Analysis of the capsid processing strategy of *Thosea asigna* virus using baculovirus expression of virus-like particles

Fiona M. Pringle, James Kalmakoff and Vernon K. Ward

Department of Microbiology, University of Otago, PO Box 56, Dunedin, New Zealand

*Thosea asigna* virus (TaV), a putative member of the genus *Betatetravirus* of the family *Tetraviridae*, is predicted to have a novel capsid expression strategy compared with other characterized tetraviruses. The capsid precursor protein is cleaved twice to generate three proteins. Two of the proteins, L (58–3 kDa) and S (6–8 kDa), are incorporated into the TaV virion. The third, non-structural protein, produced from the N terminus of the precursor protein, is up to 17 kDa in size and is of unknown function. The TaV capsid precursor protein sequence without the 17 kDa N-terminal region was modelled against the solved structure from *Nudaurelia ω* virus (NoV) using SwissModel. The TaV model was very similar to the solved structure determined for subunit A of NoV and had features that are conserved between tetraviruses and nodaviruses, including the positioning of the cleavage site between the L and S capsid proteins. The production of virus-like particles (VLPs) using the baculovirus expression system was used to analyse the capsid processing strategy employed by TaV. VLPs were formed in both the presence and absence of the 17 kDa N-terminal region of the capsid precursor. VLPs were not formed when the L and S regions were expressed from separate promoters, indicating that cleavage between the L and S capsid proteins was an essential part of TaV capsid assembly. Expression of the TaV 17 kDa protein in bacteria did not produce intracellular tubules similar to those formed by bacterial expression of the p17 protein from *Helicoverpa armigera* stunt virus.

**Introduction**

The family *Tetraviridae* is comprised of positive-sense single-stranded RNA viruses which exclusively infect lepidopteran insects (Hanzlik & Gordon, 1997). Tetraviruses have non-enveloped isometric capsids that are organized using *T* = 4 icosahedral symmetry with twelve Y-shaped trimers on each face of the capsid (Hanzlik & Gordon, 1999). The family is divided into the *Betatetravirus* and *Omegatetravirus* genera, based upon of the appearance of the virion and genome organization. There are three pits visible on each face of a *Betatetravirus* capsid, with deep grooves between capsid faces (Olson *et al.*, 1990; Pringle *et al.*, 1999). In contrast, the omegatetraviruses have smaller faces with a more compact appearance, and do not have a pronounced groove between the capsid faces (Johnson & Reddy, 1998).

Omegatetraviruses have a bipartite genome (Agrawal & Johnson, 1992), with the RNA-dependent RNA polymerase and capsid precursor protein genes encoded on approximately 5–3 kb and 2.5 kb RNA molecules, respectively. A second open reading frame on the small RNA molecule has been identified in *Helicoverpa armigera* stunt virus (HaSV), an *Omegatetravirus* (Hanzlik *et al.*, 1995). This open reading frame encodes a 17 kDa protein of unknown function which produces tubules when expressed in bacteria. Hanzlik *et al.* (1995) hypothesized that this protein has a similar function to movement proteins in plant viruses, based upon the structures formed in bacteria and the high PEST amino acid content of the HaSV protein.

Betatetraviruses have a monopartite genome with the RNA-dependent RNA polymerase and capsid precursor protein genes present on an approximately 6.5 kb RNA molecule. The capsid precursor protein gene is also present on a subgenomic RNA molecule of approximately 2.5 kb (Gordon *et al.*, 1999; Pringle *et al.*, 1999). It has been proposed that the capsid precursor protein gene is expressed from the subgenomic RNA molecule (Gordon & Hanzlik, 1998).

In both genera of the tetraviruses, the virus capsid is made from two proteins of approximately 60 kDa and 8 kDa in size. The two structural proteins are produced from a capsid precursor protein which is cleaved post-assembly (Canady *et al.*, 2000). We have previously proposed that, although *Thosea*...
asigna virus (TaV) has a capsid appearance and genome organization similar to Nudaurelia β virus (NjV). TaV employs a novel capsid expression strategy when compared to other members of the Tetraoviridae (Pringle et al., 1999). The capsid precursor protein of TaV is predicted to cleave twice to generate two structural proteins of 58-3 kDa (L) and 6-8 kDa (S), and a third protein of up to 17 kDa (p17) of unknown function which has not been detected in mature TaV particles. We have utilized the baculovirus expression system to produce virus-like particles (VLPs) by expression of different portions of the TaV capsid precursor protein gene. VLPs have been produced by the expression of the capsid precursor protein gene of an Omegatetravirus, Nudaurelia o virus (NjV; Agrawal & Johnson, 1995), and have also been utilized extensively for structure and function studies of nodaviruses (Schnemann et al., 1994).

