Arch Virol (2006) 151: 1973–1983
DOI 10.1007/s00705-006-0767-2

Archives of Virology
Printed in Austria

The role of the C-terminal region of olive latent virus 1 coat protein in host systemic infection

V. Pantaleo, F. Grieco, A. Di Franco, and **G. P. Martelli**

Istituto di Virologia Vegetale del CNR,
Sezione di Bari and Dipartimento di Protezione delle
Piante e Microbiologia Applicata, Università degli Studi, Bari, Italy

Received January 27, 2006; accepted March 13, 2006
Published online May 15, 2006

Summary. A full-length cDNA clone of olive latent virus 1 (OLV-1), a member of the genus *Necrovirus*, family *Tombusviridae*, was subjected to site-directed mutagenesis, and coat protein gene mutants were constructed. A mutant clone, denoted Δ3297, was obtained by deleting the nucleotide in position 3297, thus inducing a frameshift and replacing the last 49 amino acids of the viral coat protein (CP) by a shorter sequence of 39 amino acids. This mutant was viable, stable, able to synthesize a smaller CP, and able to give rise to the formation of apparently intact virus particles. Cell-to-cell movement of Δ3297 in *Nicotiana benthamiana* leaves was not affected, but, contrary to wild type OLV-1, it failed to spread systemically. These results indicate that virion formation is necessary but not sufficient for long-distance movement for OLV-1 and highlights the role of the CP carboxy-terminal domain in systemic infection.

Introduction

Systemic host infection by plant viruses is characterized by an initial slow movement of infectious elements from cell to cell in the inoculated leaf, followed by their rapid long-distance transfer through the vascular system. Whereas virus-encoded movement proteins (MP) are well-established determinants for cell-to-cell movement of infectivity through plasmodesmata [2], long-distance transport is not equally well understood.

Except for a few cases [31, 32, 35], viral coat protein (CP) has been shown to be essential for efficient long-distance movement of positive-strand RNA viruses. To this effect, a functional CP and/or the formation of stable virions are required, as shown for sobemoviruses [1], cucumoviruses [39], tobamoviruses [12, 33], alfamoviruses [38], potyviruses [8], and dianthoviruses [41]. Functional CP

may interact with other long-distance movement viral factors as suggested for potexviruses [22], carmoviruses [17], bromoviruses [40] and comoviruses [3]. Furthermore, regulation of systemic spread needs an interaction between viral CP and host factors as suggested for tobamoviruses [18, 21] and bromoviruses [28, 29], and for tombusviruses [6].

Olive latent virus 1 (OLV-1), a member of the genus *Necrovirus* (family *Tombusviridae*) recovered from symptomless olive trees in southern Italy and later reported from Jordan, Turkey, and Portugal [25, 11], has isometric particles *c*. 30 nm in diameter and a monopartite, positive sense ssRNA genome 3699 nt in size [14]. Viral RNA contains five open reading frames (ORFs). ORF1 encodes replication-related proteins, i.e. a 23-kDa protein ending with an amber codon, whose read-through yields an 82-kDa product (ORF2). ORF3 and ORF4 encode two small polypeptides of 8 kDa and 6 kDa, respectively, involved in cell-to-cell movement [5], and ORF5 encodes the 30-kDa coat protein (CP).

Characterisation of OLV-1 CP mutants has shown that for long-distance movement this virus requires a functional CP [30], but it was not ascertained if encapsidation was necessary for systemic viral infection. A mutant clone of OLV-1 was therefore produced by deleting a single nucleotide at the 3′end of OLV-1 genomic sequence, whereby the C-terminal portion of the CP cistron was substituted for by a slightly shorter amino acid sequence.

In the present paper, we describe the biological features of this mutant and we discuss the possible implication of the C-terminal region of OLV-1 CP in long-distance movement of the virus.

Materials and methods

Virus purification and viral RNA extraction

The OLV-1 isolate, the same used in previous studies [5, 14, 30], was propagated in *Nicotiana benthamiana* and purified as described [24]. Nucleic acid was extracted from purified virus preparations [14] and analysed by electrophoresis in 1.2% low-melting-point agarose. After staining with ethidium bromide, the band corresponding to genomic RNA was excised and eluted from the gel [34].

Preparation of the mutant clone

The full-length clone pMUC-OLV [30] was modified by site-directed mutagenesis according to Kunkel et al. [19], using a Mutagenesis Kit (Bio-Rad, Hercules, CA, USA) and following the manufacturer's instructions, except that the *ung*$^-$ and *ung*$^+$ *E. coli* strains were RZ1032 and TG1, respectively.

