Arch Virol 134 (1994)

VDN
Virology Division News

Trichovirus, a new genus of plant viruses

G. P. Martelli[1], **T. Candresse**[2], and **S. Namba**[3]

[1] Dipartimento di Protezione delle Piante, Università degli Studi and Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Bari, Italy
[2] Station de Pathologie Végétale, INRA, Villenave D'Ornon, France
[3] Experimental Farm, Faculty of Agriculture, University of Tokyo, Japan

Summary. The genus *Trichovirus* embraces five viral species (two definitive and three tentative) with similar biological, morphological, physicochemical, and ultrastructural properties. Viral replication is likely to occur in the cytoplasm, where virions accumulate in more or less loose bundles or paracrystalline aggregates. The genome is a 3' polyadenylated, positive-sense, single stranded RNA of 7.5–8.7 kb in size. In definitive species (apple chlorotic leaf spot and potato T viruses), the genome is constructed of three slightly overlapping open reading frames coding for replication-related proteins (ORF 1), a putative movement protein (ORF 2), and the coat protein (ORF 3), respectively. ORFs 2 and 3 are probably expressed through subgenomic RNAs. Grapevine virus A (GVA) and grapevine virus B (GVB), two tentative species, may express an extra small open reading frame at the 3' terminus, encoding, in the case of GVB, a polypeptide with homologies with the small RNA-binding protein of carlaviruses. The taxonomic relevance of this difference in genome organization remains to be ascertained.

Introduction

Apple chlorotic leaf spot virus (ACLSV) has particles resembling very much those of closteroviruses [13], which prompted its inclusion in the *Closterovirus* group (now genus) when it was first established [11]. However, differences in particle length, biological, physicochemical, and epidemiological properties, suggested to give ACLSV the status of possible rather than definitive member of the group [11, 15].

Capilloviruses also possess particles morphologically comparable to those of closteroviruses [14], but differ enough in other properties to deserve classification in a taxonomic groups of their own [2] (now genus *Capillovirus*, type species apple stem grooving virus, ASGV).

ACLSV was the first clostero-like virus whose complete genome sequence and structural organization was determined [9]. When molecular information on other viruses became available [1, 25], it became evident that distinct differences existed in genome properties and structure between ACLSV and both beet yellows virus (BYV), the type species of the *Closterovirus* genus and ASGV. These differences, which add up to those

involving biological and physicochemical properties, suggested the establishment of a new viral genus, denoted *Trichovirus* ("tricho" from Greek *thrix*, a hair) having ACLSV as type species, which was approved by ICTV at the 9th International Congress of Virology, Glasgow 1993.

A brief description of the genus *Trichovirus* follows.

Taxonomic structure of the genus

Currently, the *Trichovirus* genus embraces five species, two of which are definitive, i.e. ACLSV and potato virus T (PVT) [23], and three tentative, i.e. heracleum latent virus (HLV) [3], grapevine virus A (GVA) [7], and grapevine virus B (GVB) [4]. Differentiation between definitive and tentative species rests primarily on molecular information, which is complete for ACLSV and PVT [9, 17, 23, S. Namba, unpublished information], partial for GVA and GVB [16], and nil for HLV.

Biological properties

Trichoviruses infect dicotyledonous plants, and have a geographical distribution from wide to restricted, according to the species. ACLSV, GVA and GVB are probably worldwide, as they occur in most of the countries where their hosts are grown, whereas PVT was reported only from the Andean region of South America. The natural host range of individual species may be narrow (ACLSV), or restricted to a single host (PVT, HLV, GVA, GVB). Infections induce little or no symptoms (PVT, HLV, ACLSV in certain hosts), or mottling, rings and line patterns (ACLSV), or pitting and grooving of the wood (GVA, GVB).

All species are experimentally transmitted by inoculation of sap, some with difficulty (i.e. GVA), and by grafting. PVT is seed-transmitted in several hosts, including *Solanum* spp. [22]. Natural dissemination is mediated by propagative material in clonally propagated hosts and, with some viral species, by vectors. GVA and GVB are transmitted by the pseudococcid mealybugs *Pseudoccus* and *Planococcus* [4, 21], and HLV is transmitted semi-persistently by aphids with the assistance of the helper virus heracleum closterovirus 6 [3]. No natural vectors of ACLSV and PVT are known.

Some of the species (i.e. GVA and GVB) are phloem-restricted in the natural host, but invade parenchymas of artificially infected experimetal hosts, much the same as ACLSV and HLV [3, 4, 20, 21]. Virions accumulate in bundles or paracrystalline aggregates in infected cells, which produce vesicular evaginations of the tonoplast containing finely fibrillar material.

