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Potato virus Y helper component protein is associated with amorphous inclusions

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The distribution of the helper component (HC) protein of potato virus Y in tissues and cells of infected plants was studied by immunoblotting and immunogold labelling techniques. This HC protein was found in leaf blade and vein tissue but not in the petiole of leaves. In infected cells, the protein was localized in rod-shaped cytoplasmic inclusions known as amorphous inclusions.

Potyviruses are transmitted by aphids in a non-persistent manner. The transmission of these viruses by aphids is dependent on the presence in infected cells of a virus-encoded protein known as the helper component (HC) (Kassanis & Govier, 1971*a*, *b*; Govier & Kassanis, 1974*a*, *b*; Govier *et al.*, 1977; Thornbury & Pirone, 1983). The biologically active form of the HC proteins is believed to exist as a dimer with an M_r of 106K for the HC of tobacco vein mottling virus (TVMV) (Hellman *et al.*, 1983) and 116K for that of potato virus Y (PVY) (Thornbury *et al.*, 1985). However, SDS-gel electrophoretic analysis shows that the M_r of the monomeric HC is 53K for TVMV and 58K for PVY (Thornbury *et al.*, 1985). These values have been confirmed by nucleic acid sequence analysis (Domier *et al.*, 1986; Robaglia *et al.*, 1989). Serological cross-reactivity studies have shown that antisera to amphorous inclusion bodies produced by pepper mottle virus and papaya ringspot virus immunoprecipitate *in vitro* translation products which are identical to those immunoprecipitated by antisera to the HC proteins of PVY and TVMV (Hiebert *et al.*, 1984; DeMejia *et al.*, 1985*a*, *b*). These results suggest indirectly that the amorphous inclusion bodies produced by some potyviruses are pools or reservoirs of HC protein (DeMejia *et al.*, 1985*b*). However there is no evidence to link the amorphous inclusion protein directly with the HC protein. In this paper, using immunogold techniques, we report that the HC protein of PVY is localized in the rod-shaped cytoplasmic inclusions (Edwardson, 1974; Christie & Edwardson, 1977) produced by this virus in infected cells. These types of inclusions are categorized as 'amorphous inclusions' (Lesemann, 1988) although morphologically the rod-like inclusions found in PVY-infected plants are not similar to those found in other potyviruses such as papaya ringspot (Martelli & Russo, 1976) or pepper mottle virus (Edwardson, 1974). Apparently, the amorphous inclusions produced by different potyviruses take on different morphological forms.

Young tobacco plants (*Nicotiana tabacum* L. cv. Xanthi nc) were inoculated with an isolate of PVY obtained from Dr T. P. Pirone (University of Kentucky, Lexington, Ky., U.S.A.). Young systemically infected leaves were sampled for analysis of the presence of HC protein by Western blotting and immunogold labelling on day 15 after inoculation. The leaves were cut into leaf blades, veins and midribs, and petioles. The tissue samples were then processed for immunoblotting (Towbin *et al.*, 1979) and immunogold electron microscopy. For immunoblotting, tissue samples were ground in three volumes of Laemmli sample buffer (Laemmli, 1970), heated at 100 °C for 5 min and centrifuged at 12000 r.p.m. for 10 min at 20 °C using an SS34 rotor in an RC2B Sorvall centrifuge. The proteins from the supernatants were precipitated with 70% acetone at 4 °C and the precipitated proteins were dissolved in the Laemmli sample buffer by heating at 100 °C for 5 min. The dissolved proteins were subjected to electrophoresis in 12% SDS–polyacrylamide gels using a Bio-Rad minigel unit (Mini-PROTEAN II) and the proteins were transblotted electrophoretically onto nitrocellulose paper for Western blot analysis using normal rabbit serum or rabbit anti-HC serum. After washing this was followed by incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG. The blots were developed with bromochloroindolyl phosphate and nitroblue tetrazolium. For immunogold electron microscopy, we used a procedure modified from a previously published protocol (Baunoch *et al.*, 1988). Tissue samples were fixed at 4 °C overnight in 2·5% glutaraldehyde, 2% paraformaldehyde

