Assembly of virus-like particles in insect cells infected with a baculovirus containing a modified coat protein gene of potato leafroll luteovirus

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DNA encoding the coat protein (P3) of a Scottish isolate of potato leafroll virus (PLRV) was inserted into the genome of Autographa californica nucleopolyhedrovirus (AcNPV) such that the coat protein was expressed either in an unmodified form or with the addition of the amino acid sequence MHHHH-HHGDDDDKDAMG at the N terminus (P3-6H). Insect cells infected with these recombinant baculoviruses accumulated substantial amounts of P3 and P3-6H. P3 could not be recovered from cell extracts unless it was denatured in SDS but a proportion of the P3-6H was recoverable in a soluble form in non-denaturing conditions. Immunogold labelling of sections of infected cells showed that P3 accumulated in nuclei in large amorphous bodies. In contrast, although much of the P3-6H also accumulated in nuclei, it formed virus-like particles (VLP) which were often grouped in close-packed, almost crystalline arrays. When electron microscope grids coated with antibodies to PLRV were floated on cell extracts containing P3-6H, VLP were trapped which were indistinguishable from PLRV particles trapped from extracts of PLRV-infected plants. The VLP co-sedimented in sucrose gradients with PLRV particles which suggests that the VLP contained RNA. VLP collected from sucrose density gradient fractions contained protein which reacted with nickel chelated to nitrilotriacetic acid, a histidine-specific reagent. Cells infected with either recombinant baculovirus also synthesized a protein, with an M, of about 17 000, which was shown to be the translation product of the P4 gene which is in the +1 reading frame within the coat protein gene. This protein was also found in the nuclear fraction of infected cells but was more readily soluble than was P3.

Introduction

Potato leafroll luteovirus (PLRV) causes an important disease of potato crops world-wide. Like all luteoviruses (Waterhouse et al., 1987), PLRV multiplies in phloem tissue and is transmitted from plant to plant by aphids, commonly Myzus persicae, but is not transmissible mechanically. PLRV particles circulate in the haemolymph of their aphid vectors but there is no evidence that the virus multiplies in aphid tissues (Harrison, 1984). Therefore, much research on the mechanisms of aphid transmission has focused on the properties of the intact virus particles (Harrison & Murant, 1984; Gildow, 1993; van den Heuvel et al., 1993; Jolly & Mayo, 1994).

PLRV particles consist of a single-stranded positive-sense RNA of 5·8 kb encapsidated in a major coat protein of M, 23000 (P3) (Mayo et al., 1989; van der Wilk et al., 1989). PLRV RNA has six major open reading frames which are probably expressed by a variety of mechanisms (Martin et al., 1990; Miller et al., 1995; Mayo & Ziegler-Graff, 1996). Detailed study of these mechanisms in naturally infected tissues is complicated by the restriction of PLRV to phloem tissue, and various artificial systems have been used to overcome this limitation. Bahner et al. (1990) infected tobacco protoplasts with PLRV in order to investigate readthrough of the coat protein termination codon and Tacke et al. (1990) used transient expression of DNA constructs containing the gene for β-glucuronidase to study translation of different reading frames in the coat protein gene.

Viral genes have been expressed for a variety of purposes by cloning them in vectors for expression in heterologous systems. Bacterial expression has been used to prepare PLRV
proteins for antisera production (Bahner et al., 1990) or for biochemical assay (Tacke et al., 1991) but a more versatile system that has been used for several other viral genes is insertion into baculovirus DNA and subsequent expression of the recombinant DNA in infected Spodoptera cells (Stewart & Possee, 1993).

Here we report that the PLRV coat protein accumulated in insect cells that had been infected with a recombinant baculovirus containing the P3 gene, and that, when a minor modification was made to the amino acid sequence, the coat protein molecules assembled to form VLP. During the completion of this work, Tian et al. (1995) reported a similar phenomenon with the coat protein of beet western yellows luteovirus (BWYV).