The aims of this study were to analyse the capsid expression strategy employed by TaV and to determine if any of the three proteins produced by cleavage of the TaV capsid precursor protein were essential for assembly of VLPs. This paper presents evidence that supports the novel capsid processing strategy for TaV proposed previously (Pringle et al., 1999) and is the first report of the production of VLPs for a Betatetravirus. In addition, the structure of the TaV capsid protein was modelled against the solved crystal structure of subunit A from NjV.

**Methods**

**Source of TaV and antibodies.** Infected Setohosea asigna larvae from north Sumatra were supplied as frozen insect cadavers by Bernhard Zelazny, Integrated Coconut Pest Control Project, Jakarta, Indonesia. Virus was purified from frozen insect cadavers as described previously (Pringle et al., 1999) and stored at 4 °C. Antiserum against TaV was prepared as described by Kalmakoff & McMillan (1990).

**Prediction of the tertiary structure of the capsid proteins of TaV.** The structure of the TaV capsid precursor protein was modelled using SwissModel and the Swiss/PDBViewer protein modelling program (version 3.5; Guex & Peitsch, 1997), which are both available from the ExPASy website (http://www.expasy.ch/spdbv). The TaV capsid precursor protein sequence used for modelling corresponded to the construct LS and did not contain the 17 kDa N-terminal protein, which has not been detected in mature virus particles (TaV cDNA sequence asigina_virus_summary). RT–PCR of TaV RNA. The coding regions of the TaV capsid precursor were designated p17, L and S. Different combinations of the TaV capsid sequence were amplified by RT–PCR in preparation for baculovirus expression (Fig. 1). Four constructs were generated. Clone p17LS was the full-length capsid coding region which was amplified with the primers capsid-front/C-terminal. Clone LS corresponded to the capsid coding region without p17 and was amplified with the primers MPTTTVA/C-terminal. Clone p17L, the large capsid protein with p17, was amplified with capsid-front/large capsid stop (LCS). Clone L, the large capsid protein coding region without p17, was amplified with the primers MPTTTVA/LCS. Finally, the small capsid protein coding region, S, was amplified with the primers MGWG/C-terminal.

Primers were designed to the published TaV cDNA sequence (Pringle et al., 1999) and were synthesized by the Life Technologies Custom Primers service. The primers used to create constructs for baculovirus expression were capsid-front (5′ TAGGATCCAAATAGCCCGGGGC-TCCAAGTG 3′), C-terminal (5′ TTAAGAATTCCGGACTATTGTG 3′), MPTTTVA (5′ GGAGATCCCATGCCCCACCGG 3′), LCS (5′ ACCGGATCTTCAATAAGGCGGGGATACG 3′) and MGWG (5′ CGATTCCGTGCTAAAAATGCGGGGTTGAT- GTTC 3′). Bold letters represent restriction enzyme sites.

TaV RNA was purified using TRizol (Life Technologies) as described by Pringle et al. (1999). Purified RNA (5 μl) was used as template in one-step Titan RT–PCR system reactions (Roche) and the PCR products were performed as recommended by the manufacturer. The resulting PCR products were cloned into the baculovirus expression vector pAcUW51GUS (p17LS, LS) or pAcUW51 (p17L + S, L + S) by standard methods. All constructs were verified through single-strand sequence obtained by dye-termination sequencing (ABI Prism model 377 automated sequencer, Centre for Gene Research, University of Otago).

**Recombinant baculovirus production.** Insect Sf-21 cells were maintained as suspension cultures at 28 °C in SF-900 II medium (Life Technologies). Recombinant baculoviruses were generated in Sf-21 cells by standard methods. Briefly, linearized BacPAK6 DNA (Kitts & Possee, 1993) was co-transfected with the transfer vector using Cellfectin (Life Technologies) as per the manufacturer’s instructions. Recombinant baculoviruses were purified to homogeneity by plaque purification. Virus titre were increased by serial passage in Sf-21 cells.

