Symptomatology

Infection by most members of the genus *Potexvirus* is latent or causes only mild mosaic symptoms in the natural host. Symptoms vary cyclically or seasonally and may disappear soon after infection. Some viruses cause necrosis, ringspot, or dwarf symptoms in a wide range of plant species. If symptoms are evident, more severe symptoms such as mosaics appear in the early stages of infection. Some potexviruses such as PVX cause diseases that are of economic importance on their own; however, most of them are associated with more serious diseases when plants are co-infected with other viruses.

Serology

Virions are good immunogens. Most potexviruses are serologically related to several others, with the relationships varying from close to distant.

Geographical Distribution

Potexviruses are found wherever their hosts are grown, and so the geographical distribution of many species is restricted to only certain parts of the world.

Viral Epidemiology and Control

Most potexvirus-associated diseases are usually very mild or symptomless. The need for their control is often perceived as not important. However, crops such as potatoes, certain cactis, and some ornamental crops that may be infected by more damaging potexviruses require suitable control measures. Transgenic potato, tobacco, and orchid plants, which are resistant to infection by PVX and CymMV, have been developed.

See also: Allexivirus; *Capillovirus, Foveavirus, Trichovirus, Vitivirus*; *Carlavirus*; Flexiviruses; Plant Virus Vectors (Gene Expression Systems); Vector Transmission of Plant Viruses.

Further Reading

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Potyviruses

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Introduction

The existence of potyviruses as a specific group of plant pathogens with numerous members was recognized soon after the onset of virology as a scientific discipline. Historical evidence of symptoms caused in plants by potyviruses includes the color-breaking of infected tulips that was considered fashionable in the seventeenth century and abundantly reproduced in Dutch paintings from that period. Some viruses of the family were among the first plant viruses to be identified due to the importance of the diseases they cause, and relatively soon after potyviruses were grouped according to common characteristics. Electron microscopy provided a clear taxonomic criterion to allocate viruses into the group by showing the consistent presence of pinwheel-shaped cytoplasmic inclusions in plant cells infected by potyviruses. Further contributions to classification were provided by biological and serological studies, followed by deciphering of virus sequences. The finding of biological peculiarities in closely related viruses, with unusual vector organisms or genome composition, led to the current, although still provisional, classification of six genera in the family.

The large number of virus species within the family *Potyviridae* reveals its evolutionary success. Currently almost 200 definitive and tentative species are included in the genus of aphid-transmitted viruses, *Potyvirus*. Members of the family *Potyviridae* are distributed throughout the world, although each particular virus has a specific host range that limits its geographic distribution to the

area potentially occupied by susceptible hosts. Generally speaking, host ranges are constrained to a limited number of natural and experimental hosts, although several potyviruses can infect a considerable number of plant species distributed in many botanical families and some members infect the most economically important crops, including grain, legumes, forages, vegetables, fruits, and ornamentals. The fact that several potyviruses are among the most damaging plant pathogens has fueled both the interest of researchers and the sustained attention of plant breeders and agronomists seeking practical solutions to the losses they cause in many crop species.

This article focuses on current knowledge about viruses in the family *Potyviridae*, with special emphasis on the more recent advances in taxonomy, evolution, diagnostics, functional and structural aspects of viral proteins, as well as host–virus interactions, including characterization of resistance genes and defense responses based on RNA silencing mechanisms. Present and future biotechnological applications of viruses within the family are also considered.

Taxonomy and Classification

The family *Potyviridae* comprises six genera of plant viruses, including the genus *Potyvirus* that is currently the largest genus of plant viruses. Other genera in the family are *Bymovirus*, *Ipomovirus*, *Macluravirus*, *Rymovirus*, and *Tritimovirus*.

Taxonomic standards for classification into the family include: (1) properties of virus particles: long flexuous filamentous (**Figure 1(a)**), (2) cytopathological manifestations: presence of pinwheel or scroll-shaped cylindrical cytoplasmic inclusions in infected plant cells (**Figure 1(b)**), and (3) genome structure and expression strategy: positive-sense ssRNA genomes, with 5′ terminal proteins and 3′ polyA tails, translated as large polyprotein precursors (see the section titled 'Properties of the genome').

Within the family, genera are differentiated according to biological and molecular criteria. Potyviruses are aphid-transmissible and possess a monopartite genome, characteristics that they share with macluraviruses, although particles of the latter are shorter in length. Other monopartite genera include whitefly-transmitted ipomoviruses, and mite-transmitted rymoviruses and tritimoviruses, further distinguished by their vector organisms belonging to the genera *Abacarus* and *Aceria*, respectively. Rymoviruses can also be distinguished by nucleotide similarity, being more closely related to potyviruses than tritimoviruses. Finally, bymoviruses have bipartite genomes that are encapsidated separately, and are transmitted by plasmodiophoraceous fungi of the genus *Polymyxa*. **Table 1** summarizes the family classification.

Phylogenetic analysis based on the alignment of genome regions has established standard thresholds of around 50% and 75% of nucleotide identities (variable depending on the genomic region considered) as demarcation criteria for assigning viruses to genera and species, respectively. This approach has essentially confirmed the classification shown in **Table 1**. An example of phylogenetic analysis is shown in **Figure 2**. Subgrouping viruses, particularly between members of the large genus *Potyvirus*, is also contemplated as a possibility for future refinements in classification, and could follow host range restriction as biological demarcation criteria. Information about taxonomy and sequence of members of the family *Potyviridae* is available at several web-based databases (**Table 2**).