A mutant was generated using the mutagenic primer 5′-CACCTTTCTGGCATGCACCA ACATCC-3′, spanning nt 3285 to 3312 of the genomic sequence, where a C residue present in the wild-type sequence between the underlined nucleotides was deleted. The mutation introduced into pMUC-OLV restriction fragments was confirmed by sequencing before subcloning back into the full-length clone [15]. The newly obtained mutant clone was denoted pMUC-Δ3297.

In vitro transcription and inoculation of plant and protoplasts

Run-off transcription of pMUC-OLV or pMUC-Δ3297 was done by adding the *Sac*II-digested DNA to a reaction mixture (100 μl) containing 40 mM Tris–HCl, 6 mM $MgCl_2$, 40 mM spermidine, 10 mM dithiothreitol, 100 mM HPRI (Amersham, UK), 500 μM of each ribonucleotide, and 100 units of T7 RNA polymerase and incubating at 37 °C for 90 min. The transcription mixture was diluted 1:1 with inoculation buffer [16] and inoculated to *Vigna unguiculata* or *N. benthamiana* plants (10 μl per leaf). Protoplasts were prepared from *N. benthamiana* leaves as described by Nagy and Maliga [26], transfected with viral or *in vitro* transcribed RNA, and cultured under continuous light for 24 h.

RNA and Western blot analysis

Total RNA was isolated from leaf tissue and protoplasts as described by White and Kaper [43] and Dalmay et al. [6], respectively. Northern blot analysis was done according to Sambrook et al. [34], using as a probe a restriction fragment 1043 bp in size obtained by digesting pMUC-OLV with *Eco*RI and *Hin*dIII and cloning in the transcription vector pSPT19 (Boehringer, Mannheim). One microgram of this recombinant clone, denoted pSPTOLV, was linearised with *Eco*RI and *in vitro* transcribed with T7 RNA polymerase. Riboprobes labelled with ^{32}P-UTP (800 Ci/mM, Amersham) were generated with the SP6/T7 Transcription Kit (Boehringer, Mannheim) according to manufacturer's instructions. Detection in infected plant tissues, of pMUC-OLV transcripts (denoted OLV-1 wild type) and of mutant Δ3297 RNAs was done by RT-PCR on total RNA extracts as described [24].

For Western blotting, tissue pieces (100 to 200 mg) from inoculated and uninoculated leaves were homogenized in 10 volumes of Laemmli buffer [20]. Samples were boiled for 3 min, and the insoluble material was removed by centrifugation at 15,000 rpm for 2 min. Solubilized proteins were resolved by 15% SDS-PAGE, blotted, and exposed to a polyclonal antiserum to OLV-1 [24] at a dilution 1:500. Visualization of antigen-antibody complexes was done using the ECL Western Blotting Detection System (Amersham, UK) following manifacturer's instructions.

Electron microscopy

Particles of OLV-1 wild type and of mutant Δ3297 were negatively stained with 2% aqueous uranyl acetate and decorated using a polyclonal antiserum to OLV-1, diluted 1:500 [24], before observation with a Philips Morgagni 282D electron microscope.

For thin sectioning, tissue pieces were excised from local lesions in *N. benthamiana* leaves four days post inoculation (d.p.i.) with OLV-1 wild type or mutant Δ3297, and at 12 d.p.i. from upper uninoculated leaves. Tissues were processed in the cold (4 °C) according to standard procedures [22], i.e. fixation in 4% glutaraldehyde in 0.05 M phosphate buffer, post-fixation in osmium tetroxide for 2 h, staining overnight in 2% uranyl acetate, dehydration in graded ethanol, and embedding in Spurr's medium. Thin sections were stained with lead citrate prior to observation. The same procedure was used for immunogold labelling (IGL), except that osmium post-fixation was omitted and embedding was done in London White resin. IGL was carried out essentially as described by [4], using an antiserum to OLV-1 diluted 1:500 and a preparation of 15 nm colloidal gold (Amersham, UK) conjugated with goat anti-rabbit antibodies. Controls consisted of tissue pieces from healthy *N. benthamiana* processed as the infected samples.

Results

Properties of mutant Δ3297

OLV-1 CP is organised in three distinct domains: R (random, from aa 1 to 55), a (arm, from aa 56 to 79), and S (shell, from aa 80 to 270) [14]. Mutant Δ3297 was obtained by deleting the cytosine residue in position 3297 of OLV-1 sequence. This deletion induced a frameshift in the CP gene coding sequence, resulting in the replacement of the last 49 aa of viral CP with 39 aa (Fig. 1). The newly produced CP had a M_r of 29,025 Da versus 29,918 Da for wild-type CP.