**Expression of TaV proteins by recombinant baculoviruses.** Recombinant baculoviruses were used to infect Sf-21 cell monolayers at an m.o.i. of 5–10. Supernatants were harvested after 5–7 days incubation at 28 °C and analysed for protein production as described below. Virus stocks were stored at 4 °C.

**Detection of expressed proteins by SDS–PAGE and Western blotting.** To determine if expression and/or processing of the capsid precursor protein was occurring in infected cells, cell samples were analysed by electrophoresis through a 10% glycerine SDS–PAGE gel (Laemmli, 1970) and Western blotting. A sample of native TaV was included in some cases. Proteins were transferred onto Immobilon-P membrane (Millipore) by semi-dry blotting using the Pharmacia 2117 Multiphor II system. The membrane was blocked overnight at room temperature in 5% skim milk then probed with rabbit polyclonal anti-TaV antiserum reactive to the large capsid protein of TaV. Western blots were visualized using goat anti-rabbit alkaline phosphatase-conjugated IgG (Sigma ImmunoChemicals) and BCIP/NBT substrate (Sigma). Sizes of reactive proteins were estimated by comparison to the New England Biolabs Broad Range Protein Marker. Following protein transfer and prior to the blocking step, the lane corresponding to the marker was removed. The marker proteins were visualized by staining for 30 s with 0.025% Coomassie brilliant blue in 40% methanol and destaining in 50% methanol.
Fig. 1. Strategy for recombinant baculovirus expression of TaV capsid proteins. (a) Proposed capsid processing strategy of TaV. The capsid precursor protein of TaV is cleaved twice to generate three proteins. The large and small capsid proteins are incorporated into the mature virion. Protein sequences at the N terminus of the cleavage points are shown as one letter amino acid codes. The third protein, p17, has not been detected in TaV virions and is of unknown function. (b) Four recombinant baculovirus constructs were made for expression of different regions of the TaV capsid precursor protein. Each construct is represented by open boxes preceded by baculovirus p10 (striped arrows) or pol (shaded arrows) promoters. Cleavage points which occur in the expressed products are represented by vertical lines and N-terminal protein sequences are indicated. Translational stop codons are indicated by dots. Primers used to amplify each region and in PCRs of recombinant baculovirus DNA to verify the constructs are shown as black triangles. The primers used in negative control PCRs when verifying the constructs are shown as open triangles. The primer capsid-4 (grey triangle) was used as an additional positive control in PCR of the p17LS construct.

■ Purification of VLPs from infected insect cells. Supernatants from recombinant baculovirus-infected Sf-21 cells were harvested 5–7 days post-infection and clarified by centrifugation at 7740 g. VLPs were pelleted by ultracentrifugation at 100 000 g in SW41 tubes (Beckman) and the VLP pellets were resuspended in 10 mM Tris–HCl, pH 7.5.

■ Visualization of VLPs by transmission electron microscopy (TEM). Samples from each construct were analysed by TEM. Samples were stained using the double-negative staining technique described by Pringle et al. (1999) and viewed with an Akashi EM-002A transmission electron microscope.

■ Confirmation of baculovirus constructs by PCR. Supernatants from recombinant baculovirus-infected Sf-21 cells were harvested 5–7 days post-infection and clarified by centrifugation at 7740 g. The baculovirus was pelleted from the supernatant by ultracentrifugation at 100 000 g through a 1 ml cushion of 40% (w/v) sucrose in an SW41 tube (Beckman). Virus pellets were resuspended in 500 μl of distilled H2O, then treated with 25 μl of 20 mg/ml proteinase K (Roche) in the presence of 1% SDS. The sample was extracted twice with an equal volume of buffer-saturated phenol, once with an equal volume of phenol–chloroform, and twice with half volumes of chloroform. It was then ethanol-precipitated and resuspended in 20 μl of distilled H2O.

The DNA was screened by PCR for the presence of the appropriate coding sequence (Fig. 1). PCRs were performed using 1 μl of viral DNA as template in 50 μl PCRs using the Expand High Fidelity PCR system (Roche). Positive PCRs, designed to amplify a fragment corresponding to the original construct, were performed for p17LS, LS, p17L + S and L + S. A second positive control designed to amplify the region encoding the 17 kDa protein of TaV was performed for p17LS. A series of negative control PCRs designed to amplify contaminating sequences was performed on recombinant baculovirus DNA to confirm the constructs being tested. PCR products were analysed on a 1% agarose gel and the sizes were estimated by comparison to the 1 kb Plus DNA ladder (Life Technologies).

