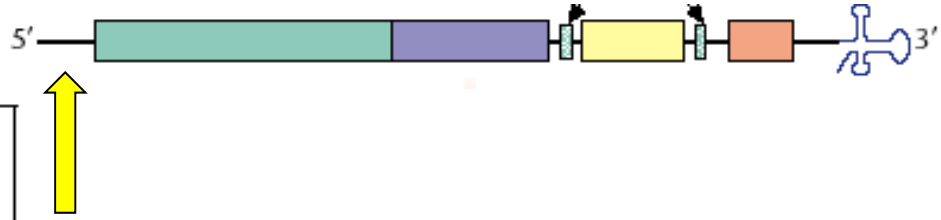
- Many proteins produced from a minimum of genetic information
- Lots of potential for regulating processing pathways
  (through varying the rates of cleavage)

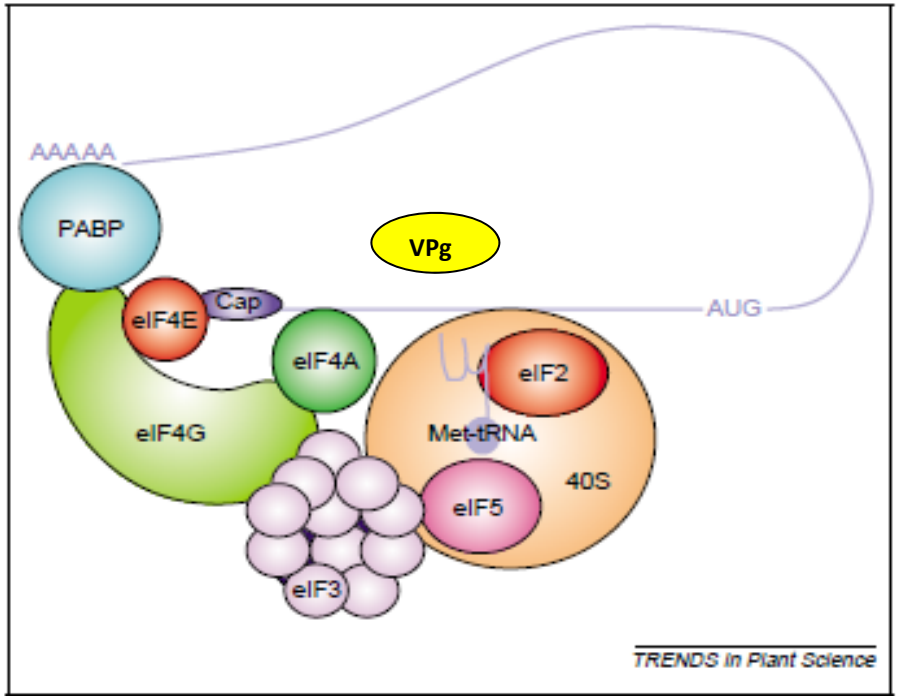
Disadvantages:

- Not clear how this strategy can be efficient
  ie for every molecule of coat protein produced, approx. one molecule of all other proteins has to be produced (2000 are needed for each virion)

- Apparently large amounts of several gene products in non-functional states accumulate in infected cells