V. unguiculata plants inoculated with Δ3297 and wild-type OLV-1 RNAs developed identical necrotic local lesions and did not show any systemic symptoms. Moreover, Northern blot analysis of *N. benthamiana* protoplasts transfected with *in-vitro*-synthesised transcripts, showed that, 24 h after transfection, mutant Δ3297 RNA accumulated in an amount comparable to that of OLV-1 wild type (Fig. 2A, lanes 1 and 2), thus suggesting that CP modification induced in mutant Δ3297 apparently did not affect infectivity or viral accumulation in plant cells.

However, when mutant Δ3297 was inoculated to 4-week-old *N. benthamiana* seedlings, it induced chlorotic local lesions similar to those induced by wild-type OLV-1 RNA, but no systemic symptoms. A Northern blot assay of total nucleic acids (TNAs) extracted from these seedlings showed that viral RNA was present only in the inoculated leaves (not shown).

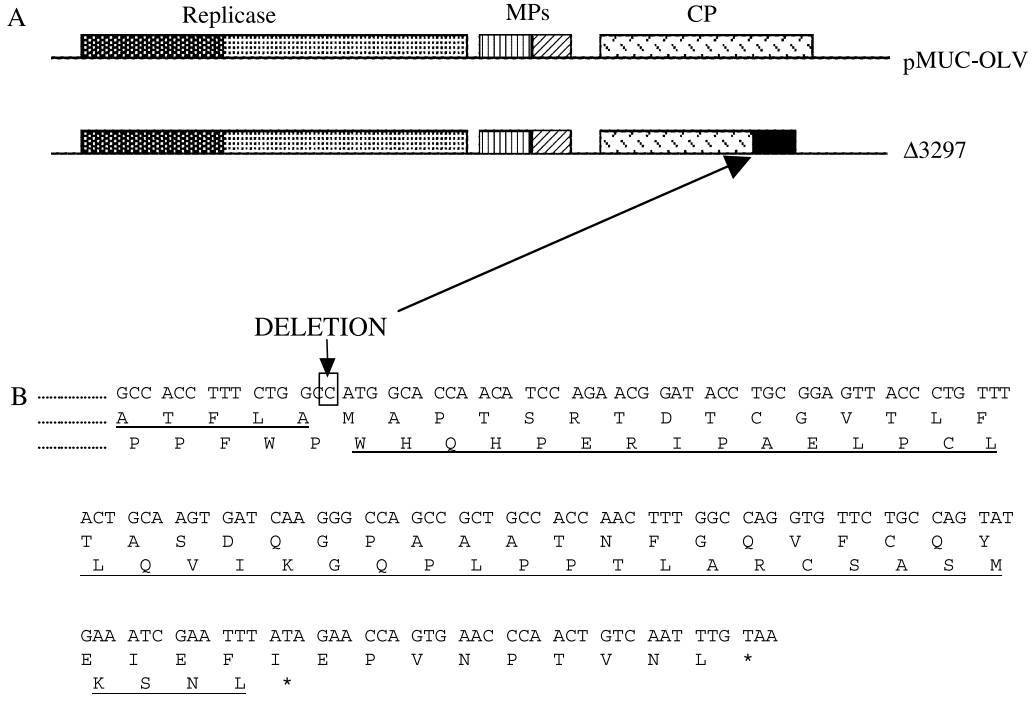

Fig. 1. Effect of the deletion of a C residue in position 3297 of the OLV-1 genome. **A** Genome organisation of OLV-1 and Δ3297 mutant. **B** Frameshift and sequence of the mutated CP 3′ terminus (underlined)