Morphological and physicochemical properties

Particles of all species are helically constructed flexuous filaments 640–800 × 12 nm in size, with a pitch of 3.3–3.5 nm and about 10 subunits per turn of the helix [24]. Customarily, virions show a distinct cross banding, but they may also exhibit criss-cross or rope-like features, according to the electron stain used.

In density gradient or analytical centrifugation virions sediment as single or two very close bands with sedimentation coefficient of 92–99. Particles of ACLSV and HLV are sensitive to ribonucleases, but resist moderately high temperatures (55–60°C) and organic solvents, much the same as those of other species.

The nucleic acid is a single molecule of linear, positive sense, single stranded RNA, accounting for c. 5% of the particle weight. It has a polyadenylated 3' terminus and a size of

2.2–2.5 × 10^6 daltons (7.5–8.7 kb). Indirect evidence suggests that the genomic RNA of ACLSV is capped at its 5' end.

Virions do not contain lipids nor carbohydrates. Their structural proteins consist of a single major polypeptide with Mr 22–27 × 10^3.

Antigenic power is moderate (ACLSV, PVT, HLV) to low (GVA, GVB). Species are not serologically interrelated.

Molecular properties

The viral genome of the definitive species ACLSV and PVT contains three slightly overlapping open reading frames (ORFs). The large 5' most ORF 1 codes for a protein of 180–220 kDa containing polymerase (GDD), nucleotide binding (helicase), and methyltransferase signature sequences typical of replication-associated proteins of the Alpha-like RNA plant viruses. ORF 2 encodes a polypeptide of 40–50 kDa with weak homologies to some plant virus movement proteins and ORF 3, which is located at the 3' terminus, is the coat protein cistron [9, 17, 23]. Whereas ORF 1 of ACLSV is directly expressed from genomic RNA, the two smaller dowstream ORFs are probably expressed from subgenomic messenger RNAs. ACLSV-infected tissues contain five dsRNA species. Three of these are 5' coterminal with genomic RNA and represent the double-stranded form of genomic RNA plus two additional very abundant species, with a size of 6.5 and 5.5 kbp, respectively. The remaining two are dsRNA forms of subgenomic RNAs [10].

The tentative species GVA and GVB have a small ORF located downstream of the coat protein cistron. This potentially encodes a polypeptide of 10–13 kDa with weak homologies with the 12–15 kDa product of carlaviruses [16], which has RNA-binding properties [8]. Tissues infected by GVA and GVB contain at least four dsRNAs with slightly different banding pattern [5].

Replication is supposed to be cytoplasmic and to involve the products of ORF 1.

Discussion

Virions of *Closterovirus* and *Capillovirus*, two of the currently recognized genera of plant viruses, exhibit the same flexuous and open particle structure that characterizes *Trichovirus* species.

At the present status of knowledge, it seems that particle morphology is the main single feature shared by all closteroviruses and trichoviruses. Similarities exist also in tissue tropism (most species of both genera are phloem-limited) and, to a lesser extent, in the type of vector and mode of transmission (some species in both genera are transmitted by mealybugs, or semi-persistently by aphids) [6]. However, striking differences occur in particle size, genome organization and structure of the 3' end. Levels of molecular similarity between proteins, both structural and non structural, of the few sequenced representatives of both genera are also very low [16, 17]. In fact, in a recent scheme of phylogenetic taxonomy based on conserved sequence motifs of viral proteins [12], ACLSV comes much closer to species of the *Carlavirus* and *Capillovirus* than of the *Closterovirus* genus.

Trichovirus and *Capillovirus* genera appear more closely related to one another because of: (i) comparable size of particles; (ii) presence of a polyadenylated 3' terminus; (iii) extensive sequence homology in the replication-associated and in the coat proteins [16–19, 23]. However, the alleged differences in genome organization and strategy of expression seem to support the establishment of two different taxa.

Most of the individual species of the *Trichovirus* genus are fairly well characterized biologically and physicochemically. The available information indicates that there is a certain amount of heterogeneity among them, for instance in the epidemiological behaviour. However, the greatest discrepancy resides in the structure of the genome as shown by the presence of an additional small ORF at the very 3' end of GVA and GVB.

The taxonomic relevance of this differential feature is difficult to assess at the moment. For a proper evaluation, it will be necessary to await the availability of more extensive molecular information on these and the other tentative species of the genus.