Primary Translation





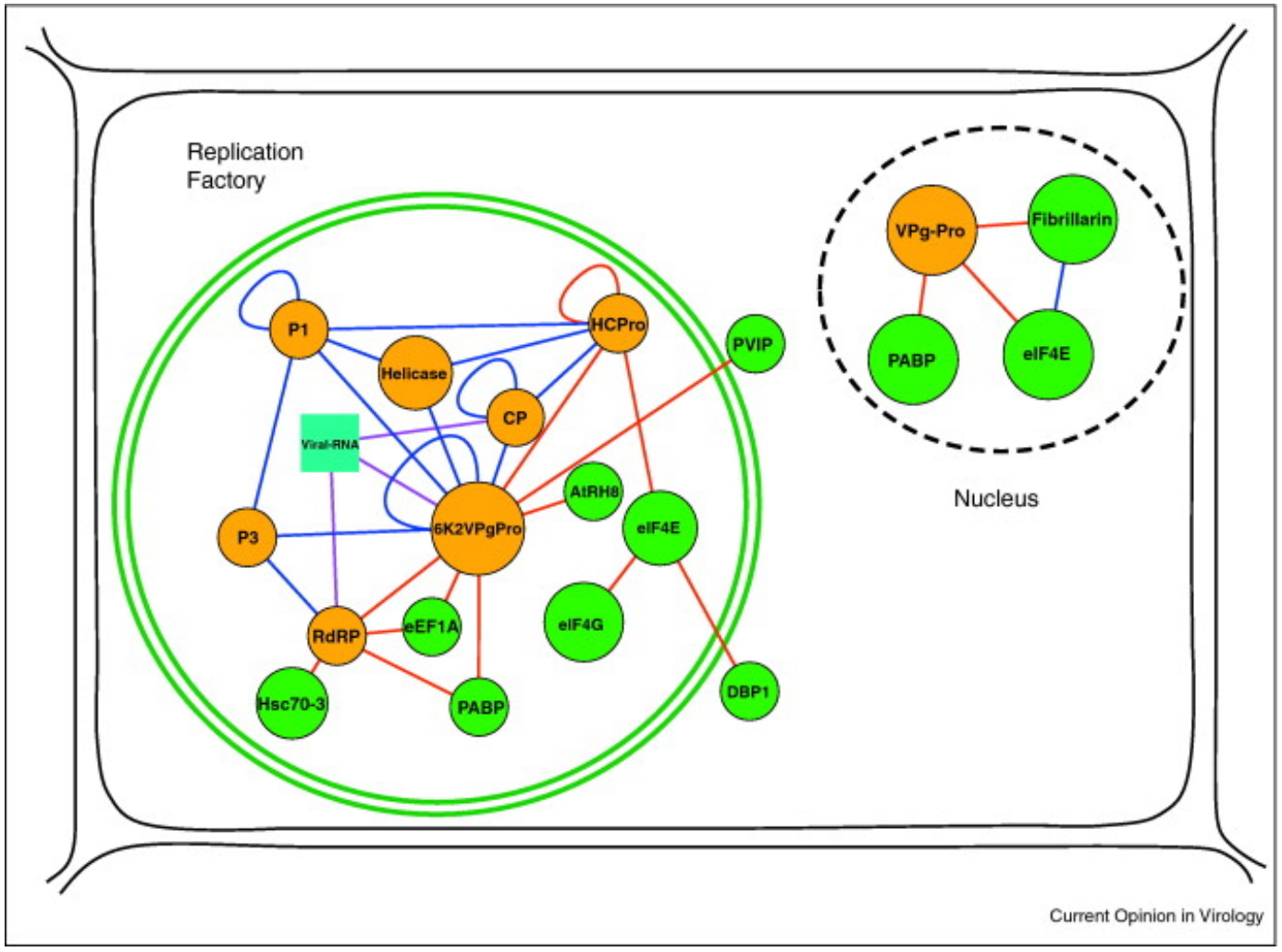
Translation occurs by binding of host proteins to 5’ untranslated region (UTR) of the genome

UTR has secondary structure

The VPg recruits (binds to) eIF4E, a host component of the cellular translation initiation complex

VPg (viral protein, genome-linked)

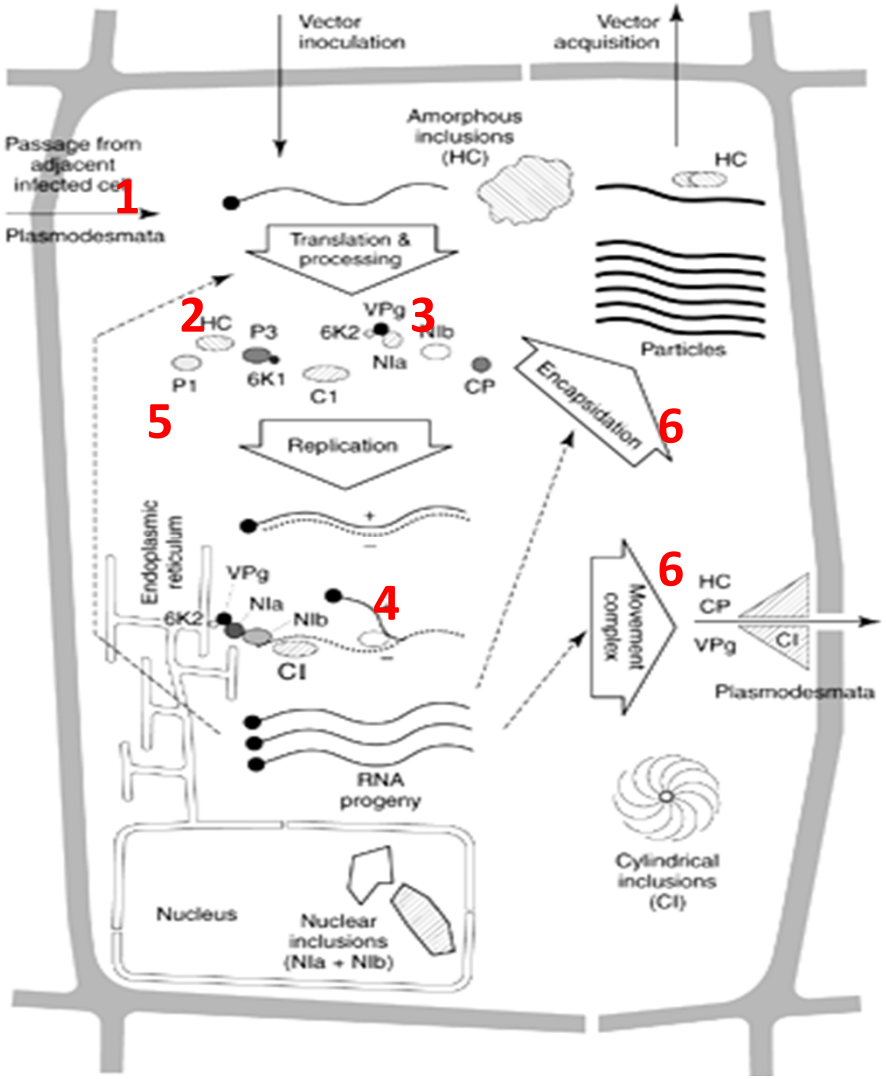
VPgs are at the center of an intricate protein interaction network residing within virus replication factories



