POSSIBILITIES FOR INTER- AND INTRACELLULAR TRANSLOCATION OF SOME ICOSAHEDRAL PLANT VIRUSES

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ABSTRACT
Southern bean mosaic virus (SBMV) and Tomato ringspot virus (TomRV) were compared with regard to possible ways of inter- and intracellular translocation. The pore complexes in the nuclear membranes of nuclei in leaf palisade and mesophyll cells of several plant species commonly used in plant virus research were studied. The pore structure resembled that earlier described. The diameter of the pores was great enough to allow icosahedral plant viruses between 25 and 30 nm wide to move through. SBMV occurred in noncrystalline form in nuclei of infected cells. Although this virus forms paracrystalline structures when partially purified, no virus crystals were seen in the cytoplasm of cells containing high concentrations of SBMV. It was established that this virus could move through nuclear pores. TomRV was found in infected leaf tissue in low concentrations. This virus showed a tendency to crystallize even when present in low concentrations. TomRV was observed only in the cytoplasm, not in nuclei. This virus was present in plasmodesmata, indicating the possibility of cell to cell translocation of whole particles through these structures.

INTRODUCTION
Plant viruses have been reported in nuclei (5, 8, 14), chloroplasts (5), and the cytoplasm (3) of infected plant cells of many different origins. Many of these viruses have been reported in association with membrane systems of these organelles and other cellular membranes like endoplasmic reticulum (9) and the dictyosomes.

The rapid translocation of plant viruses, called long distance transport (2), is thought to occur in the vascular tissue. Schneider and Worley (11) implicated tracheary elements and phloem in long distance transport of the icosahedral Southern bean mosaic virus (SBMV). Worley (16) showed, by means of fluoresceine-labeled antibody, that SBMV antigen was present in phloem elements. By electron microscopy, Esau and coworkers (4, 5) proved that beet yellows virus and tobacco mosaic virus were present in the phloem elements as well as in phloem parenchyma cells. Thus, similarities between flexuous rod-shaped, rod-shaped, and icosahedral viruses in their routes of long distance transport seemed to have been established (12).

The short distance (2), cell to cell movement, as well as movement from the cytoplasm to organelles, is a subject of considerable interest to plant virologists. Plant viruses or their products have been found in nuclei of infected cells (5, 6, 14). This does not imply that these viruses or their products are synthesized within the nuclear confines. Virus particles may be synthesized at locations in the cell other than those where they are ultimately seen.

Exchange of material between nuclei and the
surrounding cytoplasm may include transport of virus particles or products of virus synthesis through the nuclear membranes. The nuclear pore would constitute a possible route for movement of virus material between nucleus and cytoplasm. Therefore, we decided to determine whether the nuclear pore complex is a common structure observed in tangential views of nuclear membranes in plants that are used as hosts in plant virus research. Attempts were made to establish whether nuclear pores can facilitate transport of icosahedral plant viruses with sizes ranging from 25 to 30 μm.

Esau and her coworkers have presented pictorial proof that beet yellows virus can move in its assembled flexuous rodlike form from cell to cell through plasmodesmata. It seemed to be of interest to compare the short distance translocation of several icosahedral plant viruses (25-30 μm wide) with that reported for beet yellows virus (10 μm wide).

MATERIAL AND METHODS

The two principal viruses used in this study were *Southern bean mosaic virus* (SBMV) and a virus isolated from gladiolus and tentatively identified as tomato ringspot virus (TomRV) on the basis of serological relationships.

Leaf tissue of *Phaseolus vulgaris* L. infected with SBMV, and *Datura stramonum* L. (Jimson weed) infected with TomRV were cut into small pieces and fixed in 5% glutaraldehyde, rinsed in cacodylate buffer, and postfixed in osmium tetroxide. Appropriate controls were run in all experiments. *Belamcanda chinensis* (L.) DC, *Nicotiana tabacum* L., *Nicotiana megalesiphon* Heurck & Muell., *Petunia hybrida* Vilm., and *Cucumis sativus* were used in the study of nuclear pores besides the two plant species mentioned earlier.

Tissues were dehydrated in acetone and embedded in Araldite 6005. Uranyl acetate staining was done during the 70% dehydration step (70% acetone saturated with uranyl acetate for 6 hr). Sections were cut on a Reichert OMU 2 ultramicrotome (Reichert Instruments, W. J. Hacker & Co. Inc., West Caldwell, N.J.) and subsequently stained with lead citrate unless stated differently.

Markham's (10) contrast enhancement techniques were used to resolve patterns of symmetry in nuclear pores.

