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Immunogold Labeling Locates the Site of Disassembly and Transient Gene Expression of Tobacco Mosaic Virus-Like Pseudovirus Particles *In Vivo*

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Recombinant *in vitro* transcripts with a 5′ open reading frame for bacterial chloramphenicol acetyltransferase (CAT) and a 3′-proximal copy of the origin-of-assembly sequence from tobacco mosaic virus (TMV) RNA can be assembled *in vitro* into ribonucleoprotein particles ("pseudoviruses") resembling TMV. When rubbed on the lower surfaces of leaves of *Nicotiana tabacum* cv. Xanthi, both these pseudoviruses and true TMV particles were located predominantly within the cytoplasm of the epidermal cells. Cells in mock (buffer)-inoculated leaf panels, excised and fixed immediately or after 15 or 120 min, showed little or no gold-labeling when probed with polyclonal rabbit antisera to TMV coat protein, CAT, or the 126,000 Da (126 kDa) polypeptide encoded by TMV RNA. In contrast, by 15 min, the cytoplasm of epidermal cells from TMV-inoculated (control) leaf panels was strongly labeled by serum against the 126 kDa protein but not by CAT antiserum. Conversely, sections from CAT pseudovirus-inoculated leaf panels, left for 15 or 120 min, revealed particles distributed throughout the epidermal cell cytoplasm, specifically labeled by CAT antiserum. Intracellular TMV or pseudovirus particles, and those attached end-on to the outer (cuticular) surface of the cell, could be labeled with anti-TMV coat protein antiserum. At each of the three sampling times, abrasive wounds were visible in the outer walls of some epidermal cells. Clumps of TMV or pseudovirus particles had entered the cell via these wounds and, by 15 min or less, were dispersed throughout the cytoplasm. Cells in which the clumps failed to disperse, or cells that were irreparably wounded, showed little gold-labeling. Translationally active TMV or CAT mRNA remained associated with coat protein. We propose that these gold-labeled, cytoplasmic complexes may be the so-called striposomes, which can also be labeled with L-[^{35}S]methionine and isolated from epidermal cells by Cs_2SO_4 gradient centrifugation (J. G. Shaw, K. A. Plaskitt, and T. M. A. Wilson, Virology 148:326-336, 1986).

Additional key words: chloramphenicol acetyltransferase, electron microscopy, immunogold labeling, pseudovirus particles, tobacco epidermis, tobacco mosaic virus, transient gene expression

The site(s) and mechanism(s) of plant RNA virus disassembly and early gene expression *in vivo* remain unknown and the subject of much speculation (Shaw 1985; Wilson 1985; Gaard and De Zoeten 1979; De Zoeten 1981; De Zoeten and Gaard, 1984). Logic predicts, and numerous experiments (Kontaxis 1961; Fry and Matthews 1963; Fannin and Shaw 1987) confirm that, during the mechanical inoculation of a leaf, most, if not all, parental plant virus particles (i.e., those from the inoculum) interact first with the epidermal cells. Occasionally, during inoculation of the lower leaf surface, some virions may pass directly into the mesophyll cells (Sulzinski and Zaitlin 1982). Although plant viruses are considered to be wound pathogens, controversy still surrounds the role of wounding in the earliest events of infection, notably the processes of cell entry and virus uncoating (De Zoeten and Gaard 1984).

End-on attachment of tobacco mosaic virus (TMV) or tobacco rattle virus particles to the cuticle or outer surface of plant cell walls (Gerola *et al.* 1969; Gaard and De Zoeten 1979) prompted the view that these rod-shaped virus particles were destabilized and uncoated extracellularly (Gaard and De Zoeten 1979), a view apparently supported by the presence of TMV coat protein in the cell walls of infected tissues (De Zoeten and Gaard 1984). In such a process, naked viral RNA must traverse the cellulose cell wall and plasmalemma to enter the cytoplasm and initiate infection.

Contrary to this view, we believe that predominantly intact virus particles enter the epidermal cell cytoplasm directly through the sites of wounding, in agreement with earlier observations (Rawlins and Tompkins 1936; Kontaxis and Schlegel 1962). Once within the cytoplasm, components of the cellular protein synthetic machinery may then uncoat the parental viral RNA sequentially and cotranslationally (Shaw *et al.* 1986), as was first shown to occur *in vitro* in a wide range of cellfree translation systems (Wilson 1984a, 1984b, 1986).

To test this hypothesis, we constructed TMV-like ribonucleocapsids ("pseudoviruses"; Sleat *et al.* 1986) containing convenient reporter gene transcripts, the *in vivo* uncoating and expression of which can be detected enzymatically (Gallie *et al.* 1987a) or located immunologically. In this report, pseudovirus particles containing a chimeric RNA with a 5′-proximal open reading frame for bacterial (Tn9) chloramphenicol acetyltransferase (CAT) were rubbed onto the lower surfaces of healthy tobacco leaves that had been dusted with an abrasive. These pseudoviruses do not replicate, thus the distribution and location of parental rod-shaped particles can be observed unambiguously. Furthermore, transient expression of the CAT antigen may be detected by immunogold-labeling of thin tissue sections with polyclonal antiserum to CAT.

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METHODS AND MATERIALS

Virus and pseudovirus preparation. For control inoculations, TMV (U1, common or *vulgare* strain; Cambridge isolate) was prepared by the method of Leberman (1966). The recombinant plasmid pJII102 (Gallie *et al.* 1987a, 1987b) contained the bacteriophage SP6 promoter and the following contiguous cDNA sequences: a derivative of the 5′-leader of TMV RNA (Ω′; Gallie *et al.* 1987b), Tn9-derived CAT mRNA, and the origin-of-assembly sequence from TMV RNA. pJII102 was used as a transcription template *in vitro*, after linearization with *Bgl*II. Transcription was initiated using the cap analogue $G^{5'}ppp^{5'}G$ and the resulting chimeric RNAs, 1.2 kb long, were spontaneously assembled *in vitro* into TMV-like pseudovirus particles 60 nm long (Gallie *et al.* 1987a) using a disk preparation of TMV coat protein as previously described (Sleat *et al.* 1986). These ribonucleocapsids were recovered and purified by ultracentrifugation in a Beckman Type 40 rotor at 36,000 rpm for 90 min at 4° C and resuspended in water.