Potyvirus Life Cycle

1. Uncoating
2. Primary Translation
3. Transcription
4. Replication
5. Secondary Translation (?)
6. Encapsidation, Movement

Life cycle occurs in the cytoplasm



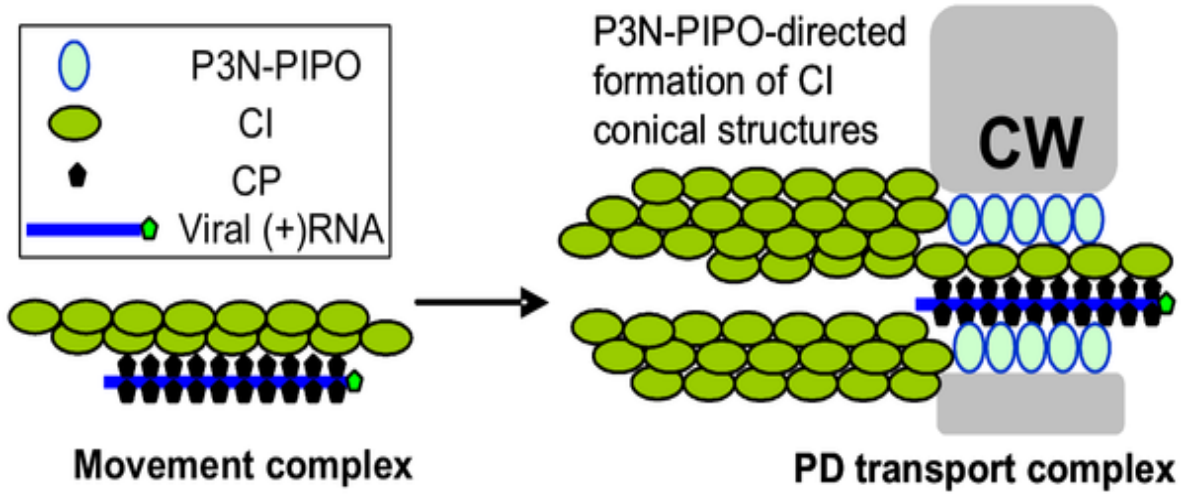
Encyclopedia of Virology, 2e, Academic Press

Model for potyvirus intercellular transport through plasmodesmata

- Cell to cell movement:

A complex of P3N-PIPO, CP, CI, and genomic RNA

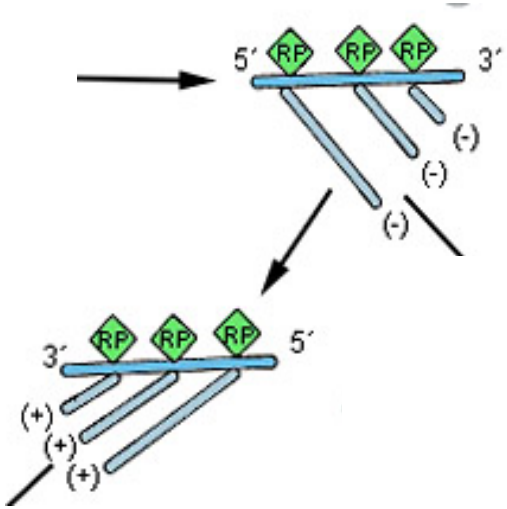
- Increases the exclusion limit of plasmodesmata



Wei T, Zhang C, Hong J, Xiong R, et al. (2010) Formation of Complexes at Plasmodesmata for Potyvirus Intercellular Movement Is Mediated by the Viral Protein P3N-PIPO. PLoS Pathog 6(6): e1000962. doi:10.1371/journal.ppat.1000962
http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000962