**Methods**

- **DNA manipulation and cloning.** DNA isolation, digestion of DNA by restriction endonucleases, DNA ligation, DNA transformation of competent *E. coli* cells and PCR amplification of DNA templates were done essentially as described by Lamb & Hay (1991) and Matthews et al. (1993). Two plasmids containing the coat protein gene cDNA (pSAB60 and pSAB61) were constructed by ligating BglII-EcoRI fragments into BglII-EcoRI-cut transfer vector pVL1392 (Invitrogen). The cDNA was obtained by amplifying DNA in a PCR using either primers A and B, or B and C, and pSCHR103 as a template. pSCHR103 is a subclone in pBluescript (Stratagene) of the PLRV-specific sequence from pPLR439 (Barker et al., 1992). Primer A was 5' ACACAGATCTATGAGTACG

  GTCGTGGTTAAAGG, primer B was 5' AGAGGAATTCCTATTTGG

  CATCACCATCACCATCACGGGGACGATGACGATAAAGACG

  (Barker et al., 1992).

- **Antisera.** Rabbit polyclonal antisera raised against PLRV particles was similar to serum G (Torrance, 1992) and was used for electron microscope studies. A mixture of monoclonal antibodies SCR-1, SCR-5, SCR-7, SCR-8 and SCR-9 (Massalski & Harrison, 1987) was used for the detection of P3 in immunoblots. A polyclonal rabbit antisera was raised against the PLRV M protein 17,000 protein (P4; see Fig. 1). The P4 gene was cloned into plasmid pGEX-2T (Smith & Johnston, 1988) in *E. coli* strain JM101 to generate a fusion protein comprising PLRV P4 linked at its N-terminus to glutathione S-transferase (GST). The fusion protein was purified from insoluble *E. coli* inclusion bodies to near homogeneity by differential extraction and sedimentation. The protein was dissolved in 8 M-urea, emulsified with Freund's complete adjuvant and injected (250 µg) into rabbits. Ten days after four further similar injections in incomplete adjuvant, rabbits were sacrificed and the serum was collected.

- **ELISA.** Samples were assayed using DAS-ELISA as described by Barker & Solomon (1990). The trapping antigen and the alkaline phosphatase-conjugated detecting globulin were obtained from polyclonal rabbit antisera.

- **Immunoblotting.** Proteins were separated in 16% polyacrylamide–SDS gels and electrobotted onto nitrocellulose membranes as described by Bjerrum & Schafer-Nielsen (1986). Membranes were blocked with PBS containing 5% non-fat milk powder (Marvel; Cadbury Schweppes) and 0-1% Tween 20 overnight at 4 °C and then incubated for 30–60 min with primary antisera diluted in blocking buffer at 1/100 (monoclonal antibodies) or 1/1000 (polyclonal anti-P4). Anti-P4 was pre-treated by mixing with an extract made in blocking buffer of *E. coli* JM101 carrying pGEX-2T. Blots were washed twice for 15 min with the blocking buffer and incubated for 30–60 min with goat anti-rabbit IgG conjugated to alkaline phosphatase (Sigma) or horseradish peroxidase (Amersham) or with peroxidase-conjugated goat anti-rabbit IgG (Amersham) diluted 1/1000, 1/3000 or 1/3000 respectively in blocking buffer. Blots were then washed twice in blocking buffer and twice with PBS containing 0-1% Tween 20 and developed with enhanced chemiluminescence (ECL; Amersham).

- **Detection of histidine tags.** Protein samples were spotted directly onto nitrocellulose or were separated by SDS–PAGE and transferred by electroblotting. The nitrocellulose filter was then blocked with 10% non-fat milk powder in 20 mM-Tris–HCl pH 7-4, 0·3 M-NaCl containing 0-1% Tween 20, for 1 h, washed in the above buffer without milk powder and then incubated with alkaline phosphatase conjugated to the nickel binding quadridentate chelator nitrilotriacetic acid (Booting & Randall, 1995: AF-NTA-Ni) (2 µg/ml) (gift from C. Botting) in the same buffer for 1 h. After further washes in the same buffer the histidine-tagged proteins were detected using AP colour development reagents (Bio-Rad) as substrate (100 µl of each reagent in 10 ml 0·1 M-Tris, pH 7-6).