Inoculation procedure. Expanding leaves (10–15 cm long) of *Nicotiana tabacum* cv. Xanthi were inoculated while attached to plants 30–45 cm tall. One leaf panel on the lower surface was dusted lightly with diatomaceous earth (Celite), and 20 μl of 10 mM sodium phosphate buffer, pH 7.0, was applied either alone (mock) or containing 8 mg/ml TMV (control) or 6–8 mg/ml CAT-pseudovirus particles. The leaf panel was rubbed gently with a gloved forefinger, excess inoculum was not rinsed off, and the tissue was excised with a razor blade either immediately or after 15 or 120 min under laboratory conditions of light and temperature.

Processing of leaf tissue. The excised, inoculated area of each leaf was immersed in 2–3 ml of 2.0% (v/v) glutaraldehyde in 0.05 M sodium cacodylate-HCl buffer, pH 7.2, at room temperature, cut into strips (approximately 0.5 × 2.0 mm), and left overnight. The minimum time from inoculation to fixation of tissue strips was between 90 and 120 sec. The strips of tissue were dehydrated in a series of precooled ethanol solutions (Hills *et al.* 1987): 30% at 0° C, 50% at −20° C, and 70–100% at −35° C and then infiltrated with increasing concentrations of L. R. White medium grade resin (London Resin Co. Ltd., Woking, Surrey) containing 0.5% benzoin methyl ether in ethanol at −20° C. The tissue blocks were polymerized by UV light at −20° C in N_2 gas for 24 hr. Polymerization was completed by UV light at room temperature for 16 hr. Embedded tissue was sectioned using a Reichert-Jung Ultracut ultramicrotome with glass knives, and the sections were mounted on 600 hexagonal mesh gold grids. Polyclonal rabbit antisera were raised against TMV coat protein, the 126,000 dalton (126 kDa) polypeptide of TMV (Hills *et al.* 1987; a gift from M. Zaitlin) or electrophoretically pure CAT prepared by

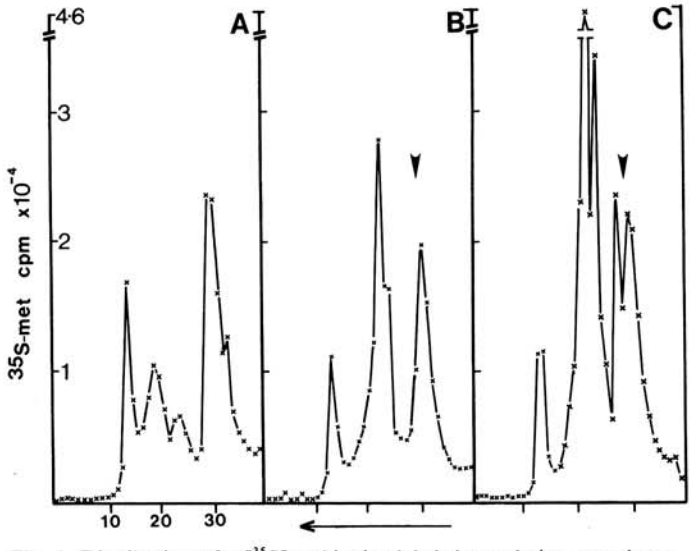

Fig. 1. Distribution of L-[^{35}S]methionine-labeled translation complexes isolated from strips of tobacco epidermis peeled and collected 15–45 min after sample application. Each sample (20 μl) contained 200 μCi L-[^{35}S]methionine and buffer, either **A**, alone or including **B**, 400 μg pseudovirus particles containing chloramphenicol acetyltransferase (CAT) mRNA **C**, 400 μg tobacco mosaic virus (TMV). Sedimentation in gradients of Cs_2SO_4 was from right to left in each case. The solid arrows mark the buoyant density position of uncomplexed pseudovirus or TMV particles, or fragments thereof, containing 5% (w/w) RNA.