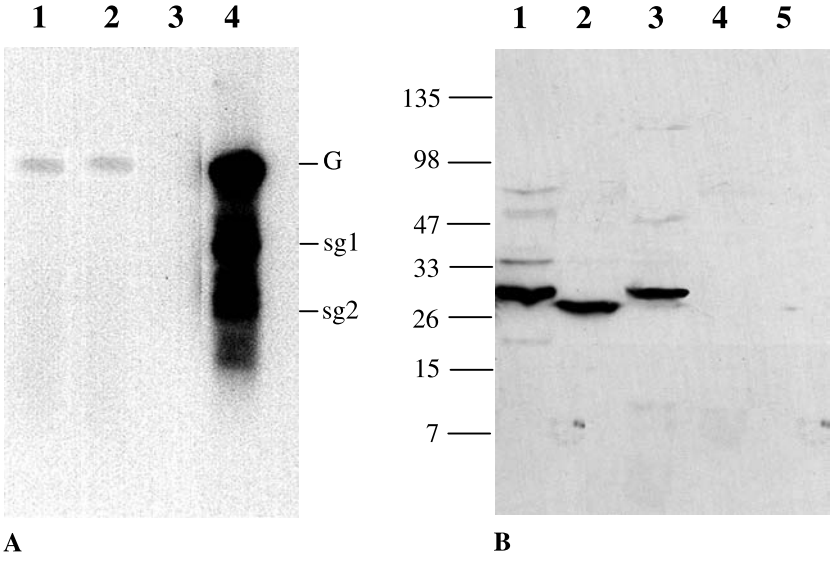

Fig. 2. A Northern blot analysis of total nucleic acids extracted from *N. benthamiana* protoplasts inoculated with pMUC-OLV (*1*), Δ3297 (*2*). Healthy control in *3*. TNA preparation from OLV-1-infected *N. benthamiana* plant in *4*. Hybridization was done with an OLV-1 RNA-specific riboprobe. G, sg1 and sg2 indicate the positions of genomic and subgenomic RNAs, respectively. **B** Western blot analysis of total protein extracted from *N. benthamiana*. Leaves infected locally (*1*) and systemically (*3*) with OLV-1 wild type. Leaves locally inoculated with Δ3297 (*2*) and upper non-inoculated leaves (*4*). Mock-inoculated leaves in *5*. Molecular weight markers are on the left

To exclude possible systemic movement of Δ3297 RNA, which would not have been detected by Northern blot analysis, *N. benthamiana* leaves inoculated with the mutant and the upper uninoculated leaves were analysed 8 d.p.i. by RT-PCR. Mutant Δ3297 was detected only in inoculated leaves, whereas wild-type OLV-1 RNA accumulated in both types of leaves (not shown). Furthermore, no symptoms developed in *V. unguiculata* leaves inoculated with sap from upper leaves of *N. benthamiana* locally inoculated with Δ3297, thus supporting RT-PCR results.

Western blot analysis of total protein extracts from tissues of inoculated and uninoculated *N. benthamiana* leaves showed that, contrary to OLV-1 wild type (Fig. 2B, lanes 1 and 3), Δ3297 CP was present only in inoculated leaves (Fig. 2B, lanes 2 and 4).

To ascertain whether a possible assembly defect of mutant Δ3297 could be the cause of its failure to spreading systemically, sap extracted from Δ3297-inoculated *N. benthamiana* leaves was incubated at 25 °C for 2 h in the presence of RNaseA (1 μg), then used to inoculate *V. unguiculata* plants. As a control, untreated sap was inoculated immediately. The number of local lesions developed at 4 d.p.i. in leaves inoculated with both samples was comparable and was about 10 times lower than that elicited by wild-type inoculum.

The stability of the Δ3297 mutant was verified by ten successive passages through *N. benthamiana.* TNAs were extracted after each subculture and subjected

to RT-PCR, and the amplicon containing the mutation site was sequenced. No revertant progeny was detected, indicating that the induced mutation was stable.

Electron microscopy

The presence of apparently intact virus particles was ascertained in dips from *N. benthamiana* leaves locally inoculated with mutant Δ3297 but not from the uninoculated upper leaves of the same plant. These virus particles apparently did not differ morphologically from those of OLV-1 wild type and were uniformly decorated by a polyclonal antiserum to OLV-1 (Fig. 3A and B).

The cytopathology of thin-sectioned *N. benthamiana* leaves locally and systemically infected with OLV-1 wild type did not differ from what has already

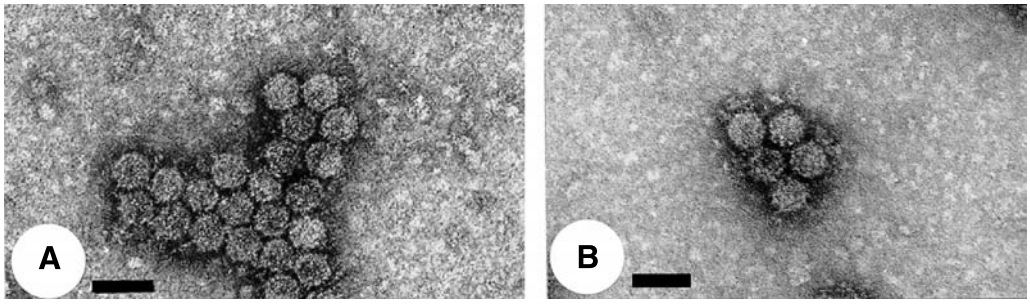

Fig. 3. OLV-1 wild type (**A**) and mutant Δ3297 (**B**) particles decorated with a polyclonal antiserum to OLV-1. Bars = 50 nm