- **Purification and expression of recombinant baculoviruses.** Purified DNA (0·5 µg) of pSAB60 or pSAB61 was co-transfected with 0·1 µg BsaI-cleaved AcRP231acZ viral DNA (Possee & Howard, 1987) into Spodoptera frugiperda (SF9) cells essentially as described by Watson & Hay (1990).

  Recombinant baculoviruses were plaque-purified from the medium of transfected cells as described by O'Reilly et al. (1992). The single plaque lysates were used in further infections to amplify the recombinant virus and isolate a viral stock of approximately 5 × 10⁷ p.f.u./ml. AcNPV-pSAB60 and AcNPV-pSAB61 are referred to as Ac60 and Ac61 respectively.

  To express a particular recombinant virus, 500 ml of a culture of mid-exponential phase SF9 cells or *Mamestra brassicae* cells were collected by centrifugation at 2000 g for 5 min and the cells were resuspended in 50 ml of viral stock. After incubation for 1 h at 28 °C, virus-infected cells were diluted to 500 ml with fresh culture medium (TC-100) and incubated at 28 °C.

- **Fractionation of cells and purification of VLP.** Nuclear and plasmidic fractions were prepared by lysis in NP40, and NaCl extraction as described by Bosher et al. (1992).

  To prepare VLP for sedimentation in sucrose gradients, cells were infected with Ac61 at an m.o.i. of 3 and incubated at 28 °C with constant agitation for 50–96 h. Cells were collected by centrifugation at 1000 r.p.m. for 10 min at 15 °C from 500 ml cultures and resuspended in 20 ml/g cells of 0·1 M-sodium citrate, pH 6, containing 0·4 M-NaCl. After homogenization for 2 min at maximum speed (Ultraturrax T-25, Merck), the extracts were centrifuged at 8000 r.p.m. for 15 min at 10 °C (Sorvall SS-34) and the supernatant fraction was centrifuged at 20 min at 10000 r.p.m. at 10 °C. Particles were recovered from the final supernatant fraction by centrifugation for 3 h at 45000 r.p.m. at 10 °C. Pellets were resuspended in 0·4 ml 20 mM-sodium phosphate, pH 7.

  In some experiments the first extract was clarified by vigorous mixing with 0·5 vol. chloroform and particles were recovered by precipitation from 8% polyethylene glycol (PEG), 0·2 M-NaCl. This procedure did not enhance the yield or the cleanliness of the particles obtained.

  Particles were also recovered from culture media by precipitation from PEG and NaCl. Yields were usually about 10 to 20% of that obtained from cell extracts.
Sucrose density gradient centrifugation. Resuspended pellets were layered onto 5 ml gradients of 10–40% (w/v) sucrose in 0.02 M-sodium phosphate buffer, pH 7.5 and centrifuged for 75 min at 45 000 r.p.m. in a Beckman 5W50.1 rotor at 10 °C. Fractions of about 0.4 ml were collected by upward displacement. Fractions of interest were pooled and particles were recovered by centrifugation and subjected to a further gradient centrifugation. The optical densities of the fractions in the first gradient were very large; discernible peaks were visible after the second gradient.

Fixation, embedding and sectioning for electron microscopy. Sf9 cells were collected from culture medium by low speed centrifugation (3 min at 2500 r.p.m.), resuspended in 5% glutaraldehyde (in TC-100), incubated at 4 °C for 5 h, and washed twice for 30 min at 4 °C in TC-100. The fixed cells were then centrifuged in 1% agar at 40–50 °C. After cooling, pieces containing agar-embedded cells were post-fixed in 0.1% OsO4 in TC-100, dehydrated and embedded in Araldite as described by Fasseas et al. (1989). Samples for immunogold labelling (IGL) were treated the same way except that they were not post-fixed.