3. Transcription

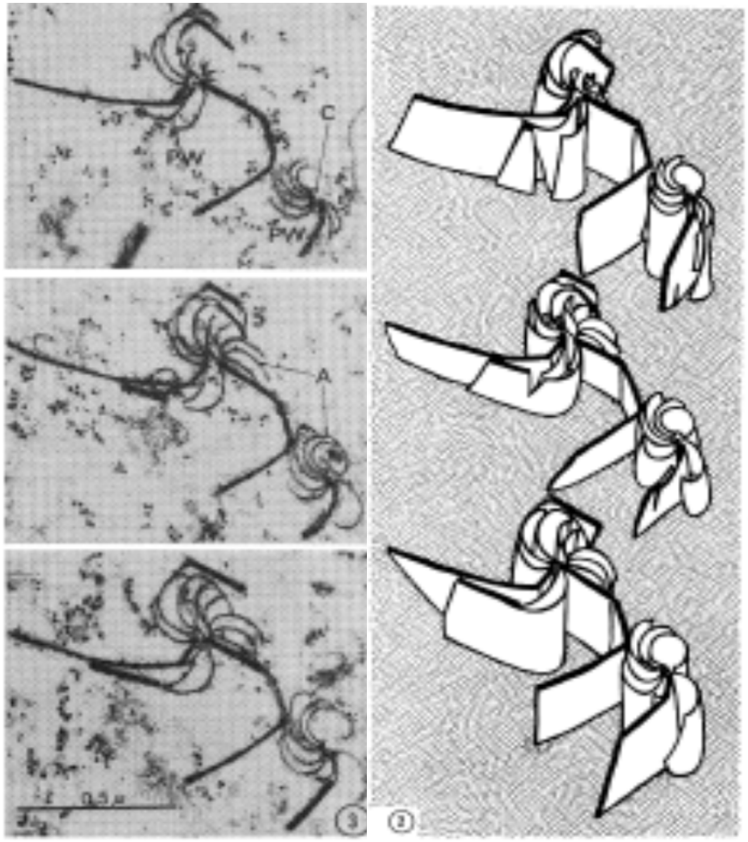
General Scheme for Synthesis of genomic RNA:



Similar to that of TMV, but without the production of subgenomic RNAs

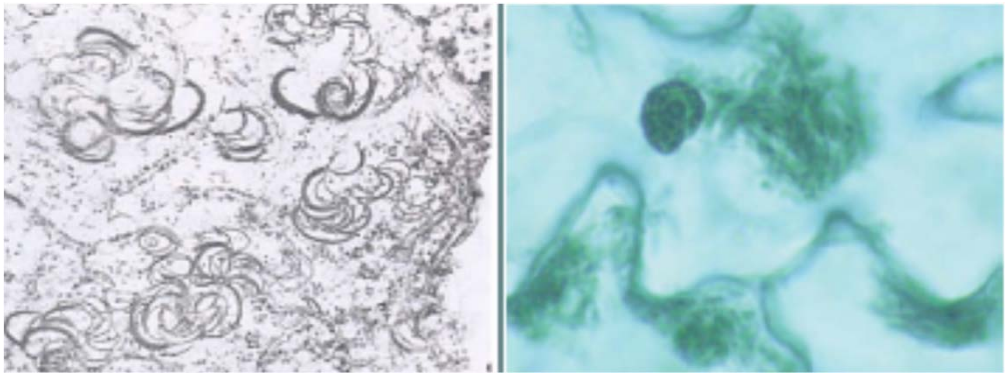
VPgs bind at specific sites in the UTR and functions the primer for viral RNA synthesis