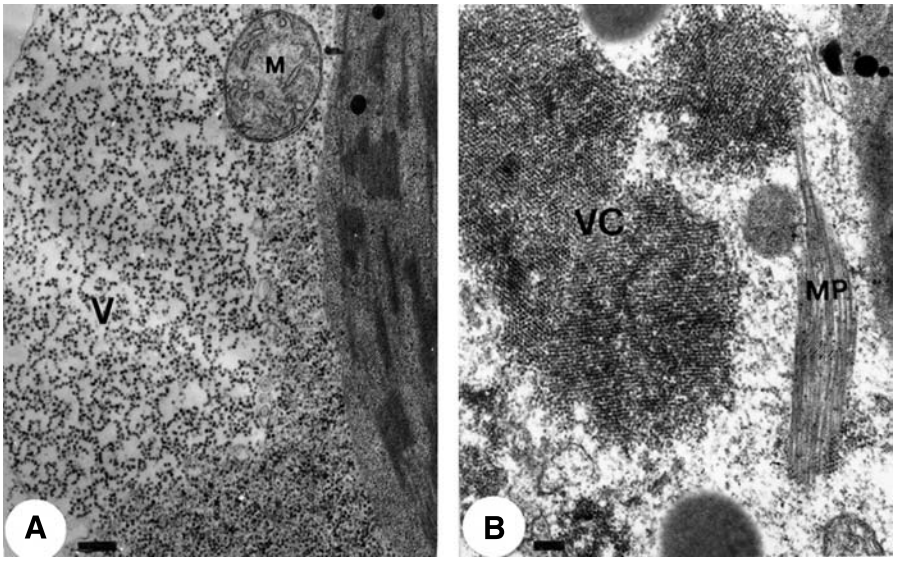

Fig. 4. Thin-sectioned *N. benthamiana* leaves locally (**A**) and systemically (**B**) infected with OLV-1 wild type. Virus particles scattered (*V*) or in crystalline aggregates (*VC*) are plentiful in both types of leaves. *M*, mitochondrion; *MP*, movement protein. Bars = 200 nm

been reported by Castellano et al. [4]. Cell architecture and major organelles were fairly well preserved, virus particles were plentiful and scattered in localized infections (Fig. 4A) but were frequently arranged in crystalline aggregates