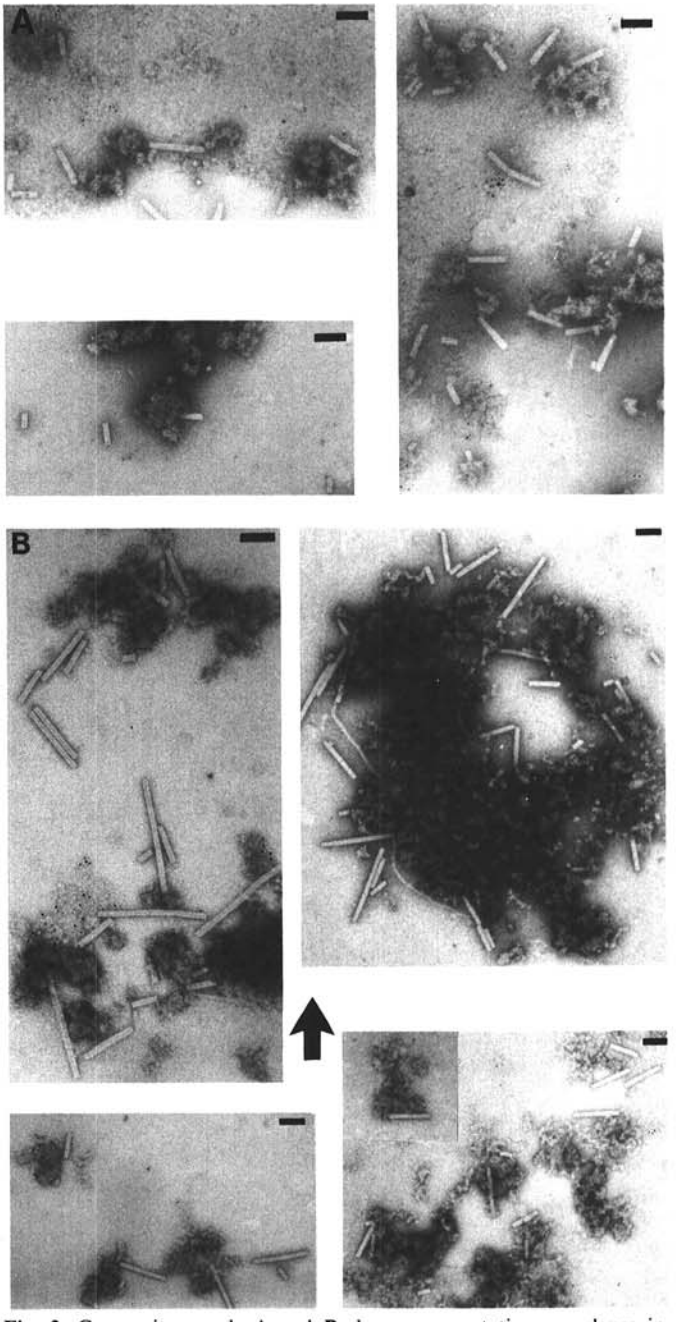

Fig. 2. Composite panels **A** and **B** show representative complexes in fractions 20–25 from gradients B and C (Fig. 1), respectively. Fractions were pooled separately, desalted, spotted directly onto carbon-coated copper grids, and negatively stained with uranyl acetate. Bars = 100 nm.

affinity chromatography (a gift from W. V. Shaw, University of Leicester, England). Before use, each of these primary antisera was diluted 1:100 in 0.2 M Tris-HCl buffer, pH 7.4, containing 1% (v/v) Tween 20, 0.1% (w/v) gelatin, and 1% (w/v) bovine serum albumin. Tissue sections (70 nm thick) were incubated overnight at 4° C in diluted primary antiserum, washed six times in water or 1% (v/v) dilution buffer (above), and finally, for immunogold-labeling, incubated for 1 hr at room temperature in a 1:30 dilution of 15 nm gold-conjugated, goat antirabbit IgG antiserum (Janssen Auroprobe EM GAR G15, Life Sciences Products, Janssen Pharmaceuticals Ltd., Grove, Wantage, Oxford). After thorough washing, the sections were poststained with uranyl acetate and lead acetate-tartrate (Millonig 1961). Tissue sections were viewed in a Siemens 1A or JEOL JEM 1200EX electron microscope.

Isolation and fractionation of ^{35}S-labeled *in vivo* complexes. The lower surfaces of three expanding tobacco leaves were each rubbed with 100 μl of 10 mM sodium phosphate buffer, pH 7.0, containing 200 μCi L-[^{35}S]methionine (New England Nuclear, $\geqslant$1,100 Ci/mmol) either alone ("mock-inoculated") or in the presence of 400 μg TMV or 400 μg CAT pseudovirus particles. Strips of epidermal cells were peeled and collected separately from each leaf 15–45 min after inoculation. ^{35}S-labeled translation complexes were then extracted and separated on isopycnic Cs_2SO_4 gradients, as described previously (Shaw *et al.* 1986).

RESULTS

***In vivo* radiolabeling of striposome-like complexes containing CAT-pseudovirus particles.** To complement recent studies on the uncoating and expression of CAT mRNA contained in TMV-like pseudovirus particles in tobacco mesophyll protoplasts, pea epidermal cells and *Xenopus laevis* oocytes (Gallie *et al.* 1987a) and to extend previous work with TMV-inoculated tobacco epidermis (Shaw *et al.* 1986), pseudovirus particles containing CAT mRNA were rubbed onto the lower surface of a tobacco leaf in the presence of L-[^{35}S]methionine. Strips of epidermis were removed, frozen, and extracted as before (Shaw *et al.* 1986). The resulting distribution of radioactively labeled complexes in a Cs_2SO_4 gradient is shown in Figure 1B. For comparison, complexes from a TMV-inoculated control leaf are shown in Figure 1C. The ^{35}S-counts peak at fractions 13–15 in each gradient, including the mock-inoculated control (Fig. 1A), probably represents plant polyribosomes. The ^{35}S-counts peak centered on fraction 30 marks the position of pigmented cellular debris at the original interface between the overlayered aqueous sample and the Cs_2SO_4 column. Material in the central peak of radioactivity (fractions 20–25 in Figs. 1B and C) had many of the physicochemical properties of putative "striposome" complexes (Shaw *et al.* 1986). As reported previously, these complexes contain nascent, high molecular weight, radiolabeled polypeptides (data not shown) in addition to numerous shorter-than-full-length TMV-like pseudovirus particles or TMV, which are easily identified in the electron microscope (Figs. 2A and B). Rodlets in radiolabeled complexes from pooled gradient fractions 20–25 (Figs. 1B and C) had a higher buoyant density than that of uncomplexed, intact or fragmented ribonucleocapsids (banding position denoted by the solid arrows in Figs. 1B and C.). We conclude that some of our CAT-pseudovirus particles had undergone structural modifications similar to