RESULTS

Nuclear Pores

In all tissues, infected or noninfected, whenever nuclei were cut tangentially across the nuclear envelope, the nuclear pore complex was found. In no case were there obvious differences between the pore complex in nuclear membranes seen in our leaf material and the pore complex described in negatively stained, isolated nuclear membranes of pea (17) and onions (7). All the pore complexes in all cases consisted of an annulus with eight subanuli (Fig. 1a, b, and d). Application of Markham's (10) rotational contrast enhancement technique resolved the eightfold symmetry in the annuli (Fig. 1d).

Although the central granules could be resolved in thin sections (Fig. 1a and b), no consistent number was observed in any of the plant species studied. One to three granules could be seen in the pores. The significance of these granules is not known. In light of Feldherr's (6) experiments with colloidal gold, it is doubtful that these central granules constitute a physical barrier for macromolecular transport. Figure 1b depicts a view of the pore complex regularly observed. The origin of the osmiophilia around the pores is not known, unless it can be considered a face view of the lipids of the nuclear membrane.

In cross-section, no structure could be resolved in the nuclear pore complex in any of the tissues sectioned (Fig. 1c). The inner and outer diameters of the nuclear pore complex measured in tangential sections ranged from 37 to 45 μm and from 90 to 108 μm, respectively.

Southern Bean Mosaic Virus

Virus distribution was determined in young systemically infected bean leaves and in mechanically inoculated primary leaves 14-21 days after inoculation.

Virus-like particles were observed in the cytoplasm (Fig. 2a and b), the nuclei (Fig. 3), and small vacuoles (Fig. 2c) of infected leaf cells. On the basis of comparative measurements, the particles were thought to represent virus particles (Fig. 4). The average size of purified negatively stained, purified embedded, intranuclear, and cytoplasmic particles was between 26 and 29 μm, indicating that the apparent size was similar. Similar particles were absent from healthy leaf tissue.

SBMV was present in large amounts in the cytoplasm of diseased mesophyll cells of primary leaves as well as in young and old secondary leaves that became systemically infected. An impression of the ribosomal population was difficult to resolve.
to obtain in those areas with abundant virus particles (Fig. 2a). Occasionally areas were found where the particle density was low and fibrillar material would be present (Fig. 2b). The nature of this material is unknown. Crystalline inclusions were seldom encountered. Small vacuoles sometimes contained paracrystalline or crystalline inclusions (Fig. 2c). As a rule, however, no SBMV crystallization was seen in either the cytoplasm or the nuclei of infected mesophyll cells.

Nuclei contained noncrystallized virus particles (Fig. 3a and b) that could be differentiated from other nuclear material by their staining characteristics and size. The virus content of nuclei seems to keep pace with that of the cytoplasm. In early infections virus is difficult to detect in both the nucleus and the cytoplasm. Virus was not seen specifically associated with either the dictyosomes, the endoplasmic reticulum, the chloroplasts, or the mitochondria.

On several occasions virus was observed in cross-sections of nuclear pores in separately fixed blocks of infected tissue (Fig. 3c). Figure 5a suggests that SBMV can also move in big surges from the nuclei. A reverse of this process has not been observed. It has not been established whether surges of this kind damage the nuclear membrane. This type of virus discharge has been described by Shikata and Maramorosch (14) for pea enation mosaic virus, the only other icosahedral plant virus between 25 and 30 m\(\mu\) that has been reported to occur in the nucleus.

SBMV has not been observed in the plasmodesmata of bean mesophyll cells. However, plasmodesmata were seldom observed in our sections of healthy or infected bean tissue.

**Tomato Ringspot Virus**

Since TomRV occurs in such low concentrations in infected plants and is similar in size to SBMV, we wished to compare its distribution with that of SBMV.

Although definite identification of TomRV in cucumber could not be made, in Jimson weed small paracrystalline inclusions (Fig. 5b) were identified as virus inclusions on the basis of particle measurements.

Plasmodesmata were abundant between mesophyll cells of Jimson weed. Therefore, this plant could be sampled easily for the occurrence of virus particles in the plasmodesmata. Fig. 5c shows a row of five particles of the size of TomRV in a plasmodesma, suggestive of transport of these particles from cell to cell. TomRV was found only in the cytoplasm of infected cells.

**DISCUSSION**

The nuclear pore complex in the plants we studied is similar to that reported in other organisms (1, 15) and the one reported for *Allium*.

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**Abbreviations**

- c, chloroplast
- cy, cytoplasm
- f, fibers
- m, mitochondrion
- n, nucleus
- nm, nuclear membrane
- p, plasmalemma
- pl, plasmodesma
- pr, polyribosone
- t, tonoplast
- v, vacuole
- w, wall

Unless indicated differently, the size of the markers in all photographs is 0.5\(\mu\).