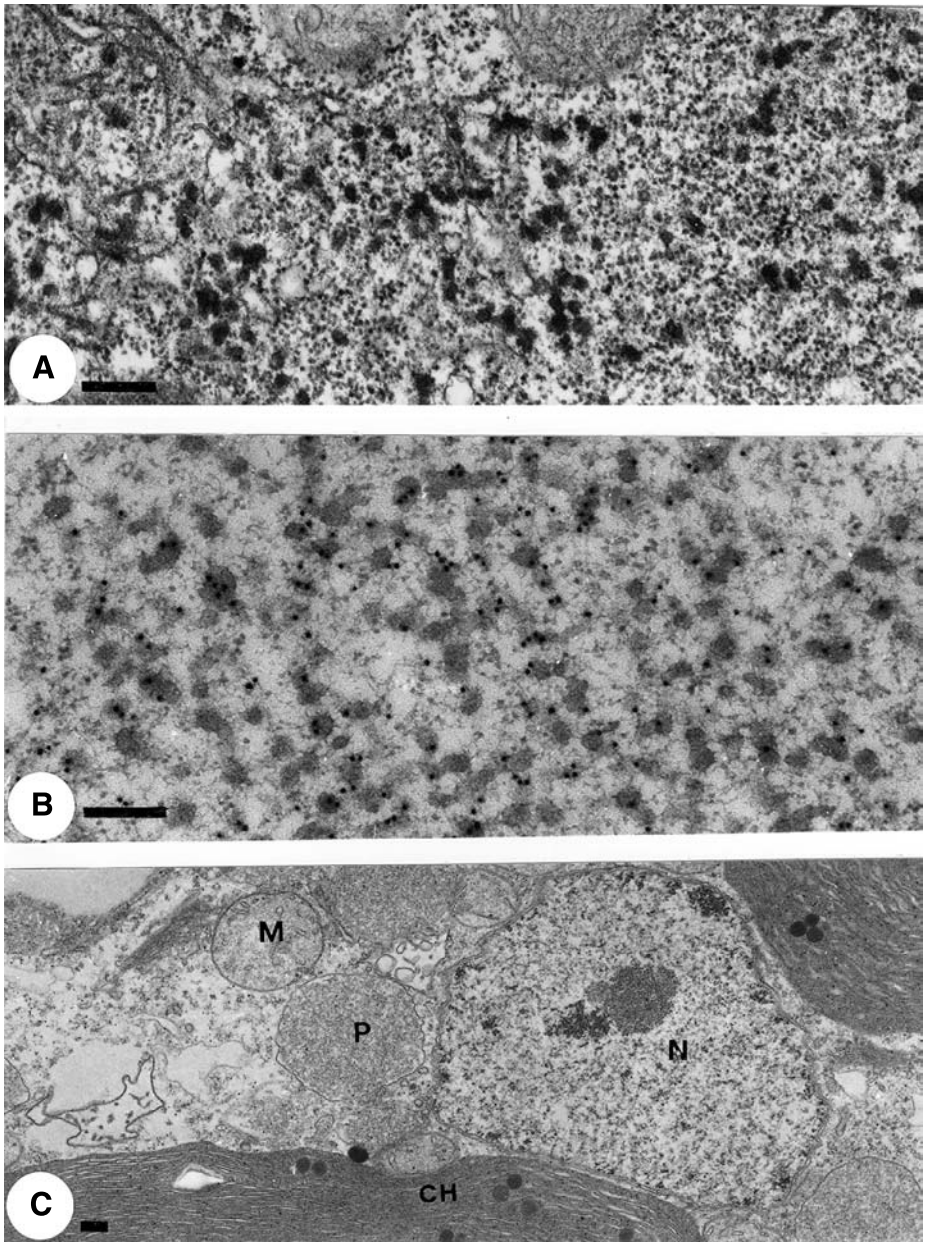

Fig. 5. Thin-sectioned *N. benthamiana* leaf locally inoculated with Δ3297. Virus particles in the ground cytoplasm are intermingled with clumps of electron-dense material (**A**) consisting of aggregated viral CP, as shown by gold immunolabelling (**B**). **C** Thin-sectioned cell from an upper uninoculated *N. benthamiana* leaf, showing a normal cytology and absence of virus particles or CP clumps. *M*, mitochondrion; *P*, peroxisome; *N*, nucleus; *CH*, chloroplast. Bars = 200 nm

in systemic infections (Fig. 4B). The same cells often contained fibrous inclusions made up of aggregates of a protein recently identified by Castellano et al. [5] as the 8K movement protein expressed by ORF 3 of OLV-1 genome (Fig. 4B).

The cytology of tissues locally infected with mutant Δ3297 did not differ much from the above except for an apparently lower amount of virus particles and the plentiful presence of clumps of electron-dense amorphous material (Fig. 5A) that was tagged by colloidal gold (Fig. 5B). Comparable material had previously been observed in cells infected by other members of the family *Tombusviridae* and identified as excess viral CP by De Stradis et al. [7] and references therein. Upper uninoculated leaves had a normal cytology and did not contain virus particles or CP clumps (Fig. 5C)

Infectivity of N. benthamiana cortical tissues above the inoculation point

The lack of systemic movement of mutant Δ3297, rather than to its inability to enter the vascular system, could depend on its failure to exit from it. To verify if this were the case, the infectivity of cortical tissues of *N. benthamiana* seedlings above the point of inoculation was tested with OLV-1 wild type and Δ3297. When extracts from cortical shavings of stem internodes were used to inoculate *V. unguiculata*, local lesions developed only in plants inoculated with sap from OLV-1 wild-type-infected *N. benthamiana*. This was taken as an indication that no virus was present in the vascular system of Δ3297-infected plants.

Discussion

Knowledge of the mechanisms underlying systemic spreading of viruses in infected hosts is limited, and much is still to be elucidated about how viruses penetrate, move through, and exit from the vascular system and, in particular, how viral CP, whether in a disassembled form or assembled in virions, mediates this trafficking.

CP expression is required for systemic movement of many plant viruses. So far, evidence of CP dispensability for long distance transfer has been obtained for a limited number of viruses such as tobacco rattle virus (TRV) barley stripe mosaic virus (BSMV), some bipartite geminiviruses (see for review [36]), and groundnut rosette virus (GRV) [32]. For tombusviruses, CP dispensability appears to be a more complex phenomenon depending on the host plant species [6, 35] and, for OLV-1, CP is required for systemic spread in *N. benthamiana* [30].

The OLV-1 capsid is constructed like that of tobacco necrosis virus (TNV), the type member of the genus *Necrovirus*, which has a CP with a surface-oriented S domain, constituting one of the two protruding domains of the particle [27]. This is likely not the case with mutant Δ3297, because its CP differs from that of OLV-1 wild type in the amino acid sequence of the S domain (Fig. 1). Notwhistanding this modification, Δ3297 was stable and infectious as OLV-1 wild type to *N. benthamiana* plants, in which it produced apparently normal particles in locally inoculated tissues, but failed to spread systemically.

Thus, the C-terminal domain of OLV-1 CP does not seem to be essential for virus particle assembly but its modification abolishes systemic virus spread in the host. In this, OLV-1 behaves like RCNMV [37], tobacco etch virus [10], and brome mosaic virus [28].