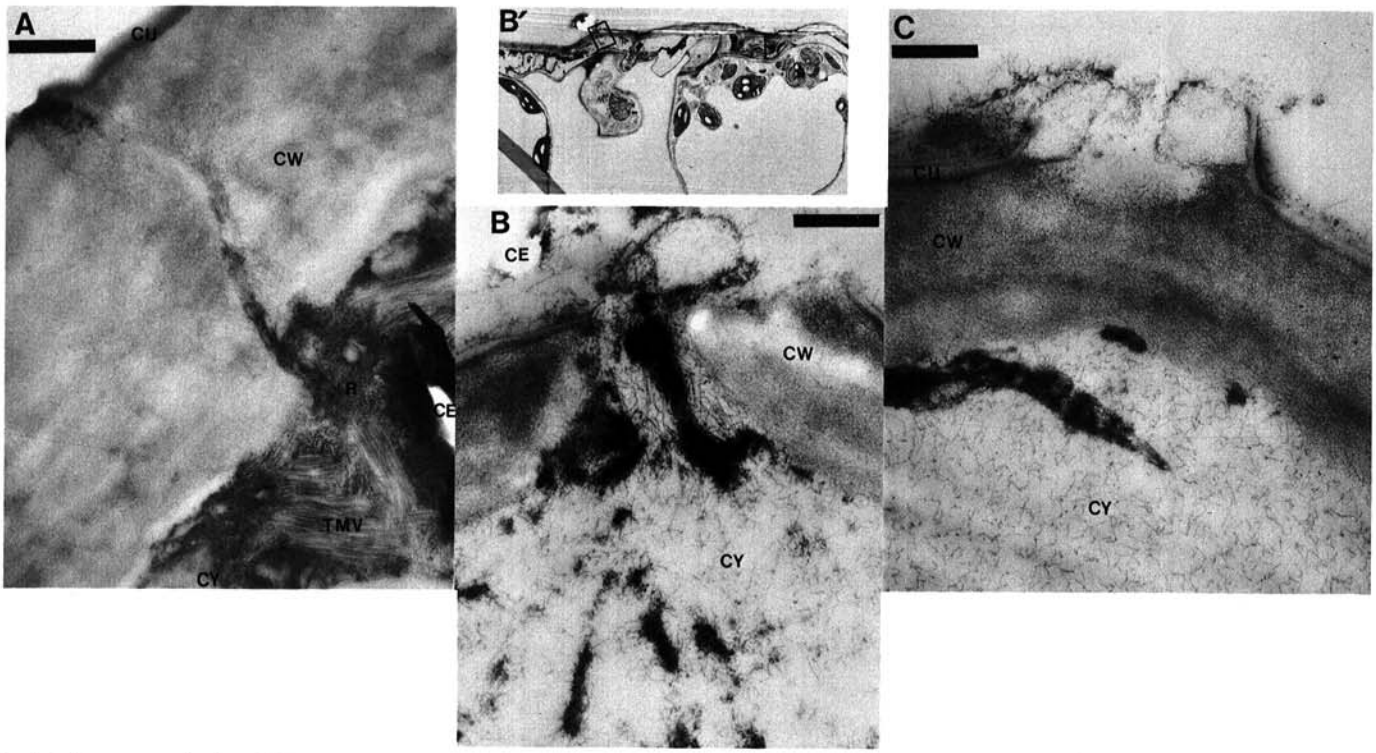

Fig. 3. Tobacco mosaic virus (TMV) or pseudoviruses enter cells through wounds caused by abrasive. The lower epidermis of a tobacco leaf was inoculated with TMV (**A**) or CAT-pseudovirus (**B′, B, C**) particles in the presence of Celite. Tissue was excised and fixed immediately (**A**) or after 120 min (**B′, B, C**). Sections were negatively stained without prior immunogold-labeling. At very low magnification of several epidermal and spongy mesophyll cells, **B′** shows scoring damage caused by the particle of abrasive. **B**, the boxed area of B′ is enlarged to show filamentous pseudovirus particles entering the cytoplasm. **C**, pseudovirus particles are well-distributed in the cytoplasm beneath what may be a partially repaired wound in the cell wall. Scale bars = 500 nm. (*CE*, Celite particle or its site after removal by the sectioning knife; *CU*, cuticle; *CW*, cell wall; *CY*, cytoplasm; *R*, ribosomes)

those reported for TMV (Shaw *et al.* 1986) during the early events of plant cell infection. Because the former do not replicate and contain a sensitive reporter gene sequence, they may be used to probe the site and possible mechanism of parental ribonucleocapsid disassembly *in vivo*.

The availability of a high-titer, monospecific antiserum to CAT, a protein alien to plant cells, provided us with an opportunity to locate the site of formation of these putative striposome complexes *in vivo* by immunogold-labeling of the nascent polypeptides *in situ*.

Location and entry route of unlabeled TMV or pseudovirus particles in inoculated leaf sections. Rod-shaped ribonucleocapsids are easily distinguished in electron micrographs of negatively stained tissue sections. In all leaf sections examined, where parental virus or pseudovirus particles were visible, they were restricted to the cytoplasm of epidermal cells. Few, if any, particles were seen in mesophyll cells. Occasionally, wounds caused by the abrasive were visible. At these positions, large numbers of ribonucleocapsids were seen to have entered the cell.

In these experiments, very high numbers of ribonucleocapsids (approximately 2×10^{12} TMV particles or 10^{13} CAT pseudovirus particles) were applied to each leaf panel to facilitate subsequent localization and maximize any gold-labeling. Consequently, the density of particles observed entering via lesions (Figs. 3A–C) probably exceeds by a factor of about 10^2 that expected in routine leaf inoculations using virus concentrations between 10 and 100 μg/ml.