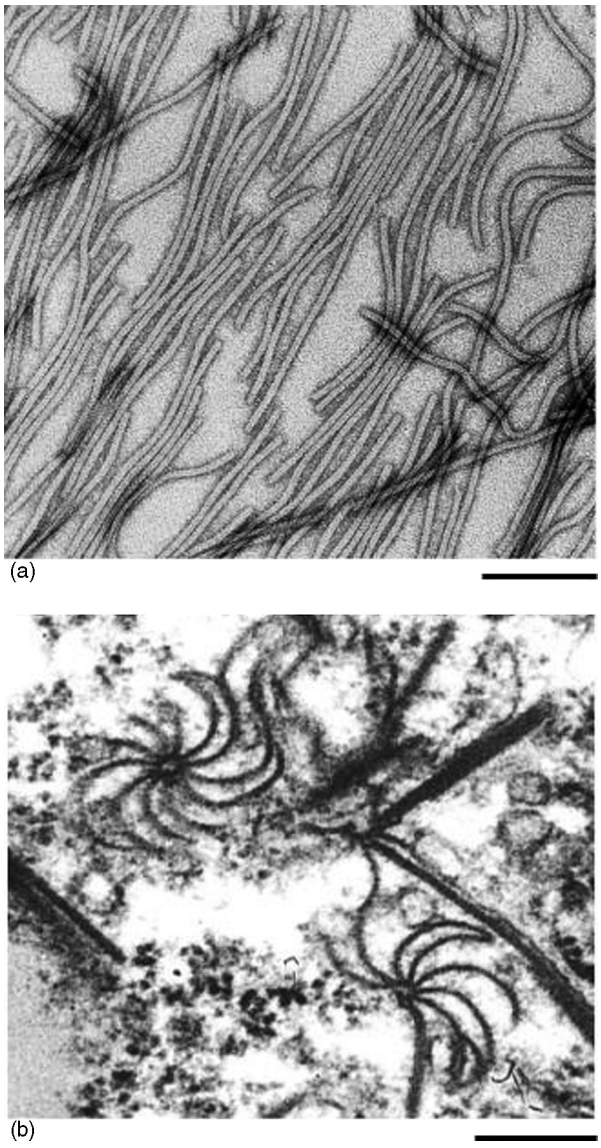


Figure 1 (a) Negative-stain preparation of purified tobacco etch potyvirus particles. (b) Thin section showing the typical pinwheel-shaped cytoplasmic inclusions present in cells of a *Nicotiana benthamiana* plant infected with plum pox potyvirus. Scale = 200 nm. Courtesy of D. López-Abella, CIB, CSIC.

Table 1 Classification of members of the family *Potyviridae* into genera, with indication of type member, number of virus species, transmission vectors, and number of genomic RNAs

		Number of species[a]				
Genus	*Type member*	*Definitive*	*Tentative*	*Genome*	*Vector*[b]	*Particle*[c] *length (nm)*
Bymovirus	Barley yellow mosaic virus (BYMV)	6		Bipartite	Fungi	250–300 500–600
Ipomovirus	Sweet potato mild mottle virus (SPMMV)	2	2	Monopartite	Whiteflies	900
Macluravirus	Maclura mosaic virus (MacMV)	3		Monopartite	Aphids	650–675
Potyvirus	Potato virus Y (PVY)	112	87	Monopartite	Aphids	700–900
Rymovirus	Ryegrass mosaic virus (RyMV)	4	1	Monopartite	Mites	700
Tritimovirus	Wheat streak mosaic virus (WSMV)	2		Monopartite	Mites	700

[a]Number of species according to the International Committee on Taxonomy of Viruses.
[b]Vector organisms responsible for transmission.
[c]Approximate length of virus particles.