It seems, then, plausible to conclude that the carboxy-terminal portion of OLV-1 capsid plays a pivotal role in the host invasion process and that virion formation is not sufficient for long-distance movement in *N. benthamiana*, likely because this domain is exposed on the external surface of the virion, and it may be necessary for vascular transport. Modifications of this region, which still allow virion production, can abolish viral long-distance movement [10, 28, 37]

The presence of viral CP-specific receptors in *N. benthamiana* phloem tissues and, more generally, the involvement of host factors in long-distance viral transport, have been hypothesised in several cases [8, 13, 18, 28, 29, 42]. However, it remains to be established whether the inability of Δ3297 to spread systemically depends on the fact that the mutated C-terminal portion of its CP lacks these determinants or on other undetermined factors.

References

1. Brugidou C, Holt C, Yassi MN, Zhang S, Beachy R, Fauquet C (1995) Synthesis of an infectious full-length cDNA clone of rice yellow mottle virus and mutagenesis of the coat protein. Virology 206(1): 108–115
2. Carrington JC, Kasschau KD, Mahajan SK, Schaad MC (1996) Cell-to-cell and long distance transport of viruses in plants. Plant Cell 8: 1669–1681
3. Carvalho CM, Wellink J, Ribeiro SG, Goldbach RW, Van Lent JW (2003) The C-terminal region of the movement protein of Cowpea mosaic virus is involved in binding to the large but not to the small coat protein. J Gen Virol 84: 2271–2277
4. Castellano MA, Di Franco A, Martelli GP (1987) Electrom microscopy of two olive viruses in host tissues. J Submicr Cytol 19: 494–508
5. Castellano MA, Loconsole G, Grieco F, Di Sansebastiano GP, Martelli GP (2005) Subcellular localization and immunodetection of movement proteins of olive latent virus 1. Arch Virol 150(7): 1369–1381
6. Dalmay T, Rubino L, Burgyan J, Kollar A, Russo M (1993) Functional analysis of cymbidium ringspot virus genome. Virology 194: 697–704
7. De Stradis A, Redinbaugh MG, Abt JJ, Martelli GP (2005) Ultrasctructure of maize necrotic streak virus infections. J Plant Pathol 87: 213–221
8. Ding X, Shintaku MH, Carter SA, Nelson RS (1996) Invasion of minor veins of tobacco leaves inoculated with tobacco mosaic virus mutants defective in phloem-dependent movement. Natl Acad Sci USA 93: 11155–11160
9. Dolja VV, Haldeman R, Robertson NL, Dougherty WG, Carrington JC (1994) Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus in plants. EMBO J 13(6): 1482–1491
10. Dolja VV, Haldeman-Cahill R, Montgomery AE, Vandenbosch KA, Carrington JC (1995) Capsid protein determinants involved in cell-to-cell and long distance movement of tobacco etch potyvirus. Virology 206: 1007–1016
11. Felix MR, Cardoso JMS, Oliveira S, Clara MIE (2005) Viral properties, primary structure and phylogenetic analysis of the coat protein of an Olive latent virus 1 isolate from *Olea europaea* L. Virus Res 108: 195–198