Ultrathin sections (silver/gold interference colours) were mounted on pyroxylin-filmed copper or nickel (for IGL). 100 mesh, hexagonal grids and post-stained in 2% uranyl acetate and Reynold’s lead citrate diluted 1/4 in 0.01 M-NaOH.

Immunogold labelling. Dilutions of normal goat serum (NGS; Biocell Research Laboratories) were made in 0.07 M-phosphate buffer, pH 6.5, containing 1% Tween 20 and 0.02% sodium azide. All steps were done at about 20 °C. Mounted sections were floated on 20 µl drops of a 1/5 dilution of NGS for 1 h and then on 20 µl drops of 10 µg/ml IgG (prepared either from rabbit polyclonal antiserum to PLRV particles, or, as a control unrelated to PLRV, from an antiserum to tobacco rattle virus) in 1/5 NGS for 18 h. Grids were individually washed on 1/20 NGS (430 µl in microtitre plate wells) drained briefly and incubated for 5 h on 20 µl drops of a 1/100 dilution of goat anti-rabbit gold conjugate (15 nm) (Amersham) in 1/5 NGS. Grids were sequentially washed (10 min each) on 430 µl of 1/20 NGS, phosphate buffer and distilled water. The grids were drained, dried and post-stained like conventional sections.

Immunooabsorbent electron microscopy (ISEM). Sf9 cells were extracted with 0.1 M-citrate buffer pH 6.0 containing 0.5% Triton X-100 using a 2 ml ‘Safe Grind’ tissue grinder (Jencon Scientific). Extracts were clarified by mixing with a 20% volume of chloroform followed by low speed centrifugation. ISEM was done essentially as described by Roberts (1986). Carbon-filmed grids were incubated for 1 h at 37 °C on 10 µl drops of rabbit polyclonal anti-PLRV serum diluted 1/1000 in 0.07 M-phosphate buffer, pH 6.5. Antiserum-coated grids were washed twice for 10 min on phosphate buffer then incubated for a further 2–4 h at 4 °C on 10 µl samples. Grids were negatively stained by washing them with eight drops of uranyl formate–sodium hydroxide (Roberts, in Barnett & Murant, 1970).

For antibody coating tests (Roberts, 1986), carbon-filmed grids were touched to 10 µl drops of the sample, drained and incubated for 15 min to 2 h at 4 °C on 10 µl drops of 1/100 dilution of rabbit polyclonal anti-PLRV serum in phosphate buffer. Grids were stained with two drops of 2% sodium phosphotungstate, pH 6.
Results

Expression of PLRV proteins in insect cells infected with recombinant baculoviruses containing the PLRV coat protein gene

To express the coat protein gene of potato leafroll virus in insect cells, two recombinant baculoviruses were constructed. Ac60 contained the DNA encoding P3 but, as it proved difficult to purify P3 from infected cells, another virus (Ac61) was made which encoded P3 modified by the addition of an N-terminal ‘tag’ with a histidine-rich sequence. The ‘tag’ was intended to allow the modified form of P3 (P3-6H) to be purified by binding to an Ni-NTA-Sepharose CL-6B column (Hochuli et al., 1987). The amino acid sequence MHHHHHHH-GDDDDDDKADMG was added to the N terminus of the P3 protein by using primer C instead of primer A in the PCR reaction to isolate DNA encoding P3 (Fig. 1).

Proteins present in uninfected Sf9 cells or Sf9 cells infected with recombinant baculoviruses containing either the bacterial lacZ gene, the P3 gene (Ac60) or the gene encoding P3-6H (Ac61) were fractionated by SDS-PAGE. Staining with Coomassie brilliant blue showed that a polypeptide with an $M_r$ of about 24000 was made in cells infected with Ac61 (Fig. 2, track 1) and Ac60 (Fig. 2, track 2) which was not made in uninfected cells (Fig. 2, track 3).