Site of Potyvirus Assembly



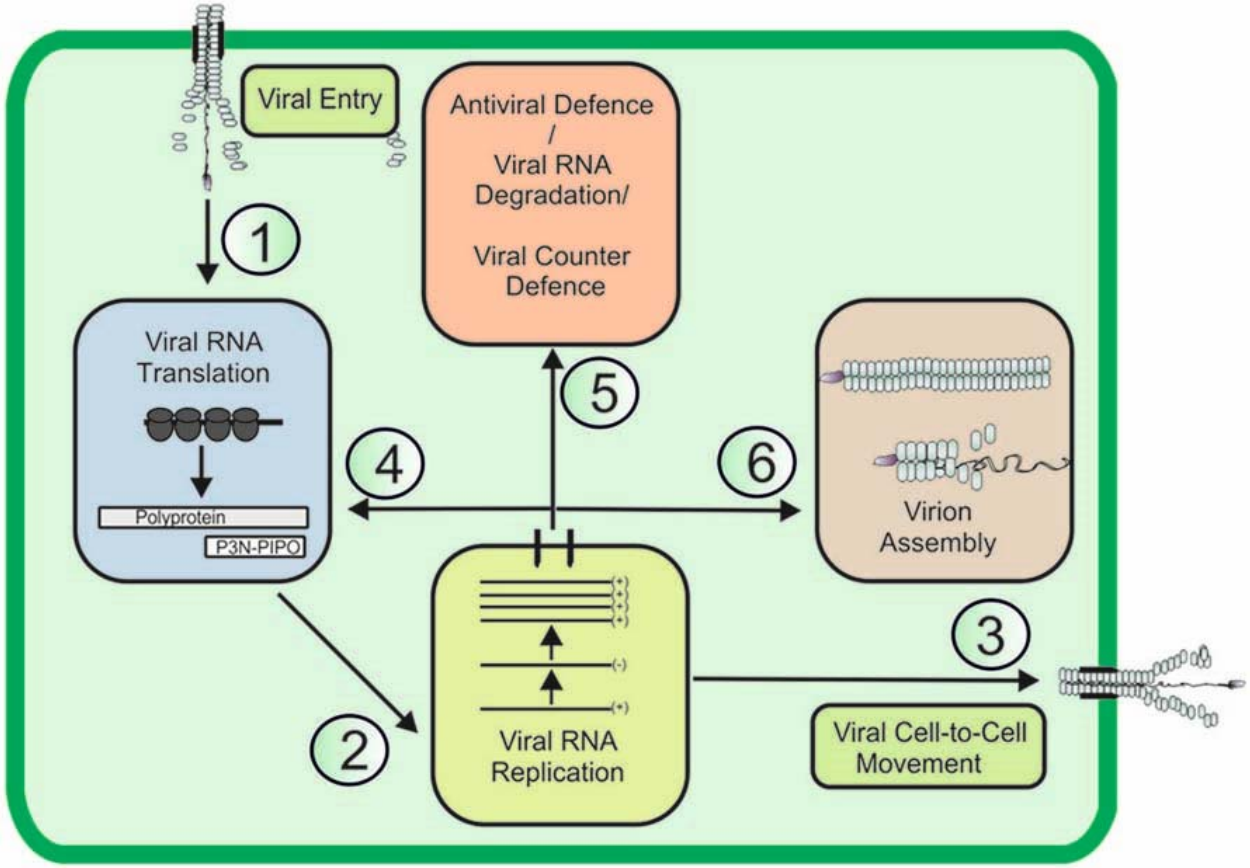
Pinwheels:
Cytoplasmic inclusions

CI (71K) protein



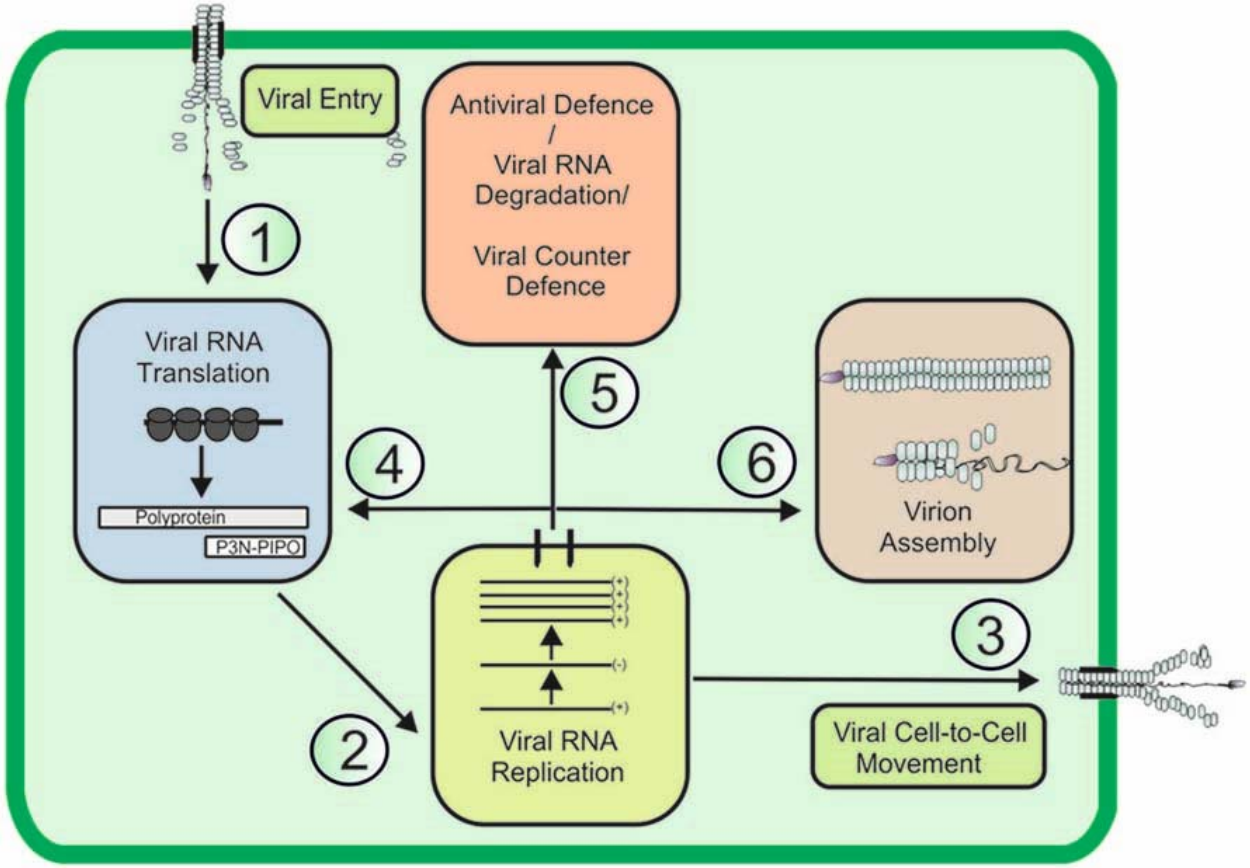
Edwardson 1966 Science 153 (Aug):883-884