Immediately after inoculation with TMV, some

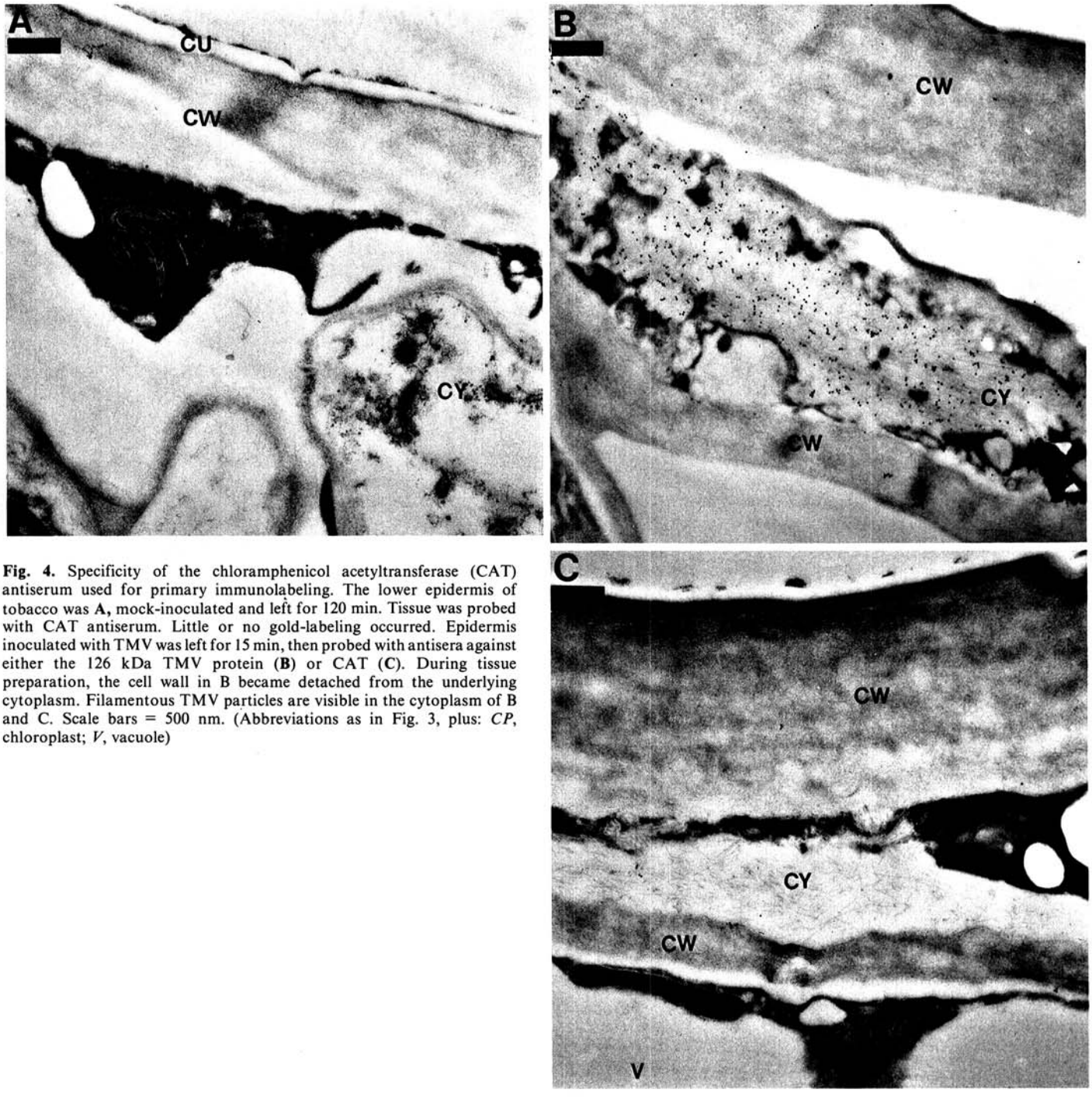

Fig. 4. Specificity of the chloramphenicol acetyltransferase (CAT) antiserum used for primary immunolabeling. The lower epidermis of tobacco was **A**, mock-inoculated and left for 120 min. Tissue was probed with CAT antiserum. Little or no gold-labeling occurred. Epidermis inoculated with TMV was left for 15 min, then probed with antisera against either the 126 kDa TMV protein (**B**) or CAT (**C**). During tissue preparation, the cell wall in B became detached from the underlying cytoplasm. Filamentous TMV particles are visible in the cytoplasm of B and C. Scale bars = 500 nm. (Abbreviations as in Fig. 3, plus: *CP*, chloroplast; *V*, vacuole)

epidermal cells showed grains of Celite within the cytoplasm (Fig. 3A). In this case, the damaged cell wall revealed a possible entry route for a dense, quasicrystalline array of TMV particles in the underlying cytoplasm.

In sections prepared 15 or 120 min after rubbing with CAT-pseudoviruses (or TMV), the ribonucleocapsids were usually dispersed more uniformly throughout the cytoplasm (Figs. 3B and C). Occasionally, a ruptured cell wall appeared to be in a state of repair (Fig. 3C).

Immunological specificity of the CAT antiserum *in vivo*. Before using the polyclonal CAT antiserum to locate the site(s) of early, transient CAT mRNA expression, several control experiments were done to confirm the specificity of the reaction. Tobacco leaf extracts have a low endogenous CAT-like enzyme activity (Gallie *et al.* 1987a, 1987b), which could prove disadvantageous for immunogold-labeling experiments with antiserum to bacterial CAT. Sections from mock-inoculated leaf panels excised immediately or after 15 or 120 min (Fig. 4A) revealed a low, background level of gold-labeling with CAT antiserum. Labeling occurred in all cell types throughout the tissue and was located mainly within chloroplasts or at the periplasmic membrane.

A leaf panel was processed 15 min after inoculation with 20 μl of 8 mg/ml TMV and probed with antisera to either the TMV 126 kDa protein or CAT. The results (Figs. 4B and C) confirmed the specificity of both the 126 kDa and CAT antisera. The high frequency of anti-126 kDa-specific gold-labeling (Fig. 4B) contrasts dramatically with the same leaf sample probed with CAT antiserum (Fig. 4C). The latter result also confirms the absence of any nonspecific, wound-induced cross-reaction with CAT antiserum (Fig. 4A).

