Immunogold Localization of Rice Stripe Virus Particle Antigen in Thin Sections of Insect Host Cells

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Rice stripe virus (RSV)\(^\text{14}\) is the type member of the tenuivirus group. The viruses in this group are transmitted by Gramineae-feeding, delphacid planthoppers\(^\text{9}\) in which they persist and are transovarially transmitted, suggesting that they multiply in their insect hosts\(^\text{8,9}\). Koganezawa et al.\(^\text{6}\) isolated branched filamentous particles from rice stripe-diseased plant tissues. Since then, similar viruses such as maize stripe virus have been shown to possess a supercoiled filament configuration, and serological and molecular biological aspects have been examined\(^\text{2,5,11-13}\). However, little is known of their localization and distribution within insect hosts. Shikata and Galvez\(^\text{10}\) reported flexuous threadlike particles in thin sections of plant and insect hosts infected with rice hoja blanca virus. It is difficult to identify such filamentous structures with certainty in thin sections by conventional methods. We describe an investigation of the location and distribution of RSV particle antigen in insect host cells using immunogold labelling of thin sections, an approach that has proved successful with several other plant viruses\(^\text{3,15}\).

The propagation of RSV (a Shiozawa isolate) and Toyama biotype of *Laodelphax striatellus* has been described previously\(^\text{7}\). Adults of insect which had fed on the infected wheat plants for more 2 weeks, were used. Non-viruliferous insects were used as control. Insect organs were dissected from planthoppers in a drop of phosphate buffer (PB, 0.1 M, pH 7.2) on a paraffin plate. The samples were fixed in a mixture of 2% paraformaldehyde and 1% glutaraldehyde in PB for 4 hr at 4°C. The pieces of insect organs were then rinsed in PB for 3-10 min, dehydrated through an acetone series and infiltrated with Spurr\’ low viscosity epoxy resin for 2-3 hr and embedded by polymerization for 18 hr at 70°C. Ultrathin sections were cut on a Reichert Jung Om4 ultramicrotome equipped with a glass knife and mounted on Formvar-filmed nickel grids (150 mesh). For immunogold labelling, immunoglobulins purified from antiserum to RSV (containing 0.5% bovine serum albumin) were used at 10 µg/ml. Protein A- gold (Auro Probe tm EM protein AG15, Janssen Life Science Products) was diluted to 1/80 and used as the gold probe. All dilutions of immunoglobulins (IgG) and gold probe were made in PB containing 0.85% NaCl (PBS). Sections were incubated with IgG performed on Parafilm in Petri dishes for 3 hr at 4°C, then floated on drops of gold probe for 1 hr at room temperature in the same way. The grids were washed sequentially with PBS and distilled water drop by drop. The grids were drained, dried and stained with uranyl acetate and lead citrate and then examined in the electron microscope (JEOL 100B 80KV). When the labels were observed over the same areas in serial sections of the same cells, they were counted as positive reaction. Insects had been individually tested for the virus by ELISA, using homogenates of the remnant after samples were taken for electron microscopy. ELISA was performed as described previously\(^\text{12}\). The terminology used for the internal organs of planthoppers is taken from Ammar\(^\text{11}\).

By using RSV antiserum, label was found over the limiting areas containing amorphous structures in the cytoplasm neighbouring the basal membrane of epithelial cells in the midgut (Plate I-1, 2 and 3). Plate I-1 and 2 show two serial sections of the same sample, in which the same areas were labelled. It
is suggested that the labelling represents RSV antigen and is not due to contamination. No other organelles or structures were labelled. Label was also found over the limiting areas in the cytoplasm neighbouring the basal lamina of the principal salivary gland (Plate I-4). No labelling was observed within cytoplasm of accessory salivary gland cells in this experiment. Areas in the cytoplasm of follicular cells of ovarioles were labelled (Plate II-1), suggesting that RSV is transovarially transmitted. In addition, labelling was intense in the cytoplasm of the fat body, in which filamentous structures were well developed (Plate II-2). The labelled areas showing amorphous or filamentous structures were never surrounded by limiting membranes and appeared to differ from the structure of neighbouring cytoplasm in which ribosomes and endoplasmic reticulum were clearly defined. Labelling appeared to be correlated with virus antigen accumulation in the cytoplasm of the viruliferous insects. No labelling was observed within cytoplasm of the midgut, the principal salivary gland, the ovarioles and fat body of non-viruliferous insects. This appears to be the first report of the positive identification of RSV antigen in sections of viruliferous vector insects after finding the particles of RSV6).

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Literature cited

Explanation of Plates

Plate I
1 and 2. Two serial sections of midgut of a viruliferous planthopper. Note the label (arrow) located in the cytoplasm neighbouring the basal membrane (B) of an epithelial cell. Bar represents 1 μm.
3. High magnification of an affected midgut cell. Note the label is distributed over the limiting areas containing amorphous structures. Bar represents 0.5 μm.
4. Section of a principal salivary gland of a viruliferous planthopper. Note the label over an limiting area containing amorphous structure in the cytoplasm neighbouring a basal lamina (B). Bar represents 0.5 μm.

Plate II
1. Section of an ovariole of a RSV-carrying planthopper. Note the label over an amorphous area in the cytoplasm of a follicular cell (FC) beside an oocyte (Oo). Bar represents 0.5 μm.
2. Section of a fat body of a RSV-carrying planthopper. Note the intense labelling over the cytoplasm in which filamentous structures develop. Bar represents 0.5 μm.

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