Viral RNA pathways in an infected cell. In a newly infected cell, polysomes translate viral RNA (vRNA, pathway 1), and it is recruited to VRCs (pathway 2). The replicated vRNA is transported to plasmodesmata to facilitate cell-to-cell movement (pathway 3). To achieve productive infection, vRNA expression continues via new rounds of translation/replication pathway.



Viral RNA pathways in an infected cell.

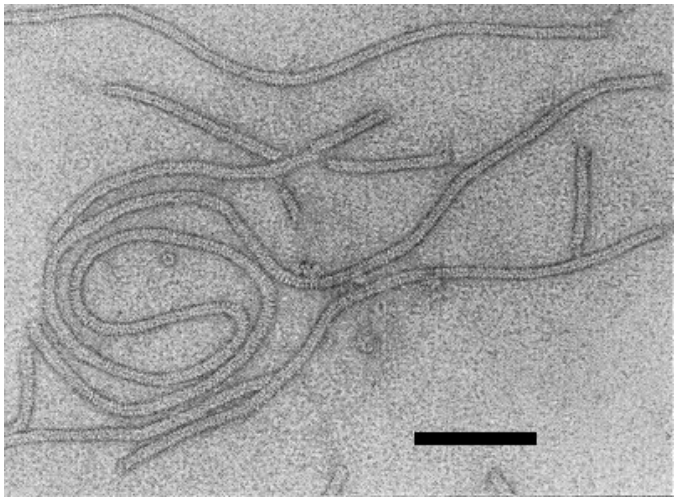
4). Host cell defense mechanisms leading to RNA degradation actively compete for vRNA substrates with viral counterdefense mechanisms (pathway 5). vRNA encapsidation completes the infection cycle (pathway 6), allowing the encapsidated virus to be transported and infect neighboring healthy plants.



***Closteroviridae* –**

(*Clostero*: from Greek *kloster*, 'spindle, thread')

3 Genera: ***Ampelovirus***
Closterovirus
Crinivirus



All *Closteroviridae* genomes are single stranded positive sense RNA

- Monopartite viruses are 1250-2200 nm
- Bipartite are 650-800 and 700-900 nm

Viruses in all 3 genera share similar biological characteristics:

- Yellowing symptoms
- Occur in very low concentrations in the host
- Are more easily found in older leaves
- Phloem-limited

Citrus tristeza virus* (CTV) Genus: *Closterovirus

Cucurbit yellow stunting disorder virus* (CYSDV) Genus*: Crinivirus

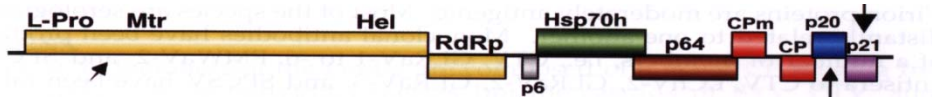
Tomato infectious chlorosis virus* (TICV) Genus: *Crinivirus

Reddening of leaves, necrotic spots, stunting, tip dieback and reduced yields associated with Pineapple mealybug wilt disease.

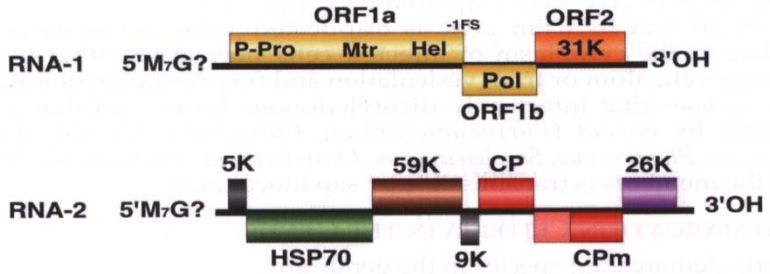
This disease is caused by at least 2 *Ampeloviruses:*
Pineapple mealybug wilt associated virus-1 (PMWaV-1)
and
Pineapple mealybug wilt associated virus-2 (PMWaV-2)

Biological Properties:

Closterovirus — **Aphids (semi-pers.) Monopartite**



Crinivirus — **Whiteflies (semi-pers.) Bipartite**



Ampelovirus — **Mealybugs (semi-pers.) Monopartite**

Closterovirus Replication

+ Sense RNA Virus genome expression

- subgenomic RNAs
- multipartite genomes (some)
- polyprotein
- translational read-through
- translational frame-shift
- ambisense RNAs