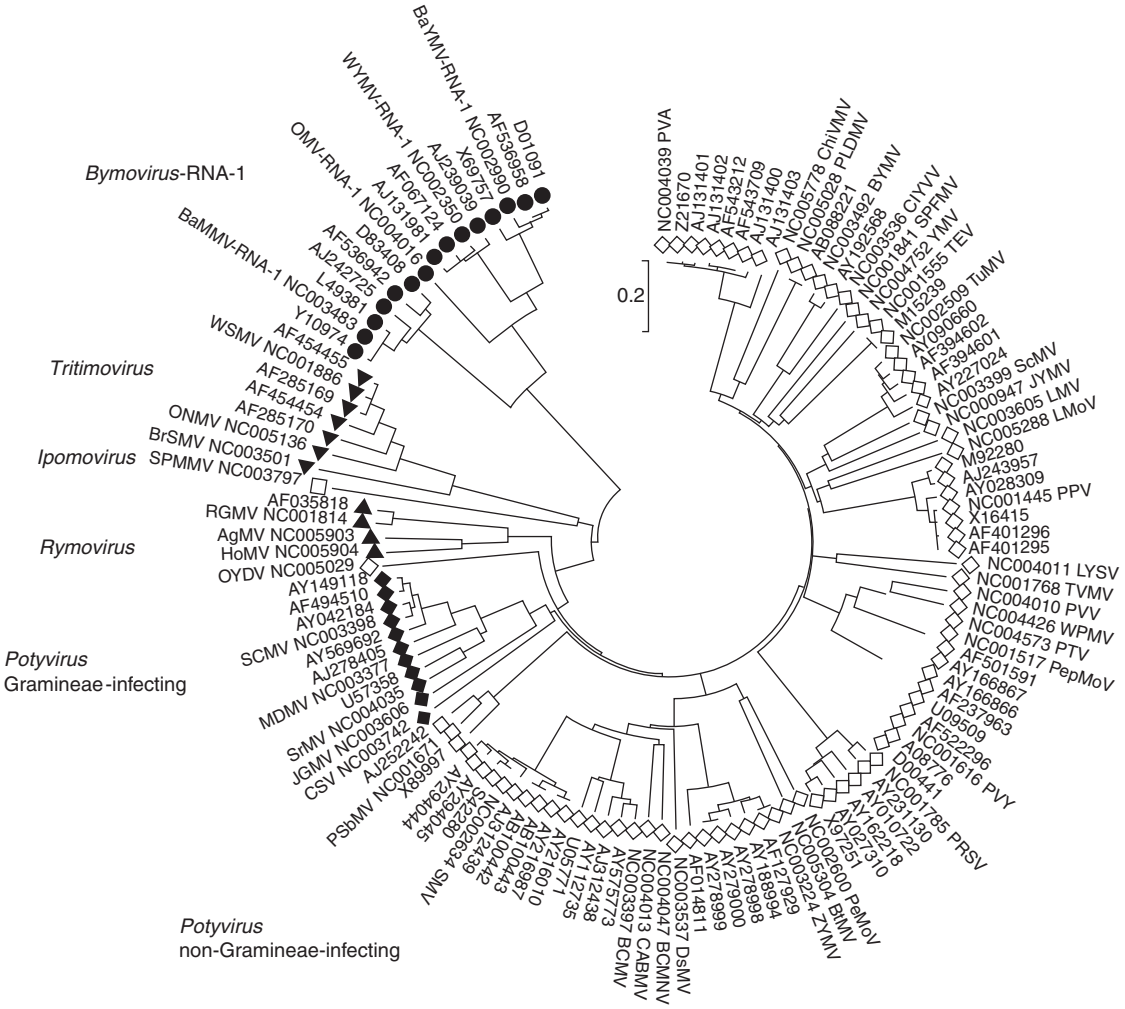


Figure 2 Phylogenetic analysis derived from the comparison of over 120 full-length viral genome sequences of 48 members of the family *Potyviridae*. The figure shows a neighbor-joining tree derived from a ClustalX alignment. Individual viral sequences, indicated by GenBank accession numbers and International Committee on Taxonomy of Viruses (ICTV) abbreviations, are grouped by genera and identified by symbols corresponding to potyviruses (diamonds, open or filled for viruses infecting non-gramineous or gramineous host plants, respectively), rymoviruses (triangles), one ipomovirus (open square), tritimoviruses (inverted triangles), and bymoviruses (circles). For bymoviruses, only the RNA-1 was used for comparison. Reproduced from Wang H, Huang LF, and Cooper JI (2006) Analysis on mutation patterns, detection of population bottlenecks, and suggestion of deleterious-compensatory evolution among members of the genus *Potyvirus*. *Archives of Virology* 151: 1625–1633, with permission from Springer-Verlag.

Table 2 Selection of resources available on the Internet (World Wide Web) for the family *Potyviridae*

Web page address (URL)	*Contents and characteristics*
http://www.ictvdb.rothamsted.ac.uk/ictv/fs_potyv.htm	ICTV (International Committe on Taxonomy of Viruses): taxonomy structure, list of species
http://image.fs.uidaho.edu/vide/genus039.htm	VIDE (Virus Identification Data Exchange) project: nomenclature, host range, virion properties
http://www.danforthcenter.org/iltab/potyviridae/index.htm	Taxonomy, references, and sequence databases of members of the family
http://www.dpvweb.net/potycleavage/index.html	Analysis of the polyprotein cleavage sites

Virion Properties

Virion particles of viruses belonging to the family *Potyviridae* are flexuous rods constituted of protein (95%) and RNA (5%) (**Figure 1(a)**). Their size is about 11–15 nm wide, with lengths ranging from less than 700 nm in the case of macluraviruses to up to 900 nm in ipomoviruses (**Table 1**). Available data on particle structure suggest an helical assembly of identical coat protein (CP) subunits (about 2000) surrounding the nucleic acid, and a distribution of 7–8 subunits per turn has been suggested for the tritimovirus wheat streak mosaic virus (WSMV).

Molecular weight of CP subunits ranges between 30–40 kDa, with differences mainly due to a variable length of the N-terminal region. A more conserved internal CP core (about 220 amino acids) is probably involved in particle architecture. Both N- and C-termini are exposed at the surface of the particle. Superficially located residues might interact with other proteins during essential processes of the virus life cycle. As an example, the conserved DAG motif near the N-terminus of the CP is required for aphid transmission of tobacco vein mottling virus (TVMV). Recent studies have established the existence of host-dependent post-translational modifications, with probable regulatory functions during the viral cycle, in the CP of several potyviruses, including phosphorylation and glycosylation.

A recently identified peculiarity of potyviral particles is the presence of unusual structures at one of the ends of the long particles, probably associated to the 5′ end of the genomic RNA, in which the VPg and HC-Pro proteins were detected in some particles of purified potato virus Y (PVY) and potato virus A (PVA).

Serology and Diagnostics

Serological tools are the preferred diagnostic system of potyviruses. While the nonstructural viral proteins have limited use in serological diagnosis, the virus particles are usually strongly immunogenic. However, serological relationships among potyviruses are complex, with unexpected and inconsistent cross-reactivities that hindered their application in taxonomy. The cause of this is the presence of common epitopes in the conserved internal CP core, while specific epitopes map in the variable N-terminus, a surface-exposed region prone to degradation. New species-specific antibodies targeted against the immunodominant N-terminal region provided excellent serological detection tools applicable for many viruses.