References

1. Agranowsky AA, Boyko VP, Karasev AV, Lunina NA, Koonin EV, Dolja W (1991) Nucleotide sequence of the 3' terminal half of beet yellows closterovirus RNA genome: unique arrangement of eight virus genes. J Gen Virol 72: 15–23
2. Bar-Joseph M, Martelli GP (1991) Capillovirus group. In: Francki RIB, Fauquet CM, Knudson DL, Brown F (eds) Classification and nomenclature of viruses. Fifth Report of the International Committee on Taxonomy of Viruses. Springer, Wien New York, pp 339–340 (Arch Virol [Suppl] 2)
3. Bem F, Murant AF (1980) Heracleum latent virus. CMI/AAB Descriptions of Plant Viruses, no. 228
4. Boscia D, Savino V, Minafra A, Namba S, Elicio V, Castellano MA, Gonsalves D, Martelli GP (1993) Properties of a filamentous virus isolated from grapevine affected by corky bark. Arch Virol 130: 109–120
5. Boscia D, Abou-Ghanem N, Saldarelli P, Minafra A, Castellano MA, Garau R, Savino V, Martelli GP (1993) A comparison of grapevine virus B isolates. Extended abstracts, 11th Meeting ICVG, Montreux 1993, Federal Research Station of Changins, Nyon, Switzerland, pp 27–28
6. Candresse T (1993) Closteroviruses and clostero-like elongated plant viruses. In: Webster RG, Granoff A (eds) Encyclopedia of virology. Academic Press, New York (in press)
7. Conti M, Milne RG, Luisoni E, Boccardo G (1980) A closterovirus from a stem pitting-diseased grapevine. Phytopathology 70: 394–399
8. Foster GD (1992) Restructure and expression of the genome of carlaviruses. Res Virol 143: 103–112
9. German S, Candresse T, Lanneau M, Huet JC, Pernollet JC, Dunez J (1990) Nucleotide sequence and genomic organization of apple chlorotic leaf spot closterovirus. Virology 179: 104–112
10. German S, Candresse T, Le Gall O, Lanneau M, Dunez J (1992) Analysis of dsRNAs of apple chlorotic leaf spot virus. J Gen Virol 73: 767–773
11. Harrison BD, Finch JT, Gibbs AJ, Hollings M, Shepherd RJ, Valenta V, Wetter C (1971) Sixteen groups of plant viruses. Virology 45: 356–363
12. Koonin EV, Dolja VV (1993) Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. Crit Rev Biochem Mol Biol (in press)
13. Lister RM (1970) Apple chlorotic leaf spot virus. CMI/AAB Descriptions of Plant Viruses, no. 30
14. Lister RM (1970) Apple stem grooving virus. CMI/AAB Descriptions of Plant Viruses, no. 31
15. Martelli GP, Bar-Joseph M (1991) Closterovirus group. In: Francki RIB, Fauquet CM, Knudson DL, Brown F (eds) Classification and nomenclature of viruses. Fifth Report of the International Committee on Taxonomy of Viruses. Springer, Wien New York, pp 345–347 (Arch Virol [Suppl] 2)
16. Minafra A, Saldarelli P, Grieco F, Martelli GP (1994) Sequence of the 3' terminal region of grapevine virus A and grapevine virus B (in prep.)
17. Ochi M, Kashiwazaki S, Hiratsuka K, Namba S, Tsuchizaki T (1992) Nucleotide sequence of the 3' terminal region of potato virus T RNA. Ann Pytopathol Soc Japan 58: 416–425
18. Ohira K, Ito T, Kawai A, Namba S, Kusumi T, Tsuchizaki T (1993) Nucleotide sequence of the 3' terminal region of citrus tatter leaf virus RNA. Virus Genes (submitted)
19. Ohira K, Namba S, Kawai A, Ito T, Kusumi T, Tsuchizaki T (1993) Nucleotide sequence of citrus tatter leaf capillovirus genome. Virus Genes (submitted)
20. Ohki ST, Yoshikawa N, Inouye N, Inouye T (1989) Comparative electron microscopyof *Chenopodium quinoa* leaves infected with apple chlorotic leaf spot, apple stem grooving and citrus tatter leaf virus. Ann Phytopathol Soc Japan 55: 245–249
21. Rosciglione B, Castellano MA, Martelli GP, Savino V, Cannizzaro G (1983) Mealybug transmission of grapevine virus A. Vitis 22: 331–347
22. Salazar LF, Harrison BD (1978) Potato virus T. CMI/AAD Descriptions of Plant Viruses, no. 187

23. Sato K, Yoshikawa N, Takahashi T (1993) Complete nucleotide sequence of the genome of an apple isolate of apple chlorotic leaf spot virus. J Gen Virol 74: 1927–1931
24. Tollin P, Wilson HR, Roberts IM, Murant AF (1992) Diffraction studies of the particles of two closteroviruses: heracleum latent virus and heracleum virus 6. J Gen Virol 73: 3045–3048
25. Yoshikawa N, Sasaki E, Kato M, Takahashi T (1992) The nucleotide sequence of apple stem grooving capillovirus genome. Virology 191: 98–105