Replication Strategy of *Beet yellows virus* (*Closterovirus*)

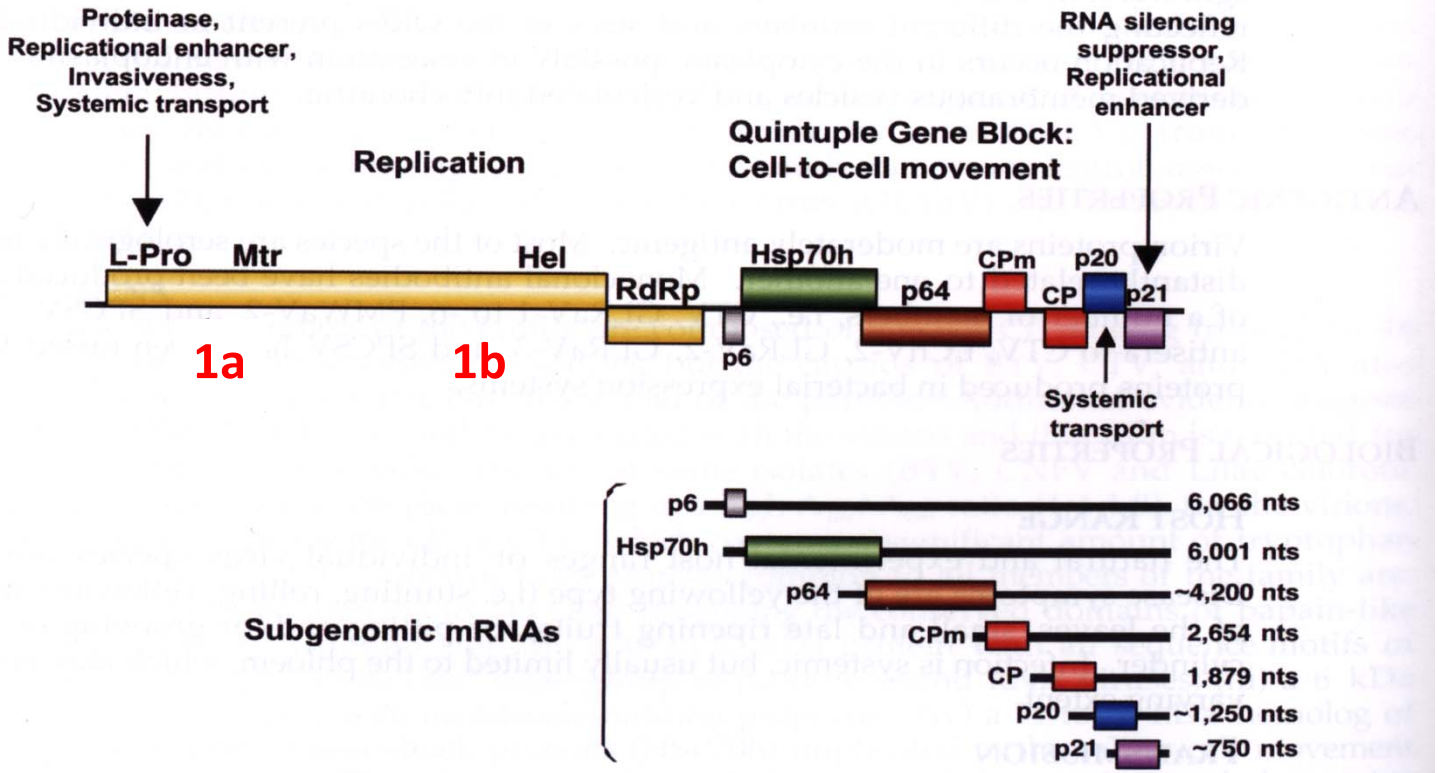


Figure 2: Genome organization and strategy of replication characteristic of Beet yellows virus (BYV) showing the relative position of the ORFs, their expression products, and the 3' nested set of sgRNAs. L-Pro, leader proteinase; Mtr, methyltransferase;Hel, helicase; RdRp, RNA polymerase; HSP70h, heat shock protein homologue; CP coat protein; CPm, minor capsid protein. The five boxes under "cell-to-cell movement" represent the five gene block conserved among closteroviruses (from Dolja, 2003).

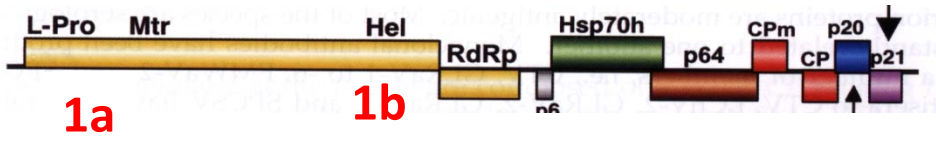
Gene Products of most Closteroviruses:

Protein **Function**

- Small 4-6 kDa hydrophobic protein…….Transmembrane Protein
- HSP70 homolog (HSP70h)…… Cell to cell movement, virus assembly
- 60 kDa protein ……Cell to cell movement
- Coat protein (CP) …… Coat protein for most of virion
- Minor coat protein (CPm) …… Coat protein for 5’ end of the virion
- P21 ……. Silencing suppressor

Polymerase?