The easy acquisition of sequence information after reverse transcription-polymerase chain reaction (RT-PCR) amplification is another way to deal with diagnosis. Degenerated primers able to amplify virtually any potyvirus have been described, for instance, targeted against conserved regions flanking the variable CP N-terminus. In combination with specific hybridization techniques or sequencing, the diagnosis is unequivocal. These molecular tools have been extensively used to identify new viruses infecting new hosts. Also, the combination of serological capture of particles with high sensitivity RT-PCR methods has resulted in robust detection systems. The application of modern molecular tools to diagnostics is being continually updated with new, more sensitive and specific approaches.

Properties of the Genome

The genome of monopartite potyviruses is an ssRNA molecule of + (messenger) sense. In all members studied so far, genomic RNA presents a 5′ terminal protein (VPg) and a 3′ polyA tail of variable length. Typical genome sizes range from 9.4 to 10.3 kbp. The genome comprises a single ORF coding for a long polyprotein (340–370 kDa) that generates mature products after ongoing an autoproteolytic processing cascade. The bipartite genome of bymoviruses is divided into two RNAs: a long RNA-1 homologous to the 3′ three-quarters portion of the monopartite potyvirus genome, and a short RNA-2 with partial similarity to portions of the 5′ region of the genome of monopartite potyviruses. Three virus-encoded proteinases are involved in the proteolytic processing of the potyviral polyprotein. The serine proteinase P1 and the cysteine proteinase HC-Pro are responsible for autocatalytic cleavages at their C-ends, and the serine proteinase NIaPro cleaves all other sites. Information on processing cleavage sites for all known viruses of the family is

available on a web-based database (Table 2). The dynamics of the process might have regulatory implications for the sequential appearance and accumulation of intermediate and final products (Figure 3).

A typical potyvirus genome starts with a 5′ noncoding region (NCR), less than 200 bp long. This leader acts as an enhancer of translation, and although the general mechanism is not fully understood, there is evidence that regulatory elements lie in this region. Studies in plum pox virus (PPV) showed that most of this region is dispensable for infectivity, although it contributes to viral competitiveness and pathogenesis, and that translation takes place by a cap-independent leaky scanning mechanism. In the case of the 5′ leader of tobacco etch virus (TEV), the existence of an internal initiation site has been suggested, with the presence of an RNA pseudoknot domain that conferred cap-independent translation.

Another NCR of about 200 bp is located at the 3′-end of the genome before the polyA tail. Putative RNA structures in this region confer pathogenic properties in TVMV, and work with TEV and clover yellow vein virus (ClYVV) demonstrated the existence of *cis* acting elements necessary for infectivity.

Properties and Functions of Gene Products

The polyprotein coded in the genome of a potyvirus comprises the following gene products (from N-terminus to C-terminus): P1, HC-Pro, P3, 6K1, CI, 6K2, NIaVPg, NIaPro, NIb, and CP. For bymoviruses, RNA-2 encodes two proteins P2-1 and P2-2, while RNA-1 encodes the remaining products starting with P3. Besides the information provided in Figure 3, some characteristics of these products are given in this section.

The P1 protein is a serine protease, extremely variable in size (about 30–60 kDa). In TEV, it was shown to act as an accessory factor for genome amplification. This region contains important sequence differences among potyviruses. In the ipomovirus cucumber vein yellowing virus (CVYV) two duplicated P1-like serine proteinases are found, with the second one acting as a suppressor of gene silencing to compensate for the absence of HC-Pro in this virus.

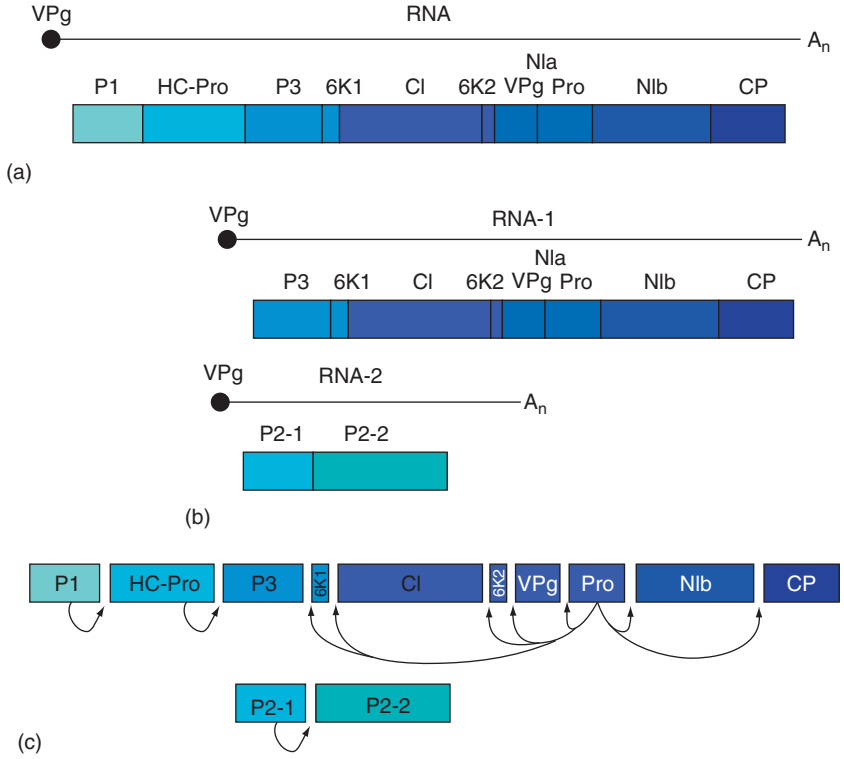
The helper component HC-Pro is a multifunctional protein originally described as a factor required for aphid transmission ('HC' in the name of the protein stands for 'helper component' and refers to this function). Recently it was shown that the HC-Pro of tritimoviruses is also implicated in mite transmission. A modular distribution of functions has been proposed in this product: the N-terminus is essential for vector transmission, the central region is involved in suppression of silencing, and the C-terminal part is a papain-like cysteine proteinase ('Pro' in the name of the protein stands for 'proteinase' and refers to this function). HC-Pro can interact with RNA, and recent data indicate that it binds to double-stranded small interfering RNA (siRNA) molecules, a feature mechanistically coincidental with other silencing suppressors of viral origin. Structural studies with lettuce mosaic virus (LMV) and TEV have demonstrated HC-Pro in pairwise oligomeric forms. Interaction of HC-Pro with several host factors has been described.