Site of pseudovirus disassembly and 5′-reporter gene expression *in vivo*. To locate the site of 5′-gene expression, and hence the likely site of ribonucleocapsid disassembly, pseudovirus particles containing CAT mRNA were rubbed onto tobacco leaf panels with abrasive. After 120 min, the distribution of pseudovirus particles and any released coat

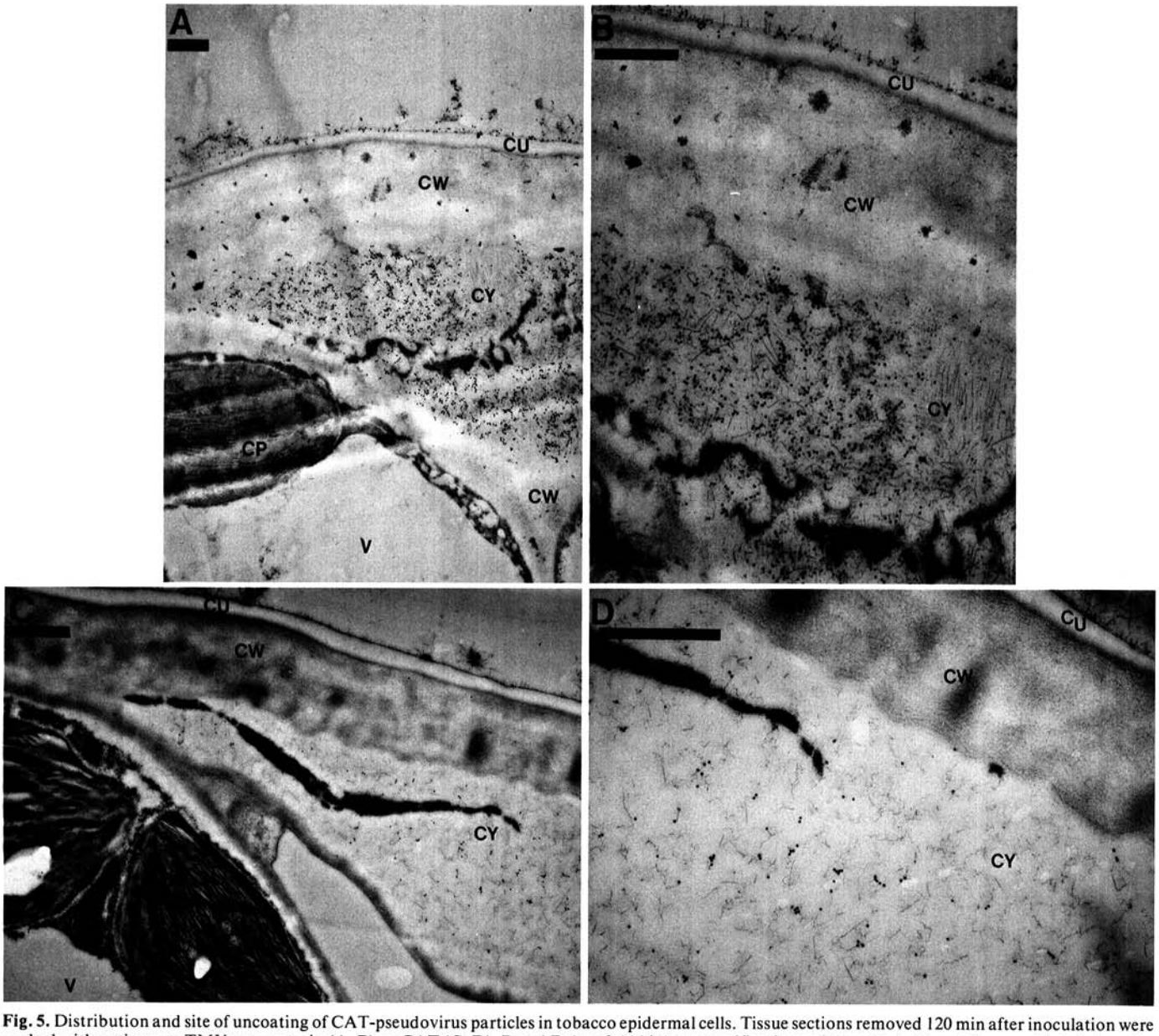

Fig. 5. Distribution and site of uncoating of CAT-pseudovirus particles in tobacco epidermal cells. Tissue sections removed 120 min after inoculation were probed with antisera to TMV coat protein (**A, B**) or CAT (**C, D**). **B** and **D** (twofold higher magnifications of the cells in **A** and **C**, respectively) show the association of colloidal gold with rod-shaped particles. Particles attached end-on to the outside of the cells were labeled by coat protein (A,B) but not by CAT (C,D) antiserum. Scale bars = 500 nm. (Abbreviations as in Figs. 2 and 3)

protein subunits could be observed using antiserum to purified TMV capsid protein (Figs. 5A and B). Particles attached predominantly end-on to the outer waxy (cuticular) surface of the cell wall, as well as those within the cytoplasm of the epidermal cells, became heavily labeled with gold. Very little coat-specific gold-label was seen in cells of the spongy mesophyll or within the epidermal cell wall itself. When similar tissue sections were probed with anti-CAT serum, only material in the cytoplasm of the epidermal cells became gold-labeled (Figs. 5C and D). Pseudovirus particles outside the cell did not express CAT antigen and there was no significant gold-labeling of the mesophyll cells after 120 min, apart from a low background signal in the chloroplast compartment (Fig. 5C). The latter observations provide a valuable internal control, in addition to Figures 4A and 4C, for the specificity of the localized reponse to CAT-antiserum shown in Figures 5C and D.

Tissue sections removed and fixed immediately (Fig. 3A) or after 15 min (Fig. 6A) often revealed clumps of ribonucleocapsids within the epidermal cells. When probed with anti-CAT antiserum (Fig. 6A) and compared with inoculated tissue that had been left for 120 min (Figs. 5C and D), cells with clumps of particles showed relatively little gold-labeling.

Expression of CAT antigen (Figs. 5C and D) or TMV 126 kDa protein (Fig. 4B) occurred in cells exhibiting little or no overt damage to the cell wall. While we cannot exclude the possibility that serial sections of these cells might reveal an open or repairing wound as seen in Figures 3A–C, seriously damaged epidermal cells (with disrupted metabolism) may be unable to uncoat TMV or pseudovirus particles and so fail to support 5′ gene expression. Figure 6B shows a section of an epidermal cell that retained a severe wound 120 min after rubbing with CAT-pseudovirus particles and abrasive. The density of CAT-specific gold-labeling is much lower than in Figures 5C and D and approaches the background levels seen in the mock- or TMV-inoculated controls (Figs. 4A and C).