Gene Products and Functions of most Closteroviruses:



Read from the viral genome:

- **1a Polyprotein (includes methyltransferase, proteinase and helicase functions)**
- **RdRp (1b) usually expressed as a fusion protein with the 1a ORF through a +1 frameshift during translation**

Closterovirus polymerase: believed to include the 2 large proteins expressed from ORF 1a and ORF 1a+1b

Closterovirus Polymerase:

Other Polymerase functions:

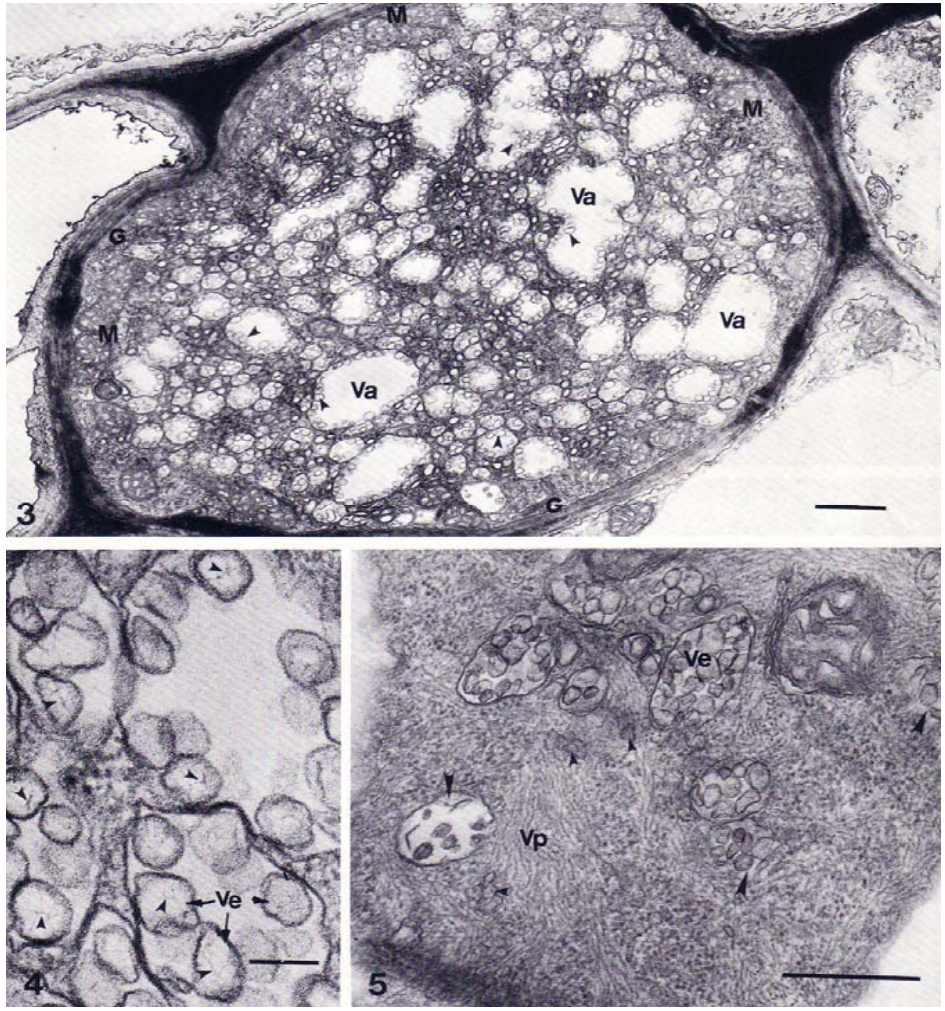
- **Forms a protein shell enveloped by a membrane derived from the host endoplasmic reticulum**
- **Causes restructuring of host endoplasmic reticulum into a large vesicular factory for viral RNA**

Dolja et al 2006 Comparative and functional genomics of closteroviruses. Virus Research 117:38–51

Images of *Diodia vein chlorosis virus* found in phloem companion cells of *Diodia virginiana*

Va – vacuoles (more than normal)
Ve – vesicles
Vp – virus particles
M – mitochondria

Closterovirus replication causes increases in the number of vacuoles in cells as well as organelles, and causes the appearance of vesicles in phloem associated cells



Larson et al 1991 Phytopathology 81:227 -232

Closteroviruses:

2 coat proteins: CP and CPm

IgG to CPm (labeled with gold) shows the location of CPm (5’ terminus of the virion)