In the case of bymoviruses, very limited information regarding roles for P2-1 and P2-2 is available. It has been postulated that P2-2 might play a role in vector transmission, since nontransmissible variants exhibited deletions in this region. However, sequence alignments indicate that P2-1, a putative cysteine proteinase, is more closely related to HC-Pro than P2-2.

Except for P1 and HC-Pro, all gene products are excised from the polyprotein by the action of the proteinase domain of NIa (nuclear inclusion a). The third gene product is P3, a protein of unknown functions, which is followed by a peptide called 6K1. Despite the presence of a typical cleavage sequence, cleavage between P3 and 6K1 of TEV does not occur *in vitro*, and excision does not seem to be required for viability of PPV. Immunology studies found both P3 and the precursor P3-6K1 in infected cells, and the latter is perhaps the functional product, although recently a tagged 6K1 product was also found in infected plants. Products of the P3-6K1 region play roles in host range definition and pathogenicity of several potyviruses.

The CI protein is the largest potyviral gene product. It forms very distinctive pinwheel-shaped cylindrical inclusions in the cytoplasm of infected cells, with high taxonomic value because they are unique to members of the family *Potyviridae* (Figure 1(b)). CI exhibits RNA helicase activity, and is supposed to act during RNA replication. Host factors interacting with CI of PPV and TEV have been recently identified. A second small peptide, 6K2, follows CI. This product has been implicated in virus replication, probably through a proposed anchoring capability which may serve to retain the replication complex in virus-induced membrane structures in the cytoplasm. Separation of 6K2 from NIa was also proposed to regulate nuclear targeting, and recent results in PVA reveals roles for 6K2 in movement and symptom induction.

The NIa protein has about 49 kDa, although it is subjected to an internal suboptimal cleavage to originate an N-terminal VPg (21 kDa) and a C-terminal proteinase fragment (28 kDa). VPg is the protein covalently attached to the 5′ end of viral RNA. VPg can be uridylylated *in vitro* by the RNA replicase NIb, suggesting that it can be used as a primer during RNA replication as in the case of picornaviruses. VPg might be associated with host factors being a common pathogenicity determinant that also participates in long-distance movement. In PVA, VPg is translocated as a 'phloem protein' that specifically acts in companion cells to facilitate virus unloading. NIa is a



Protein	Size kDa	Properties and possible function(s)
P1	30–60	Serine proteinase S30. RNA binding. Pathogenicity and suppression of RNA silencing. Host range definition.
HC-Pro	56	Cysteine proteinase C6. RNA and siRNA binding. Vector transmission. Virus movement. Pathogenicity and suppression of RNA silencing.
P3 6K1	40 + 6	Pathogenicity. Host range definition. RNA replication? Membrane anchoring?
CI	70	RNA helicase. RNA binding. Cell-to-cell movement. RNA replication.
6K2	6	Membrane anchoring? RNA replication?
VPg Pro Nla	21 + 28	Covalently linked to RNA. Priming of RNA synthesis. RNA binding. Pathogenicity. Virus movement. Host range definition. Cysteine proteinase C4. Deoxyribonuclease.
Nlb	58	RNA replicase.
CP	30–36	RNA encapsidation. Vector transmission. Virus movement. Symptom determination.
P2-1	25–28	Cysteine proteinase C6.
P2-2	70	Vector transmission?

(d)

Figure 3 Representative genomic maps for viruses in the family *Potyviridae*. A monopartite virus characteristic of a potyvirus is shown in (a), while a bipartite bymovirus is depicted in (b). Genomic structures of ipomo-, maclura-, rymo-, and tritimoviruses are essentially similar to that of potyviruses. ssRNA genomes are shown as solid horizontal lines with VPgs represented by solid circles at each 5′ end, and polyA tails located at each 3′ end. Viral ORFs are depicted below as boxes divided in the different viral products, with names of gene products indicated. The proteolytic processing (rendering functional proteins or regulating their activity) of a typical potyvirus ORF is shown in (c), with indication of the three protease-specific cleavages sites indicated by arrows. Processing of the ORF encoded by the RNA-2 of bymoviruses is also presented. Properties of the different potyviral proteins are shown in (d). Abbreviation names, approximate size (kDa), and some properties and possible function(s) of each protein are indicated.

serine protease with a cysteine at the active site, responsible for the majority of cleavages in the polyprotein, acting in *cis* and *trans* at specific processing sites defined by characteristic heptapeptides. The protease domain of NIa shows nonspecific RNA binding activity, and was shown to exhibit nonspecific double-stranded DNA degradation activity in pepper vein banding virus (PVBV). NIa can form, together with NIb, inclusion bodies in the nucleus (hence the name of these proteins), where it is translocated responding to a bipartite signal sequence. The crystal structure of TEV NIa has recently been solved, providing clues on its mode of action.