DISCUSSION

Studies on many independent leaf samples, including those shown here, indicate that very large numbers of TMV or CAT-pseudovirus particles enter the epidermal cells, almost exclusively. Entry probably occurred via abrasive lesions, sometimes still visible in the outer cell wall (Figs. 3A–C). Antiserum to TMV coat protein detected ribonucleocapsids attached predominantly end-on to the outer surface of the epidermal cell wall and distributed throughout the epidermal cell cytoplasm (Fig. 5A and B), without detectable labeling of adjacent mesophyll cells. In contrast to the conclusions of De Zoeten and Gaard (1984), neither the cell wall nor the apoplast became labeled.

After inoculation, clusters of small spherical electron-dense structures, possibly ribosomes, were seen at the site of entry of clumps of TMV (Fig. 3A) or pseudovirus particles (Fig. 6A). At later times (15 min or more), the rod-shaped particles usually became more dispersed throughout the cytoplasm of the epidermal cells (Figs. 3B and C, 4B and C, 5A–D, 6B), even when there was little evidence of particle uncoating and mRNA expression due to serious wounding (Fig. 6B).

The role of abrasive injury in plant virus infection has long been a subject for speculation (Yarwood 1957). Logic suggests that extracellular abrasive particles injure the cell wall (Figs. 3A–C, 6B) and may actually enter the cell (Fig. 3A). In either case, if the damage is reparable (Fig. 3C), the cellular metabolism may recover sufficiently to allow early viral functions (uncoating and 5′-gene expression) to proceed (Figs. 4B, 5C and D). If the damage is too severe (Figs. 3B, 6B), then little or no early viral gene expression may occur (Fig. 6B).

Expression of either the 126 kDa polypeptide of TMV (Fig. 4B) or the pseudoviral CAT antigen (Figs. 5C and D) was first detected throughout the cytoplasm of infected or "pseudo-infected" epidermal cells 15 min after inoculation. Our control experiment with TMV and anti-126 kDa

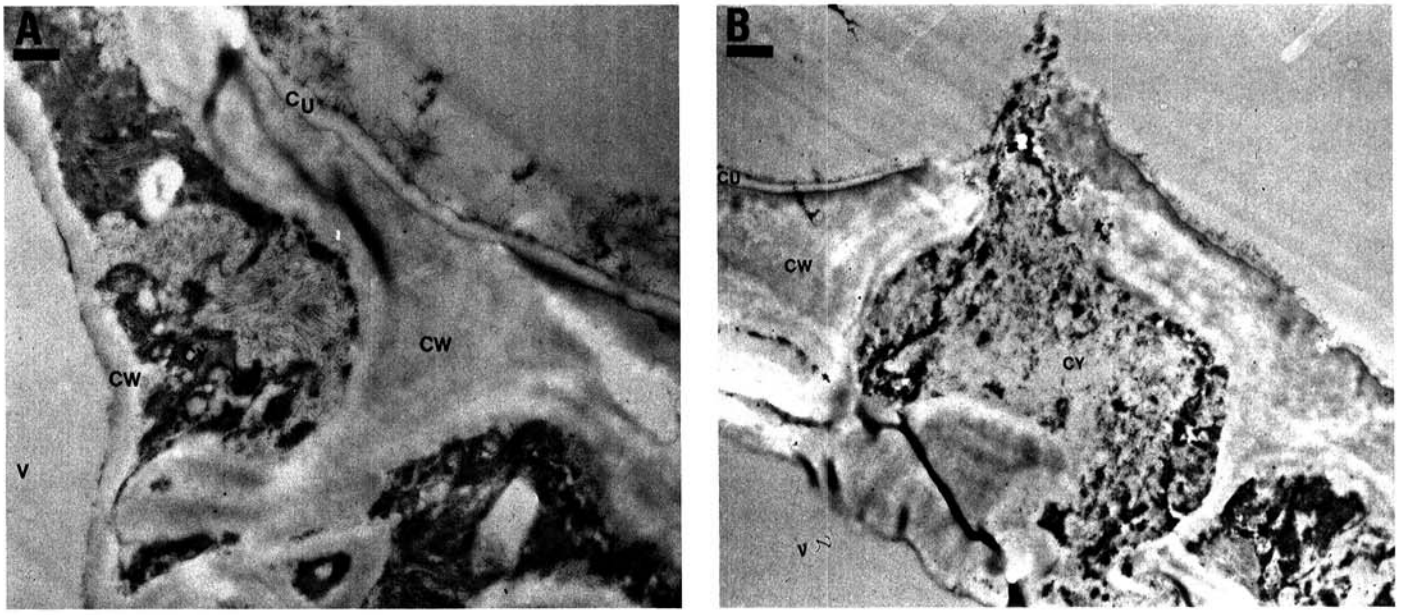

Fig. 6A. Cells in tissue sections removed and probed with CAT antiserum 15 min after inoculation occasionally retained intracellular clumps of pseudovirus particles with low levels of gold-labeling. **B,** Irreparably damaged epidermal cells exhibited low levels of CAT antigen, even 120 min after inoculation with CAT pseudovirus particles. Scale bars = 500 nm. (Abbreviations as in Figs. 3 and 4)

protein serum (Fig. 4B) confirms the tissue-specific and subcellular location of particle disassembly and early gene expression observed in independent experiments with laboratory-made pseudovirus particles and CAT-antiserum (Figs. 5C and D). In Figure 5C, the absence of gold-labeling in the mesophyll cell on the left provides a useful internal control for the specificity of our probe.

The precise distribution of many of the colloidal gold spheres in these micrographs (Figs. 4B, 5C and D) suggests that intermediate translation complexes consisting of partially uncoated virus or pseudovirus particles ("striposomes"; Wilson 1984a) are the principal site of antigen synthesis. We attempted, therefore, to immunogold-decorate the ^{35}S-labeled, "striposome"-like complexes (Figs. 2A and B) recovered from Cs_2SO_4 gradient fractions 20–25 (Figs. 1B and C). So far this approach has been unsuccessful, despite attempts to remove salt ions.