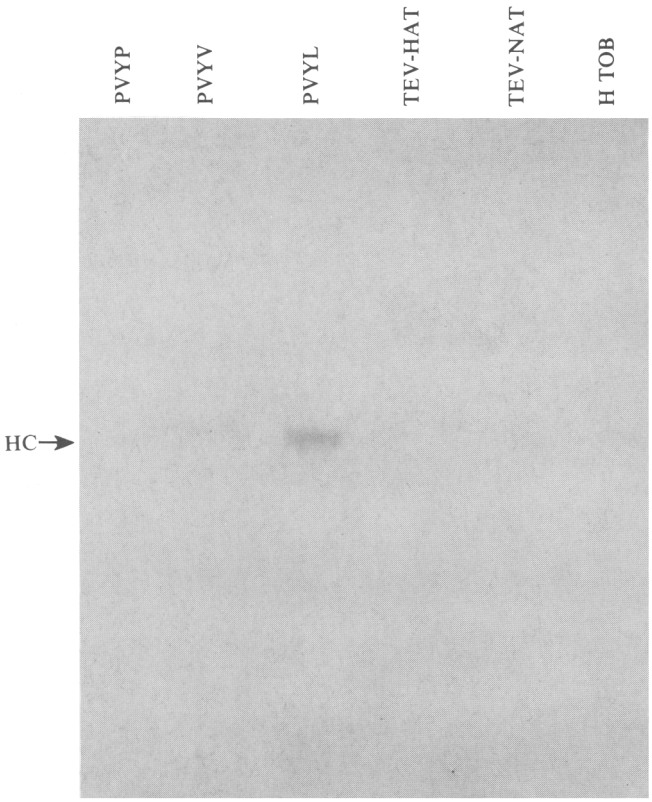

Fig. 1. Western blot analysis of tissue extracts from: petioles (PVYP), veins/midribs (PVYV) and leaf blades (PVYL) of plants infected by PVY; leaves of plants infected by an aphid-transmissible strain (TEV-HAT) and an aphid non-transmissible strain (TEV-NAT) of TEV and uninfected tobacco leaves (H TOB). The M_r of proteins was identified using the M_r of a mixture of known proteins electrophoresed simultaneously. The position of HC is marked.

in 0·2 M-cacodylate buffer pH 7·4 containing 1% sucrose. After overnight fixation, the samples were washed for 30 min in three changes of 0·2 M-cacodylate buffer pH 7·4 containing 3% sucrose. The samples were then dehydrated in a graded series of alcohol as follows: three changes, 10 min each in 50% alcohol at 4 °C, followed by three changes, 10 min each in 70% alcohol at −20 °C, three changes, 20 min each in 95% and 100% alcohol at −20 °C. After dehydration, the samples were incubated for 10 min in three changes each of 30%, 70% and 100% methyl acetate at −20 °C. They were then infiltrated sequentially, with a 1:1, 2:1 and 3:1 mixture of Lowicryl K11M (Polysciences):methyl acetate for 1 h each, at −20 °C. Finally the tissue samples were incubated for 48 h in three or four changes of Lowicryl K11M at −20 °C. During all operations from fixation to embedding in Lowicryl, the tissue samples were equilibrated in the respective solutions/reagents by gently turning the bottles containing the samples in solution by rotary shaking with the aid of a home-made tissue rotator. After embedding, the samples were photopolymerized with the aid of a u.v. light (360 nm) at −20 °C. The blocks were polymerized for 48 h. The polymerized blocks were cured at room temperature in a desiccator for 24 h before cutting. Thin sections were cut with a diamond knife and processed for immunogold labelling and electron microscopy as done previously (Baunoch *et al.*, 1988).

Preliminary immunoblotting and immunogold labelling experiments revealed that the PVY HC antiserum cross-reacted with a protein found in mitochondria of healthy plants. To remove the antibodies to the plant proteins from the HC antiserum, the serum was absorbed repeatedly with a protein extract prepared from tobacco leaves. Tobacco leaf proteins were prepared by grinding leaves in three volumes of Laemmli sample buffer followed by heating the extract at 100 °C for 15 min and centrifuging down the insoluble material. The soluble proteins were then precipitated repeatedly with cold 70% acetone and the precipitated proteins were resuspended in water. Aliquots of the plant proteins were added to the PVY HC serum, incubated at 22 °C for 1 h and centrifuged to remove insoluble material. These absorptions were repeated until the HC antiserum showed no cross-reactivity to plant proteins as tested by immunoblotting experiments. The cross-absorbed antiserum was used in all experiments reported here.

Immunoblotting experiments (Fig. 1) showed that the cross-absorbed serum reacted specifically with a protein of approximate M_r 60K found in leaf blade cells and vein and midrib cells of PVY-infected plants. The leaf petioles contained no detectable HC protein. The PVY HC antiserum did not cross-react with leaf extracts from healthy tobacco plants or from those plants infected by an aphid-transmissible strain of tobacco etch virus (TEV-HAT) or an aphid non-transmissible strain of TEV (TEV-NAT). The PVY HC antiserum did not cross-react with extracts from leaves infected by TVMV also (data not shown).