12. Fuentes AL, Hamilton RI (1993) Failure of long-distance movement of southern bean mosaic virus in a resistant host is correlated with lack of normal virion formation. J Gen Virol 74: 1903–1910
13. Gilbertson RL, Lucas WJ (1996) How do viruses traffic on the "vascular highway"? Trends Plant Sci 1: 260–268
14. Grieco F, Dell'Orco M, Martelli GP (1996) The nucleotide sequence of RNA1 and RNA2 of olive latent virus 2 and its relationships in the family Bromoviridae. J Gen Virol 77: 2637–2644
15. Grieco F, Savino V, Martelli GP (1996) Nucleotide sequence of the genome of citrus isolate of olive latent virus 1. Arch Virol 141: 825–838
16. Heaton LA, Carrington JC, Morris TJ (1989) Turnip crinkle virus infection from RNA synthetised in vitro. Virology 170: 214–218
17. Heaton LA, Lee TC, Wei N, Morris TJ (1991) Point mutations in the turnip crinkle virus capsid protein affect the symptoms expressed by *Nicotiana benthamiana*. Virology 183: 143–150
18. Hilf ME, Dawson WO (1993) The tobamovirus capsid protein functions as a host-specific determinant of long-distance movement. Virology 193: 106–114
19. Kunkel TA, Roberts JD, Zakour RA (1987) Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc Natl Acad Sci USA 82: 488–492
20. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
21. Li Y, Wu MY, Song HH, Hu X, Qiu BS (2005) Identification of tobacco protein interacting with tomato mosaic virus coat protein and facilitating long-distance movement of virus. Arch Virol 150(10): 1993–2008
22. Lough TJ, Emerson SJ, Lucas WJ, Forster RL (2001) Trans-complementation of long-distance movement of White clover mosaic virus triple gene block (TGB) mutants: phloem-associated movement of TGBp1. Virology 288(1): 18–28
23. Martelli GP, Russo M (1984) Use of thin sectioning for the visualization and identification of plant viruses. Methods Virol 8: 143–224
24. Martelli GP, Yilmaz MA, Savino V, Baloglou S, Grieco F, Güldür MA, Boscia D, Greco N, Lafortezza R (1996) Properties of a citrus isolate of olive latent virus 1, a seemingly new necrovirus. Eur J Plant Pathol 102: 527–536
25. Martelli GP (1999) Infectious diseases and certification of olive: an overview. EPPO Bull 29: 127–134
26. Nagy JI, Maliga P (1976) Callus induction and plant regeneration from mesophyll protoplasts of Nicotiana sylvestris. Z Pflanzenphysiol 78: 453–455
27. Oda Y, Saeki K, Takahashi Y, Maeda T, Naitow H, Tsukihara T, Fukuyama K (2000) Crystal structure of tobacco necrosis virus at 2.25 A resolution. J Mol Biol 300: 153–169
28. Okinaka Y, Mise K, Suzuki E, Okuno T, Furusawa I (2001) The C terminus of brome mosaic virus coat protein controls viral cell-to-cell and long-distance. J Virol 75: 5385–5390
29. Okinaka Y, Mise K, Okuno T, Furosawa I (2003) Characterization of a Novel Barley Protein, HCP1, That Interacts with the Brome mosaic virus Coat Protein. Mol Plant Microbe Interact 16: 352–359
30. Pantaleo V, Grieco F, Castellano MA, Martelli GP (1999) Synthesis of infectious transcripts of olive latent virus 1: genes required for RNA replication and virus movement. Arch Virol 144: 1071–1079
31. Petty IT, Jackson AO (1990) Mutational analysis of barley stripe mosaic virus RNA beta. Virology 179: 712–718

32. Ryabov EV, Robinson DJ, Taliansky ME (1999) A plant virus-encoded protein facilitates long-distance movement of heterologous viral RNA. Proc Natl Acad Sci 96(4): 1212–1217
33. Saito T, Yamanaka K, Okada Y (1990) Long-distance movement and viral assembly of tobacco mosaic virus mutants. Virology 176(2): 329–336
34. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York
35. Scholthof HB, Morris TJ, Jacson AO (1993) The capsid protein gene of tomato bushy stunt virus is dispensable for systemic movement and can be replaced for localized expression of foreign genes. Mol Plant Microbe Interact 6: 309–322
36. Seron K, Haenni AL (1996) Vascular movement of plant viruses. Mol Plant Microbe Interact 9: 435–442
37. Sit TL, Haikal PR, Callaway AS, Lommel SA (2001) A single amino acid mutation in the carnation ringspot virus capsid protein allows virion formation but prevents systemic infection. J Virol 75: 9538–9542
38. Spitsin S, Steplewski K, Fleysh N, Belanger H, Mikheeva T, Shivprasad S, Dawson W, Koprowski H, Yusibov V (1999) Expression of alfalfa mosaic virus coat protein in tobacco mosaic virus (TMV) deficient in the production of its native coat protein supports long-distance movement of a chimeric TMV. Proc Natl Acad Sci USA 96(5): 2549–2553
39. Suzuki M, Kuwata S, Kataoka J, Masuta C, Nitta N, Takanami Y (1991) Functional analysis of deletion mutants of cucumber mosaic virus RNA3 using an *in vitro* transcription system. Virology 183: 106–113
40. Takeda A, Kaido M, Okuno T, Mise K (2004) The C terminus of the movement protein of Brome mosaic virus controls the requirement for coat protein in cell-to-cell movement and plays a role in long-distance movement. J Gen Virol 85: 1751–1761
41. Vaewhongs AA, Lommel SA (1995) Virion formation is required for the long-distance movement of red clover necrotic mosaic virus in movement protein transgenic plants. Virology 212(2): 607–613
42. Wisniewski LA, Powell PA, Nelson RS, Beachy RN (1990) Local and systemic spread of tobacco mosaic virus in transgenic tobacco. Cell 2: 559–567
43. White JL, Kaper JM (1989) A simple method for detection of viral satellite RNAs in small plant tissue samples. J Virol Methods 23(2): 83–93

Author's address: Vitantonio Pantaleo, PhD, Istituto Virologia Vegetale, Sezione di Bari, Consiglio Nazionale delle Ricerche, via Amendola 165a, 70126 Bari, Italy; e-mail: v.pantaleo@ba.ivv.cnr.it