■ Bacterial expression of the 17 kDa protein from TaV. The 17 kDa N-terminal region of the TaV capsid protein (p17) was amplified from purified TaV RNA using the primers p17-F (5’ GAAGTTATA ACATATGATAGTG 3’) and p17-stop (5’ CCTGGATCCGTGTTCTAGCCGGATTTTCC 3’). p17 was cloned into pET-22b(+) (Novagen) using standard techniques. Intact plasmid DNA from this clone and a negative control consisting of empty pET-22b(+) vector was transformed into electrocompeotent BL21(DE3)pLysS E. coli cells and tested for protein expression as described in the pET System Manual (Novagen). Samples from test and control cell pellets were analysed on a 16–5% Tricine SDS–PAGE gel (Schagger & von Jagow, 1987).

■ Analysis of pET-22b(+)/p17 bacterial cells by TEM. Test and control cells were pelleted 2 h post-induction and were then resuspended...
in 0.25 culture vols of cold 50 mM Tris-HCl, pH 8.2, 2 mM EDTA and re-pelleted by centrifugation in a microcentrifuge. The bacterial cells were fixed in 2.5% glutaraldehyde in phosphate buffer (100 mM sodium phosphate, pH 7.2, 150 mM sucrose) for 2 h then washed in phosphate buffer and incubated with 1% osmium tetroxide in phosphate buffer for 60 min. The fixed cells were dehydrated by washing with a series of solutions of ascending concentrations of ethanol up to 100%. The pellets were embedded in LR White resin and cured overnight at 60 °C then sectioned. Sections were collected on paralodion-coated copper grids and stained with 2% uranyl acetate and Reynold’s lead citrate before examination by TEM on a Philips CM100 transmission electron microscope. Test grids were compared with control grids under the same conditions.

**Results**

**Tertiary structure prediction of the TaV capsid proteins**

The proposed capsid protein processing strategy for TaV removes an approximately 17 kDa protein, which has not been detected in mature virions, from the N terminus of the capsid precursor (Fig. 1a; Pringle et al., 1999). To determine if the L and S structural proteins were comparable to those of NωV after the N-terminal cleavage that removes p17, the structure of the TaV capsid precursor protein from the mature PTTVA cleavage point to the C terminus was predicted by comparison...
to the solved crystal structure of subunit A from NooV. The structure predicted for the TaV capsid precursor protein (Fig. 2a) is very similar to the structure determined by X-ray crystallography for NooV (Fig. 2b). The presence of an immunoglobulin-like fold in the large capsid protein is a conserved feature between the TaV model and the structure determined for NooV. In addition, the Asn/Gly cleavage point between the large and small capsid proteins of TaV (Pringle et al., 1999) has been placed in the same position as the Asn/Phe cleavage point in NooV and an alpha helix similar to the one formed by the small capsid protein in NooV is predicted to form in TaV. In the TaV model, cleavage of the precursor protein is not shown because the structure was modelled using sequence for the uncleaved precursor protein.

Eight beta-sheets that make up a jelly roll motif are a feature of all isometric virus capsids (Chelvanayagam et al., 1992). These beta-sheets are predicted to be present in the TaV model. The similarity of the TaV model to the NooV structure was viewed by overlaying the two protein ribbon drawings (Fig. 2c). As a test of the validity of the predicted model of TaV to the solved crystal structure of subunit A from NooV, Ramachandran plots for both structures were made. The plots for NooV (Fig. 2d) and the TaV (Fig. 2e) model demonstrate that the majority of residues in both structures fall within allowed conformational areas.

**Verification of recombinant baculovirus constructs by PCR**

Recombinant baculoviruses expressing different combinations of the TaV capsid proteins were produced in insect Sf-21 cells. To verify that the baculovirus constructs used for expression experiments contained the same DNA as the original transfer vector, DNA purified from the recombinant baculoviruses was analysed by PCR using the scheme shown in Fig. 1(b). All positive reactions resulted in products of the correct size being detected. There were no products detected in the negative control PCRs.