The immunogold labelling experiments showed that the HC antibodies specifically labelled rod-shaped inclusion bodies found in PVY-infected cells (Fig. 2*a, b* and *c*). These inclusion bodies were similar in appearance to the rod-like elements reportedly found in the cytoplasm of PVY-infected cells (Edwardson, 1974; Christie & Edwardson, 1977; Lesemann, 1988). The specificity of the labelling was shown by, first, the specific labelling of amorphous inclusions in PVY-infected cells by PVY HC antibodies (Fig. 2), second, the absence of immunogold labelling of amorphous inclusions or other parts of PVY-infected cells when these cells were exposed to preimmune rabbit antibodies (Fig. 3), third, the absence of immunogold labelling of cells

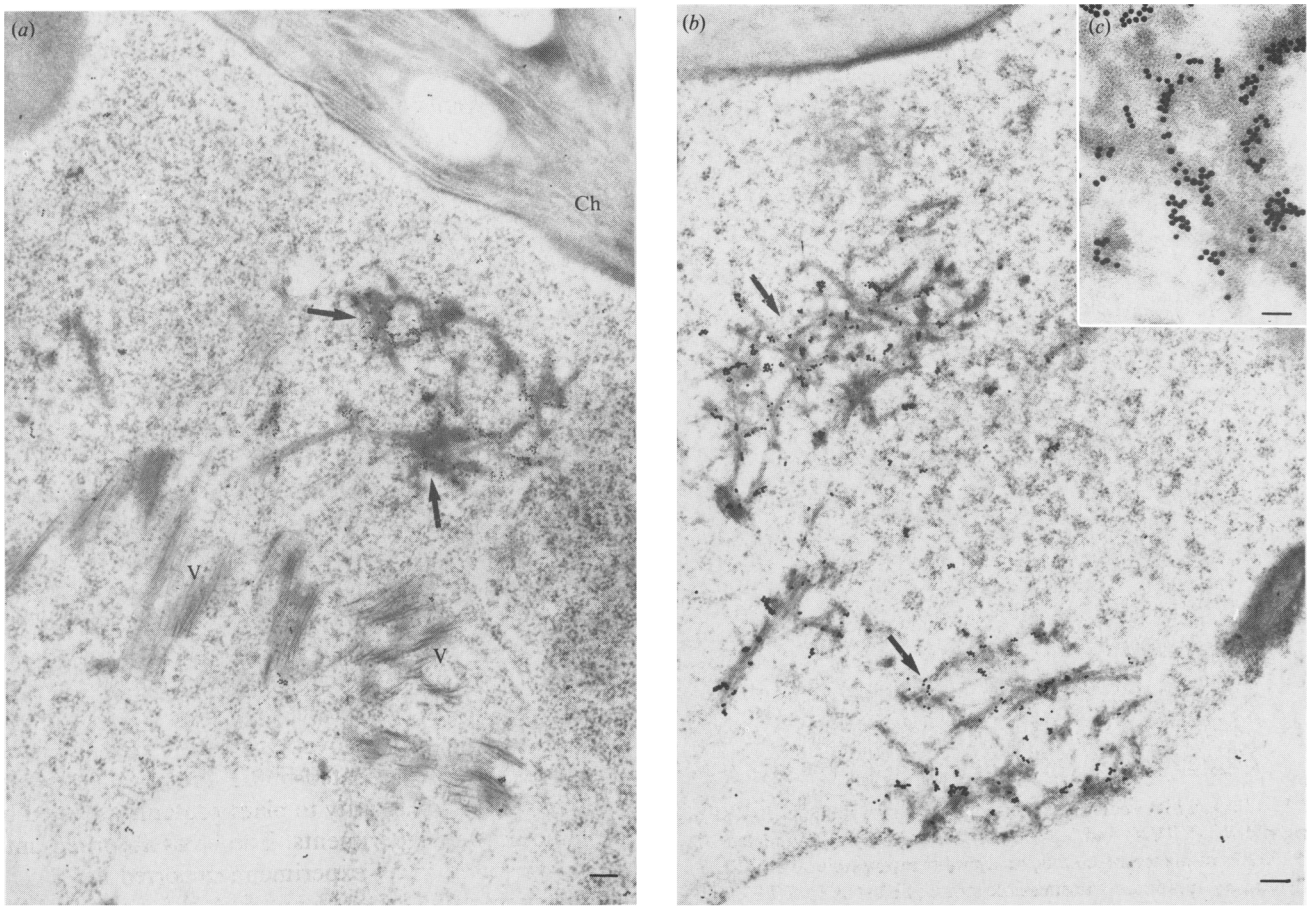

Fig. 2. Localization of HC in rod-like amorphous inclusions of PVY-infected leaves as shown by immunogold labelling. Thin sections were incubated with anti-PVY HC serum followed by gold-labelled goat anti-rabbit serum. In (*a*) virus bundles (V) as well as amorphous inclusions are seen; the latter are immunolabelled; (*b*) shows additional inclusions and (*c*) shows an enlargement of the amorphous inclusion. Bar markers represent (*a*) 125 nm, (*b*) 85 nm and (*c*) 50 nm. Arrows indicate the amorphous inclusions. Ch, Chloroplasts.

infected by TEV-HAT, TEV-NAT and TVMV and finally, the lack of labelling of uninfected cells by PVY HC antibodies.