In immunoblotting experiments an infection-specific polypeptide with an $M_r$ of about 24000 reacted with antiserum to PLRV particles and a polypeptide with an $M_r$ of about 17000 reacted with antiserum to P4. Fig. 3 shows blots of extracts of cells infected with Ac60 or Ac61 before and after lysis in non-ionic detergent and fractionation into cytoplasmic and nuclear components. The PLRV-specific protein in extracts of Ac60-infected cells (P3) was largely insoluble whereas modification to P3-6H resulted in the protein being recovered in part in the cytoplasmic as well as both salt-soluble and salt-insoluble

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\text{Track 1: cells infected with Ac61; track 2: cells infected with Ac60; track 3: uninfected cells. The gel was stained with Coomassie blue.}
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\text{Fig. 3. Cellular distribution of PLRV gene products in insect cells infected with recombinant baculovirus containing the coat protein gene. Sf9 cells were infected with Ac60 (labelled P3) or Ac61 (labelled P3-6H). Cells were collected 50 h after infection and fractionated into cytoplasmic components (cyto), 0.4 \text{-NaCl-soluble nuclear material (nuc) and 0.4 \text{-NaCl-insoluble nuclear material (insol). Proteins from unfractionated cells (cells), or from each fraction, were subjected to SDS-PAGE and were immunoblotted using antisera specific to P3 (anti-P3) or P4 (anti-P4).}
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Fig. 4. Electron microscopy of sections of Sf9 cells infected with Ac61 (a, b) or Ac60 (c). (a) Inclusions in nuclei containing crystal-like arrays of PLRV-like particles (VLP) distinct from baculovirus particles (BV). (b, c) Immunogold labelling of sections of cells infected with Ac61 (b) or Ac60 (c). Sections were treated with antibodies specific to PLRV coat protein. Bars indicate 150 nm (a) or 100 nm (b, c)
Fig. 5. ISEM of extracts of (a, c) Sf9 cells infected with Ac61 or (b) potato leaves infected with PLRV. Particles were trapped on grids coated with antibodies to PLRV particles (a, b) or on carbon-filmed grids and then reacted with antibodies to PLRV (c). Grids were negatively stained with uranyl formate-NaOH (a, b) or 2% ammonium molybdate (c). Bars indicate 50 nm.

Fig. 6. Detection of protein tagged with six histidine residues. VLP were recovered by sedimenting an extract of Ac61-infected Sf9 cells through two successive sucrose density gradients and pelleting material in the fraction of the second gradient which gave the greatest signal in an ELISA test. PLRV particles were from a preparation of purified virus. Drops of 2-fold dilutions of VLP or PLRV particles (V) were applied to nitrocellulose and the filter was probed with (a) antiserum to PLRV followed by alkaline phosphatase-conjugated anti-rabbit antibodies, or (b) with Ni-NTA-AP, and then reacted with alkaline phosphatase substrate.

Detection of P3 and P3-6H in Sf9 cells by electron microscopy

Thin sections of Sf9 cells infected with Ac60 or Ac61 showed that many nuclei contained similar electron-dense inclusions which were not present in the nuclei of cells infected with AcRP23lacZ. At high magnification, the inclusions in cells infected with Ac61 were granular and contained many isometric particles about 20 nm in diameter which resembled PLRV particles (VLP in Fig. 4a). These were often close-packed in crystal-like arrays. In Ac60-infected cells, inclusions were also found in nuclei but they were amorphous. The nuclei in all infected cells contained many baculovirus particles (BV in Fig. 4a).
Serological identification of P3 gene products in Sf9 cells by electron microscopy