**Detection of expressed proteins in recombinant baculovirus-infected cells**

Western blots of proteins from cells infected with each expression construct demonstrated the presence of proteins reactive to TaV antiserum. The estimated size of the reactive protein from all of the constructs was 59 kDa (Fig. 3). The size of the large capsid protein in native TaV as determined by sequencing is 58.3 kDa (Pringle et al., 1999). In order to compare samples directly, proteins from p17LS, LS, and native TaV virions were analysed by Western blotting (Fig. 3a). The large capsid protein was the same size in all of the samples. The sizes of the large capsid proteins from the p17L+S and L+S constructs were the same size as TaV when analysed by Western blotting (Fig. 3b). The proteins were of similar sizes to TaV and to p17LS and LS in these constructs and were estimated to be approximately 59 kDa. In constructs p17LS and p17L+S, the presence of the mature 59 kDa form of the large capsid protein indicated that processing of p17 was occurring.

**Visualization of VLPs by TEM**

VLPs were only observed in samples from p17LS (Fig. 4a). Native TaV particles (b) and c) VLPs from p17LS recombinant baculovirus-infected cells. (d) VLPs from LS recombinant baculovirus-infected cells. The arrow in (d) indicates the chain-like structures that were associated with this preparation of VLPs. The bar in each frame represents 50 nm.
protein reactive to the TaV antiserum, no VLPs were detected in these samples. The VLPs produced by p17LS and LS were structurally similar to each other and to native TaV particles (Fig. 4a). The concentration of VLPs from LS was lower than from p17LS, and chain-like structures were seen in the LS preparations but were not detected in p17LS preparations (Fig. 4d). The 38 nm estimated size of the VLPs from both constructs was the same as that of native TaV (Pringle et al., 1999).

**Bacterial expression of the TaV 17 kDa protein**

The 17 kDa protein from TaV was expressed in bacteria (Fig. 5a) and the cells were examined by sectioning and TEM (Fig. 5b). A protein of approximately 17 kDa was expressed in test cells. This protein was not detected in control cells. When cells were examined by sectioning and TEM, inclusions were noted but no structures analogous to the tubules formed by expression of the p17 protein of HaSV (Hanzlik et al., 1995) were observed (Fig. 5b). Test cell sections exhibited a large amount of extracellular material and had aggregates, possibly of expressed protein, visible inside the cells.

**Discussion**

The aim of this study was to investigate the capsid protein processing strategy for TaV that had been proposed from sequence studies (Pringle et al., 1999). The proposed processing strategy of TaV is that the capsid precursor protein is cleaved twice to generate two structural proteins of 58 kDa and 6 kDa and a third protein of up to 17 kDa in size which has not been detected in mature virus particles. The ability of VLPs to assemble in the absence of the 17 kDa protein from TaV, and the fact that it has not been detected in TaV virions, indicates that the 17 kDa protein is a non-structural protein. This hypothesis is supported by the tertiary structure prediction of the TaV capsid precursor protein. The TaV sequence used to generate the model did not include the 17 kDa protein region, but was generated using the TaV sequence which corresponds to proteins L and S. The resulting model was very similar to the solved crystal structure of subunit A from NoV. Eight β-sheets (β-B to β-I) that form the jelly roll found in isometric virus capsids were predicted in TaV previously by sequence alignments (Pringle et al., 1999). These β-sheets are present in the TaV model in similar positions to the β-sheets in NoV. There is a region of very low homology in tetraviruses between the β-sheets, β-E and β-F (Gordon & Hanzlik, 1998). This region has been shown to form an immunoglobulin-like fold in NoV (Munshi et al., 1996). A protruding surface loop, which corresponds to the region between β-E and β-F in TaV, is present on the surface of the TaV structural subunit in the model. We predict that this protrusion is an immunoglobulin-like fold as found in NoV. Another conserved feature is the location of the cleavage point between the large and small capsid proteins in NoV and TaV. Although the uncleaved precursor protein has been used for the TaV model, the predicted model places the previously determined Asn/Gly cleavage point (Pringle et al., 1999) in a similar location to the Asn/Phe cleavage point in NoV. The location of the cleavage point in the interior of the capsid is also conserved between NoV and nodaviruses (Munshi et al., 1996). The model of TaV predicts that an α-helix will be formed by the small capsid protein. The α-helical structure of the small capsid protein is conserved between NoV and nodaviruses (Johnson & Reddy, 1998; Munshi et al., 1996). These α-helices have been predicted to form pentameric bundles which interact with RNA (Fisher & Johnson, 1993; Munshi et al., 1996).