NIb (nuclear inclusion b) is the second component of nuclear inclusions, being directed to the nucleus by specific signals in its sequence. NIb is the RNA-dependent RNA polymerase (RdRp) responsible for viral replication. Recruitment for the replication complex is postulated to occur via interaction with NIa. All NIb functions essential for RNA amplification may be provided in *trans* since NIb supplied in transgenic plants complement TEV deletion mutants.

The last product is CP, a protein with many roles in addition to the encapsidation of virus particles, being implicated in aphid transmission through interaction with HC-Pro, and in cell-to-cell and long-distance movement. The importance of maintaining the net charge of the CP N-terminus for infectivity has been shown in TVMV and zucchini yellow mosaic virus (ZYMV).

Replication and Propagation

Potyviruses replicate in the cytoplasm of infected cells, as schematically shown in Figure 4. After entering the cell, the viral genomic RNA must first be translated in the cytoplasm. In general, the early events during infection are still poorly understood. Disassembly of particles might

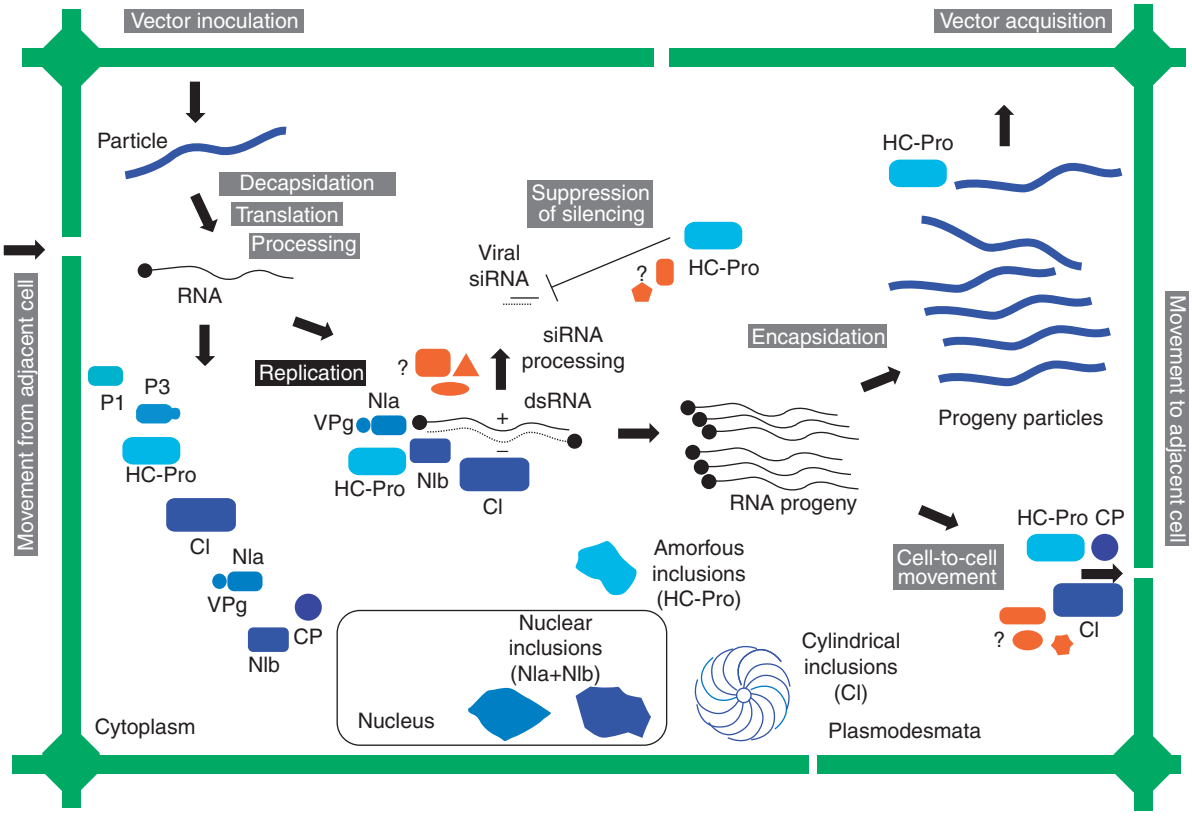


Figure 4 Schematic representation of different events during potyvirus infection of a plant cell. Colors and patterns of gene products match those displayed in Figure 3. The possible accumulation of viral proteins as inclusions in different compartments is also indicated. Putative host factors acting during the cycle are depicted as red color objects. The cycle begins (left upper corner) when the viral RNA enters the cell from an adjacent infected cell or a particle is initially inoculated by its vector and the genomic RNA undergoes decapsidation, translation, and processing to originate mature products. The replication complex is assembled with participation of NIb, CI, VPg, 6K2, and NIa, and probably other host-derived factors. The replication complex uses the genomic RNA (+ sense) to generate a complementary chain (− sense), which serves as a template for the synthesis of numerous genomic RNAs. Different mechanisms of plant defense might be activated during infection, including activation of the RNA silencing machinery that will produce virus-specific siRNA. A suppressor of RNA silencing, the HC-Pro in potyviruses, allows the virus to overcome this plant defense (indicated by the T-shaped symbol). After replication, the RNA progeny can move to adjacent cells through plasmodesmata, in a form not totally identified with the involvement of HC-Pro, CI, VPg, and CP, or it can be encapsidated and acquired by a vector organism to be transmitted again, in a process requiring HC-Pro.