Sf9 cells were collected about 60 h after infection and prepared for sectioning without OsO4 treatment. Sections were treated with rabbit polyclonal antibodies to P3 and then with gold-conjugated goat anti-rabbit γ-globulin. No label was detected on sections of cells infected with AcRP231lacZ or on sections treated with heterologous primary antibodies. When anti-P3 was used, gold labelling of sections of cells infected with Ac61 was only over electron-dense inclusions which contained VLP (Fig. 4 b). Labelling of Ac60-infected cells was over the amorphous inclusions (Fig. 4 c). In ISEM tests with buffer extracts of Sf9 cells infected with Ac61 (Fig. 5 a), VLP were trapped which closely resembled particles bound to antibody-coated grids which had been floated on extracts of PLRV-infected potato tissue (Fig. 5 b). Heterologous antisera did not specifically trap the VLP. When VLP were trapped on carbon-filmed grids and these grids were floated on anti-PLRV serum, the VLP became coated with antibody and clumped (Fig. 5 c).

Detection of coat protein tagged with six histidine residues

Samples of a preparation of VLP purified by centrifugation through two sucrose gradients and of PLRV particles of known concentration were applied in a 2-fold dilution series to nitrocellulose. The blots were reacted either with antibodies to PLRV followed by alkaline phosphatase-conjugated anti-rabbit antibodies or with AP-NTA-Ni, and then with alkaline phosphatase substrate. The result (Fig. 6) shows that the PLRV spots (V) contained about twice the amount of PLRV protein as the VLP spots, but that only VLP bound AP-NTA-Ni.

Individual fractions were collected from the second of successive sucrose gradients of a VLP preparation and assayed by ELISA. Fractions which reacted with antibodies to PLRV in ELISA corresponded to those of a parallel control gradient which contained purified particles of PLRV. Thus the VLP sedimented at a similar rate to PLRV particles and are therefore assumed to have contained RNA approximately equivalent in mass to PLRV genome RNA. Samples from each fraction of the sucrose gradient were also denatured in SDS, subjected to PAGE, blotted to nitrocellulose and reacted with Ni-NTA-AP. PLRV particles in a parallel gradient sedimented at the same rate as the ELISA-positive VLP in the gradient shown. The positions of the bands of the M23 000 PLRV coat protein and the M53 000 readthrough protein (Bahner et al., 1990) in protein from purified particles of PLRV are indicated by arrows.

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Discussion

PLRV is one of several plant viruses which, although they are major pathogens responsible for significant crop losses, have proved difficult to study, either because the amount of virus that can be recovered from their host plants is small, or because biochemical and genetic studies are difficult in the relevant hosts. One way to circumvent some of these problems has been to duplicate parts of the virus replication cycle in
convenient heterologous cell systems. In this paper, we report the assembly of PLRV-like particles in insect cells following cloning of the coat protein gene of PLRV in baculovirus DNA. Recent examples of this approach to examining plant virus coat proteins have been the assembly of VLP of the nepoviruses arabis mosaic (Bertioli et al., 1991) and tobacco ringspot (Singh et al., 1995).

PLRV particles have an icosahedral geometry and are believed to contain 180 P3 subunits. The PLRV coat protein P3 has a basic region near its N terminus (Mayo et al., 1989) which is thought to interact with the virus RNA and thus be located on the inside of the particle (Torrance, 1992). However, the extreme N-terminal seven amino acids of the coat protein have been identified as the major epitope on PLRV particles and antibodies specific to this epitope react with intact PLRV particles. Either the extreme N terminus is exposed on the surface of the particles or it is revealed when particles swell during the assay (Torrance, 1992).

Although apparently empty particles have been detected in preparations of BWYV (Hewings & D'Arcy, 1986), none have been detected in PLRV preparations. Our results suggest that, at least in S9 cells, PLRV coat protein alone will not assemble into VLP. In our experiments the addition of MHHHHHGD-DDDKDAMG at the N terminus of P3 yielded protein that could form PLRV-like particles. The added sequence contains two distinct regions and it is possible that the effect on particle formation is caused by the six histidine residues, or the acidic sequence DDDDKD, or both.