Earlier experiments (DeMejia *et al.*, 1985*b*) had suggested that the amorphous inclusions of potyviruses may represent pools or reservoirs of the potyviral HC protein. The experiments reported here show that the HC protein synthesized by PVY in infected cells is associated with rod-like inclusion bodies which are dissimilar in morphology to the amorphous inclusions produced by other potyviruses (Edwardson, 1974; Christie & Edwardson, 1977; Lesemann, 1988). Structural differences in the morphology of amorphous inclusions produced by different potyviruses had been reported earlier (Lesemann, 1988). The lack of cross-reactivity of the PVY HC antiserum with any protein from tobacco plants infected by two strains of TEV (Fig. 1) and one of TVMV is in agreement with results of immunoprecipitation experiments wherein *in vitro* translation products programmed by TEV or TVMV RNA were not immunoprecipitated by PVY HC antiserum (Hiebert *et al.*, 1984). It is not clear where the HC proteins are located in potyviruses such as TEV where the amorphous type inclusions are not formed.

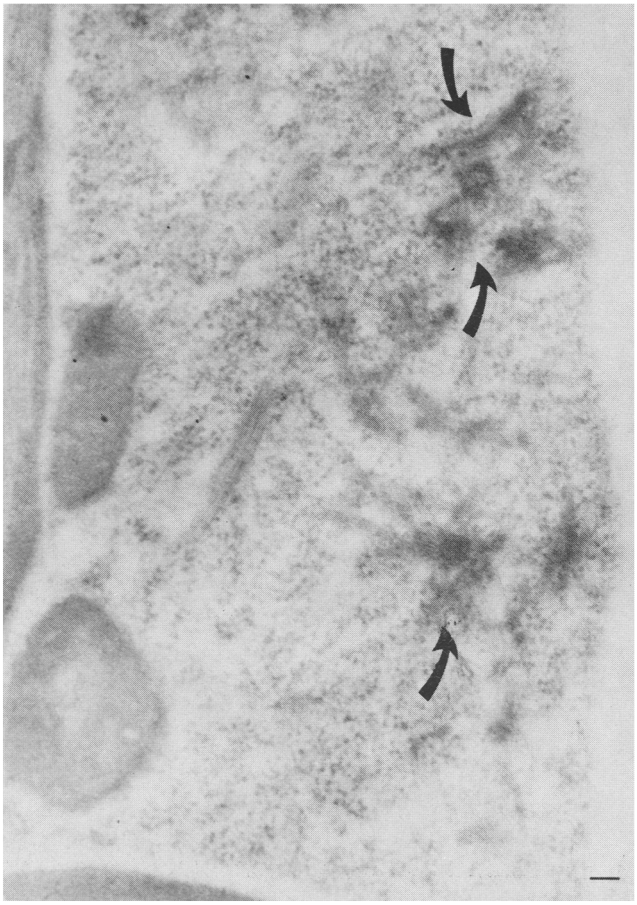

Fig. 3. Lack of immunogold labelling of amorphous inclusions (arrows) by preimmune rabbit sera. Thin sections of PVY-infected leaves were incubated with preimmune rabbit serum followed by gold-labelled goat anti-rabbit antiserum. Note that the inclusions, as well as other parts of the cell, are not immunolabelled. Arrows indicate the amorphous inclusions. Bar marker represents 100 nm.

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