occur co-translationally, and after translation the replication complex must be formed with participation of several viral products and probably host factors. This complex uses the genomic RNA (+ sense) as template to generate a complementary chain (− sense) through a dsRNA intermediate, and proceed with the asymmetric synthesis of numerous genomic RNAs. Virus specific siRNA accumulate during potyvirus infections, revealing the induction of a RNA silencing-mediated antiviral defense. Therefore, the virus needs to counterattack using a silencing suppressor, which in potyviruses was demonstrated to be the HC-Pro protein, although this function might be shared or displaced to other products in other genera. The need for several virus-encoded functions, including replication and silencing suppression, at very early stages of infection, points to the importance of viral RNA translation. Studies performed with pea seed-borne mosaic virus (PSbMV) served to identify a translation shut-off affecting many host proteins during infection. The potyvirus expression strategy through a polyprotein implies production of equimolar amounts of all gene products, giving rise to large excesses of some proteins which might remain soluble, be degraded or secreted, or end up in inclusion bodies. It is interesting that, whereas CI pinwheels are always formed, HC-Pro amorphous or NIa/NIb crystalline inclusions are present in some, but not all, potyviral infections.

Potyvirus RNA replication takes place in membranous structures probably derived mainly from the endoplasmic reticulum. NIb forms the core of the replication machinery, with involvement of CI, VPg, 6K2, and NIa. Other factors encoded by the virus or by the host might also be involved. For instance, NIa of turnip mosaic virus (TuMV) can interact with initiation factors eIF4E and eIF(iso)4E, and also with the PolyA binding protein. The fact that many potyvirus-specific resistance genes encode initiation factors points to the importance of these interactions.

Another essential process for virus infection is transport of the genomic RNA to adjacent cells. All tissues in the plant finally become invaded by the virus, with the probable exception of meristems, although a few seed-transmissible potyviruses might be capable of invading them. CP, but probably not virion formation, is essential for virus movement. In the process of local and systemic spread, again the silencing defense must be confronted by the virus in a race to avoid blockage by the transitivity diffusion of specific silencing signals.

The final step of the replicative cycle is spread of the virus to new plants, which rely on acquisition by the corresponding vector organisms and transmission. Two viral proteins, CP and HC-Pro, are involved in nonpersistent aphid transmission of potyviruses. Available data support a hypothesis in which HC-Pro serves as a reversible bridge to retain virus particles in aphid mouthparts. A conserved PTK domain in the central portion of HC-Pro could participate in binding to the DAG motif of the N-terminal region of CP, while a KITC domain at the N-terminal region of HC-Pro would be involved in binding to unknown structures of the aphid stylet. HC-Pro is also involved in semipersistent transmission of tritimoviruses by eryophid mites. Molecular information about the transmission of macluraviruses by aphids, bymoviruses by plasmodiophorids, rymoviruses by eryophid mites, and ipomoviruses by whiteflies is rather scarce.

Multifunctionality is observed in most potyviral proteins, illustrated, for instance, by HC-Pro. This capacity to participate in multiple processes suggests that potyvirus infection is not a consecutive succession of independent events, but a tightly regulated network of complex interactions between viral and host factors, still to be elucidated.

Pathogenicity

There is little information about how potyviruses cause diseases in their host plants. Several studies have served to identify sequences and/or products in the genome of potyviruses directly implicated in the production of symptoms. As mentioned above, symptom determinants are present in the 5′- and 3′-NCR. In addition, HC-Pro, P3, CI, 6K2, and VPg of different viruses have been described as determinants of pathogenicity, although single products were not always responsible for the different pathogenic responses.

As already mentioned, the P1-HC-Pro region is responsible for the synergistic effect in mixed infections of potyviruses with unrelated viruses, perhaps reflecting the capacity to interfere with RNA silencing-mediated defense. The discovery that HC-Pro is also able to affect miRNA regulated functions in plants provided a molecular explanation to some virus-induced symptoms. However, although it is tempting to regard interference with the metabolism of small RNAs in susceptible plants as a major element of pathogenicity, many other processes may also be affected. For example, specific features of the soybean mosaic virus (SMV) elicitor of the *Rsv1* resistance gene appears to be responsible for induction of either systemic mosaic or lethal systemic hypersensitive response in soybean. The fact that the HC-Pro protein of LMV targets and affects the proteasome might exemplify another way by which potyviruses can cause disease symptoms. Despite the fact that in most cases the mechanisms of pathogenicity remain uncertain, it is reasonable to conclude that the final macroscopic effects caused by potyviruses might be a combination of additive interference with several host functions.

Evolution

Members of the family *Potyviridae* are considered to belong to the picorna-like supergroup, characterized

by the same genome expression strategy, and by a well-conserved set of replication-related proteins present at equivalent positions in the genome, which could be the result of cassette evolution.

Genera in the family show a close relationship, as indicated by the homologies between gene products and their conserved order in the polyprotein. Members of each genus within the family seem to be adapted to specific vector organisms. Moreover, the adaptation to particular host species might have contributed to speciation.