The weakly basic sequence H_6 is known to bind to cations such as Ni^{2+} and Zn^{2+} attached to acidic matrices, such as NTA-Sepharose. The chelated Ni^{2+} ion strongly binds proteins which contain H_6 sequences. This binding involves lone pairs of electrons from both the amino groups of the histidine residues and from the nitrogen atom of the NTA group which co-ordinates the positive ion. It may be that the histidine residues on P3-6H are co-ordinating the metal ions in the insect cell nucleus, perhaps in combination with other electron donating groups on P3 or in the nuclear environment. If metal ions are required for the assembly of PLRV in insect cells, this process may be affected by the composition of the medium in which the cells are grown. Alternatively, it may be that the acidic domain of the added amino acids is responsible for VLP assembly, perhaps because of interaction with basic amino acids in the P3 molecule.

The VLP formed in Ac61-infected cells sedimented at a rate similar to that of intact PLRV particles and we conclude that the VLP contained RNA. No hybridization was detected when nucleic acid extracted from a preparation of VLP was annealed with DNA complementary to the coat protein gene of PLRV. It is known that in doubly infected cells, PLRV coat protein can encapsidate RNA of carrot mottle virus (Waterhouse & Murant, 1983) and we assume that the VLP formed in Ac61-infected cells had encapsidated insect cell RNA in an analogous fashion.

Martin et al. (1990) suggested that one role for the readthrough protein of luteoviruses is to assist in the assembly of virus particles. Our results show that for PLRV, at least in the heterologous insect cell system, the readthrough protein is not necessary for the assembly of PLRV coat protein into virus-like particles. However, we cannot exclude the possibility that the extra N-terminal sequence in P3-6H could act in a similar way to the readthrough domain. However, the latter is attached to the C terminus, rather than the N terminus, of P3 and there are no stretches of histidine residues or strongly acidic regions in the sequence of the readthrough protein. With BWYV, Reutenauer et al. (1993) have shown that virus particles are formed in cells infected with mutants which, because of deletion, lack the readthrough protein.

The P4 sequence lies entirely within the P3 gene in a different translational reading frame. Immunoblots with antisera to P4 showed that P4 was synthesized in cells infected with either Ac60 or Ac61.

In S9 cells infected with either Ac60 or Ac61, the P3 or P3-6H accumulated in the nucleus. This result was not unexpected because the amino acid sequence of P3 contains the nuclear localization sequence PRRRR (Hanover, 1992). There are no reports of PLRV particles being present in the nuclei of infected cells (Shepardson et al., 1980) although infection-specific vesicles were thought to move from the cytoplasm into the nuclei of these cells. Particles of BWYV (Esau & Hoefert, 1972) and barley yellow dwarf virus (Gill & Chong, 1975) have been found in nuclei, which suggests that particles of these luteoviruses were assembled inside the nuclei. Our results show that PLRV P3 and P3-6H accumulate in the nuclei of S9 cells and suggest that assembly of VLP from P3-6H in this system is an intra-nuclear event. The coat proteins of all luteoviruses have the putative nuclear location signal and intra-nuclear assembly may therefore be a general property of these viruses. If true, the significance of this feature in the biology of luteoviruses is obscure.

In this paper, we have shown that the P3 and P4 genes of PLRV can be expressed efficiently from a recombinant baculovirus vector. A small modification to the P3 gene made it possible to produce VLP which could be purified and stored. This finding has significant potential for several fields of research with PLRV such as defining the factors needed for PLRV particles to assemble in vitro and in vivo, making physical measurements on the VLP as a close approximation to PLRV particles and biological experimentation on the functions of different parts of virus particles in their interaction with vector aphids. It was reported recently that VLP were formed in cells of Bombyx mori infected with a recombinant baculovirus containing the coat protein gene of BWYV (Tian et al., 1995), but it was not possible to purify the particles from these cells. Possibly the presence of the histidine-containing tag in P3-6H, which renders PLRV coat protein capable of forming particles, also contributes to the stability of the particles such that they can be purified.
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