The 5′-leader sequence of the encapsidated CAT mRNA construct differed from that of TMV RNA in several important respects (Gallie *et al.* 1987b, Fig. 1), although the former contained a derivative of the TMV RNA leader sequence (Ω). It appears that the CAT pseudovirus particles disassembled efficiently, judged by the level of immunogold-labeling of the resulting CAT antigen (Figs. 5C and D). However, as a consequence of their modified 5′-sequence, these pseudoviruses may be less efficient than TMV in forming "striposomes." This could be judged by the different extents of labeling of nascent polypeptides with L-[^{35}S]methionine in Figures 1B and C (fractions 20–25), despite the fivefold molar excess of pseudovirus particles originally applied to the leaf (Fig. 1B). CAT (24 kDa) and the TMV 126 kDa protein contain 0.38 and 0.27 methionine residues per kilodalton, respectively. The absolute efficiency of (cotranslational) disassembly of pseudovirus particles, as a function of the precise 5′-leader sequence, remains to be studied. The immunological detection of CAT mRNA expression reported here supports results from the enzymatic approach previously used *in vivo* (Gallie *et al.* 1987a).

We believe that the technique of immunogold-labeling, in conjunction with nonreplicating pseudovirus particles containing mRNA for a sensitive reporter gene, provides evidence for the physiological site of TMV-like ribonucleocapsid disassembly and 5′-proximal gene expression *in vivo*. Our results do not support the hypothesis of extracellular disassembly of parental rod-shaped plant viruses in the cell wall or apoplast (Gaard and De Zoeten 1979; De Zoeten and Gaard 1984).

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LITERATURE CITED

De Zoeten, G. 1981. Early events in plant virus infection. In: Plant Diseases and Vectors. K. Maramorosch and K. F. Harris, eds., pp. 221-239. Academic Press, New York.

De Zoeten, G., and Gaard, G. 1984. The presence of viral antigen in the apoplast of systemically virus-infected plants. Virus Res. 1:713-725.

Fannin, F. F., and Shaw, J. G. 1987. Evidence for concurrent spread of tobacco mosaic virus from infected epidermal cells to neighboring epidermal and mesophyll cells. Plant Sci. In press.

Fry, P. R., and Matthews, R. E. F. 1963. Timing of some early events following inoculation with tobacco mosaic virus. Virology 19:461-469.

Gaard, G., and De Zoeten, G. 1979. Plant virus uncoating as a result of virus-cell wall interactions. Virology 96:21-31.

Gallie, D. R., Sleat, D. E., Watts, J. W., Turner, P. C., and Wilson, T. M. A. 1987a. In vivo uncoating and efficient expression of foreign mRNAs packaged in TMV-like particles. Science 236:1122-1124.

Gallie, D. R., Sleat, D. E., Watts, J. W., Turner, P. C., and Wilson, T. M. A. 1987b. The 5′-leader sequence of tobacco mosaic virus RNA enhances the expression of foreign gene transcripts in vitro and in vivo. Nucleic Acids Res. 15:3257-3273.

Gerola, F. M., Bassi, M., Favali, M. A., and Betto, E. 1969. An electron microscopic study of the penetration of tobacco mosaic virus into leaves following experimental inoculation. Virology 68:380-386.

Hills, G. J., Plaskitt, K. A., Young, N. D., Dunigan, D. D., Watts, J. W., Wilson, T. M. A., and Zaitlin, M. 1987. Immunogold localization of the intracellular sites of structural and non-structural tobacco mosaic virus proteins. Virology. In press.

Kontaxis, D. G. 1961. Movement of tobacco mosaic virus through epidermis of *Nicotiana glutinosa* leaves. Nature 192:581-582.

Kontaxis, D. G., and Schlegel, D. E. 1962. Basal septa of broken trichomes in *Nicotiana* as possible infection sites for tobacco mosaic virus. Virology 16:244-247.

Leberman, R. 1966. The isolation of plant viruses by means of "simple" coacervates. Virology 30:341-347.

Millonig, G. 1961. Modified procedure for lead staining of thin sections. J. Biophys. Biochem. Cytol. 11:736-739.

Rawlins, T. E., and Tompkins, C. M. 1936. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology 26: 578-58 .

Shaw, J. G. 1985. Early events in plant virus infections. In: Plant Molecular Virology. J. W. Davies ed., Vol. 2. pp. 1-21. CRC Press, Boca Raton, FL.

Shaw, J. G., Plaskitt, K. A., and Wilson, T. M. A. 1986. Evidence that tobacco mosaic virus particles disassemble cotranslationally in vivo. Virology 148:326-336.

Sleat, D. E., Turner, P. C., Finch, J. T., Butler, P. J. G., and Wilson, T. M. A. 1986. Packaging of recombinant RNA molecules into pseudovirus particles directed by the origin-of-assembly sequence from tobacco mosaic virus RNA. Virology 155:299-308.

Sulzinski, M. A., and Zaitlin, M. 1982. Tobacco mosaic virus replication in resistant and susceptible plants: in some resistant species virus is confined to a small number of initially infected cells. Virology 121:12-19.

Wilson, T. M. A. 1984a. Cotranslational disassembly of tobacco mosaic virus in vitro. Virology 137:255-265.

Wilson, T. M. A. 1984b. Cotranslational disassembly increases the efficiency of expression of TMV RNA in wheat germ cell-free extracts. Virology 138:353-356.

Wilson, T. M. A. 1985. Nucleocapsid disassembly and early gene expression by positive-strand RNA viruses. J. Gen. Virol. 66:1201-1207.

Wilson, T. M. A. 1986. Expression of the large 5′-proximal cistron of tobacco mosaic virus by 70S ribosomes during cotranslational disassembly in a prokaryotic cell-free system. Virology 152:277-279.

Yarwood, C. E. 1957. Mechanical transmission of plant viruses. Adv. Virus Res. 4:243-278.