One intriguing issue is the amazingly large number of aphid-transmitted potyviruses. A combination of a very efficient transmission system and easy adaptation to new hosts must have contributed to this large expansion. In particular, vector transmission acting as bottlenecks might lead to speciation events. Together with recombination, switching events, radiation, and host and geographical adaptation are proposed as major traits of evolution. Existence of subpopulations able to differentiate and evolve independently within a single infected perennial plant was observed in PPV, and similar phenomena can happen in epidemics of viruses affecting annual hosts. Experimental evidence in PVA showed that recombination of nearly identical, phenotypically similar virus genomes can give rise to new viral strains with novel virulence and symptom phenotypes. Indications of recombination events, partial duplications, point mutations that apparently confer different host responses, as well as other factors, might help to explain the extraordinary variability observed among potyviruses.

Epidemiology and Control

Strategies currently applicable for potyvirus control are diverse, from cultural practices to the use of genetic resistance. Insecticide treatments against vectors are frequently considered unsatisfactory and have limited use because of the transmission type.

Severity of outbreaks is commonly related to abundance of initial foci of infection, dynamics of vector populations, and other factors, such as the presence of weeds acting as reservoirs of viruses. Human intervention is responsible in many cases for the introduction of emerging diseases into new territories, while vector organisms are mainly involved in propagation within particular regions. In a few potyviruses, seed transmission is also an important means of dissemination. A typical example of well-documented spread of a potyvirus over a territory and over time is the progressive emergence of Sharka disease, caused by PPV, in European countries during the twentieth century, and its recent diffusion to other continents to become nowadays a global pandemic.

The exploitation of pathogen-derived resistance and RNA silencing is yielding promising results for potyvirus control. Transgenic plants incorporating viral sequences were found to be resistant to several potyviruses. An example of success is provided by the engineering of papaya varieties resistant to papaya ringspot virus (PRSV) by expression of a viral CP transgene. Nowadays, RNA silencing is being further exploited for resistance by designing specific transgenes with hairpin structures, or even by direct application of dsRNAs. Cross-protection has also been related to RNA silencing mechanisms. Other approaches which are being explored include expression of ribozymes, plantibodies or inhibitors of proteinases. Stability and biosafety issues are under evaluation in all strategies.

The identification of potyvirus-specific resistance genes is leading to important advances. Characterization of new systems suggested that canonical resistance genes might be operating, for instance TIR-NBS-LRR class in PVY, or NBS-LRR class in SMV. As mentioned, mutations in eukaryotic initiation factors are frequently found as the responsible factor for recessive resistance against potyviruses. The generation of variability in candidate genes, using TILLING or equivalent platforms, could, therefore, be a promising strategy for generation of resistant plants.

Biotechnological Applications

Biotechnological uses of potyviruses are being pursued, both for the expression of foreign sequences in plants, and as a source of genetic elements and products of potential biotechnological utility. In this second aspect, extensive use of the TEV NIa protease to remove affinity tags from fusion proteins is a good example.

Regarding the use of potyviruses as expression vectors, several systems have been tested. Small peptides can be expressed fused to the CP, allowing the chimeras to be used as antigen presentation systems for immunization or diagnosis. Complete foreign genes have been expressed in vectors based on a quite large list of potyviruses which covers an important range of potential hosts. The seminal work with TEV pointed to the P1-HC-Pro junction as an adequate insertion site, but later new sites were exploited, such as the NIb-CP junction, or the P1 region. Recent reports also demonstrated the capacity to use potyviruses as double vectors expressing simultaneously two foreign proteins. The stability of the inserted genes seems to respond to characteristics of the foreign sequence still not completely defined.

The understanding of RNA silencing phenomena and the discovery of viral suppressors can serve as a technological basis to boost expression based on the simultaneous expression of a replicating virus plus a silencing suppressor.

Transgenic plants expressing HC-Pro are being used to improve the expression levels of viral vectors.

Concluding Remarks

Analysis of potyvirus molecular biology has been extraordinarily successful in many aspects, and is likely to continue and provide interesting data. The knowledge currently available about potyvirus replication, movement, and transmission should eventually permit new control strategies to be designed to interfere with these key stages in the virus life cycle. Structure resolution of virus components might also serve to explore new resistance strategies. Diagnostic tools are continually being improved, and future developments will certainly supply specific and sensitive means of virus identification. Current understanding of the selective involvement of host factors in resistance and pathogenesis is fuelling work on virus–plant interactions, a challenging research area. Besides, peculiarities of particular viruses are being unravelled, and will help to understand both generic features and specificities of viruses in the family. Finally, the biotechnological application of potyviruses is also a field in expansion, which will provide in the future more exciting developments.

See also: Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus; Papaya Ringspot Virus; Plum Pox Virus; Potato Virus Y; Watermelon Mosaic Virus and Zucchini Yellow Mosaic Virus; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Antiviral Defense: Gene Silencing Pathway.

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Poxviruses

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Introduction

Poxviruses have been isolated from birds, insects, reptiles, marsupials, and mammals. The best known is variola virus (VARV), the cause of smallpox, an extinct disease that claimed millions of victims and influenced human history. All poxviruses have complex, enveloped virions that are large enough to be visible by the light microscope and contain double-stranded DNA (dsDNA) genomes with terminal hairpins linking the two DNA strands into a single polynucleotide chain. Poxvirus genes are transcribed by the virus-encoded RNA polymerase and associated transcriptional enzymes, which are packaged into the virion. Virus morphogenesis and entry have unique features, such as the possession of a thiol-oxidoreductase system to enable disulfide bond formation and morphogenesis in the cytoplasm, and a complex of several proteins for the fusion of infecting virions with the cell membrane. The large genome enables poxviruses to encode many virulence factors that are nonessential for virus replication in cell culture but which influence the outcome of infection *in vivo*. Diseases caused by poxviruses