No crystal structure information exists for a Betatetravirus capsid, and until such information does exist, the validity of the
modelling results cannot be tested. However, given that the model of the TaV capsid protein is only marginally different from the solved structure of NodV, despite similarity of only 23.3% between the protein sequences of these viruses (Pringle et al., 1999), the modelled structure of TaV is likely to prove robust. The validity of the model is also supported by Ramachandran plots of the TaV model and NodV, which demonstrate that there are very few residues which lie outside acceptable ϕ/ψ angles in the TaV model.

The hypothesis that the 17 kDa protein from TaV is not required for the assembly of virions is supported by the results from expression of the TaV capsid proteins using recombinant baculoviruses. The formation of VLPs was detected when either the full-length capsid precursor protein (p17LS) or the capsid precursor protein without the 17 kDa protein (LS) was expressed. These VLPs were structurally similar to each other and to native TaV particles. In cells infected with either p17LS or LS, the large capsid protein from each construct, when detected by Western blotting, was of equivalent size, indicating that cleavage of the 17 kDa protein from the capsid precursor protein was occurring in p17LS-infected cells. The detected protein was also similar in size to the mature large capsid protein from native TaV. While VLPs from both the p17LS and LS constructs were similar in appearance, some differences between the two constructs were observed. The concentration of intact VLPs produced by the LS construct was much lower than the concentration of p17LS VLPs even though the m.o.i.s of the recombinant baculoviruses used to infect the cells were comparable. It is possible that the TaV 17 kDa protein has a role in stabilization of the capsid during assembly, hence the lower concentration of VLPs in the LS construct. This hypothesis may be supported by the observation that Nodamura virus, when dissociated in the presence of CaCl₂, forms chain-like structures (Newman & Brown, 1978). These structures bear a strong resemblance to the chain-like structures observed by TEM in preparations from the LS construct, suggesting that the chain-like structures may be the result of VLP malformation.

In the p17LS preparation, VLPs that were half-shells and shells with holes were observed. Assuming that these structures represent assembly intermediates, this raises the question of how TaV assembles. The assembly pathway for tetravirus is not known. It has been hypothesized for NodV that the protein subunits form trimers which are then assembled into the capsid (Finch et al., 1974). This theory was supported by cryoelectron microscopy of NodV (Olson et al., 1990). However, recent studies on the procapsid of NodV have demonstrated that the procapsid is extremely different from the mature capsid and is formed from dimers which then differentiate into trimers following a conformational change (Canady et al., 2000). It remains to be determined whether TaV assembles via a similar pathway.

Although protein reactive to the TaV antiserum could be detected in p17L+S and L+S, the constructs used for separate expression of the capsid proteins, no VLPs were detected. These results imply that the cleavage between the large and small capsid proteins is required for the formation of VLPs or that the precursor undergoes inter-subunit reactions which are required for assembly. Although the production of very low levels of VLPs by the separate expression constructs cannot be ruled out, VLPs were never observed in preparations from these constructs. This result corresponds to NodV and nodavirus where the cleavage event between the large and small capsid proteins is essential for assembly (Canady et al., 2000; Zlotnick et al., 1994).

A role for the 17 kDa protein from TaV was not elucidated in this study. Although there were differences between test and control sectioned bacterial cells when the TaV 17 kDa protein was expressed, there were no structures that resembled the tubules observed by Hanzlik et al. (1995) when p17 from HaSV was expressed in bacterial cells. A role similar to the movement proteins of plant viruses was suggested for p17 of HaSV based upon the tubule structures formed and on the high PEST content of this protein. Neither of these features are shared by the 17 kDa protein of TaV, which has not been detected in TaV virions and is probably not encapsidated (Pringle et al., 1999). It is likely that the 17 kDa protein from TaV has a role at some other point in the virus lifecycle. Two possibilities include a role in particle stabilization during virus assembly, as implied by the chain-like structures observed in LS preparations, or acting as a regulatory protein during RNA replication as has been proposed for protein B from nodaviruses (Ball, 1995).

This paper presents evidence that supports the capsid processing strategy proposed for TaV by Pringle et al. (1999). VLPs were formed by recombinant baculoviruses expressing the full-length capsid precursor protein and the capsid precursor protein without the 17 kDa protein, but were not formed by constructs where the large and small capsid proteins were expressed separately, regardless of the presence or absence of the 17 kDa protein. A role for the 17 kDa protein has not been established, but it may have a stabilizing role during virus assembly.

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