**Figure 1**

- a. Nuclear pores in the nuclear envelope of a nucleus in a mesophyll cell of *Petunia hybrida*. Note the annulus with subannuli (arrow). \(\times\) 70,000.
- b. Nuclear pores in face view in the nuclear envelope of a mesophyll cell of *Belamcanda chinensis*. The osmiophilic area around the pores is a face view of the inside of one of the membranes of the nuclear envelope. Note the nonosmiophilic area directly around the pores. \(\times\) 30,000.
- c. Cross-section of a nuclear envelope showing pores. No ordered substructures are visible in the pores. \(\times\) 100,000.
- d. A face view of a tangentially sectioned nuclear pore. Applying Markham’s technique (10), it was established that 4 and 8 times turning during print exposure gave the best contrast enhancement, proving the existence of eight-fold symmetry in the annulus. \(\times\) 270,000.
FIGURE 2  a, Southern bean mosaic virus in the cytoplasm of an infected cell (arrows). Note the high concentration of particles and the absence of crystallization. × 44,000. b, Southern bean mosaic virus in the cytoplasm of an infected cell. Note the relatively low concentration of virus particles and the fibrils (f). Function of these fibrils is unknown; however, some fibrils appear to end in SBMV particles. × 33,000. c, Southern bean mosaic virus in a small vacuole of an infected leaf cell. The formation of crystalline structures of SBMV is apparent (arrows). × 30,000.
FIGURE 3  a, Southern bean mosaic virus in the nucleus (n) of an infected cell (arrows). The random order of the SBMV particles can be observed. × 10,000. b, Nuclear SBMV inclusion at a late stage of infection. Closest approximation of aggregated virus ever observed in a nucleus. × 44,000. c, Nucleus (n) of an infected cell stained with uranyl acetate only. Note virus particles (arrows) within the nucleus and the nuclear pores. × 51,000.
cepae L. (6) and Pisum sativum L. (17). The inside and outside diameters of the pores that we measured agree with those compiled and reported by Yoo and Bayley (17).

We have not found evidence for cylindrical extensions of the pore as described by Afzelius (1) and Wischnitzer (15). Others also failed to find this in higher plants (6, 17). The reason for the absence of these earlier described structures is not known and may be peculiar to the specimens studied.

It could not be established whether or not the central granule seen in tangential views of the nuclear pores constitutes an obstruction to movement through these pores.

In light of Feldherr's (6) studies and the structural intricacies of the nuclear pore complex as studied here, a control mechanism in conjunction with other physical forces in the pore may regulate translocation of macromolecules. Based on the measurements of the pore it seems possible that plant viruses with a maximum diameter of 35 μm can move through a nuclear pore. Pea enation mosaic virus and SBMV, both reported to occur in the nucleus of infected cells, have diameters between 25 and 30 μm. This size is larger than the largest gold particles reported by Feldherr (6) to pass through a nuclear pore. Notwithstanding our observation of a close association of pores and virus in SBMV-infected plants, movement of virus in disassembled form through nuclear pores cannot be discounted completely.

The fact that in sectional views no structure was observed within the nuclear pore may cast some doubt on the reality of these structures. The discrepancies between the face view and sectional appearance of the nuclear pore complex remain to be resolved.

Southern bean mosaic virus has been reported here for the first time to occur in the nucleus and...
FIGURE 5  

a, Pores and direct spillage of particles from nuclei may constitute routes of exchange of virus particles from nuclei. Spillage of SBMV from a nucleus (n). × 50,000.  
b, A micro crystal of Tomato ringspot virus between arrows. × 50,000.  
c, Short distance translocation of icosahedral virus particles may take place in the form of fully assembled particles through plasmodesmata (pl). TomRV particles in a plasmodesma (arrows). × 60,000.
is only the second plant virus of this size and shape for which this has been shown. It is present as individual particles widely spread and not aggregated or crystallized, which seems to indicate that these particles can move freely through the cell cytoplasm and the nucleus. The presence of SBMV particles in the nuclear pore has been demonstrated. The frequency of this phenomenon, however, was low. One of the difficulties in studying SBMV in the nuclear pore is that the staining procedure to make virus visible results in a densely stained pore. This makes it impossible to resolve the virus in the pore. When only uranyl acetate was used, the virus stained uncharacteristically and lightly, but the nuclear pore became more electron-transparent so that now virus could be observed.

The direction of movement could not be established and the possibility of virus presence in the nuclear pore without movement cannot be excluded. However, it has been shown that SBMV is found in the nucleus and in the cytoplasm and that this virus is present occasionally in the nuclear pores, implying that transport may occur.

The forces behind this translocation are not known, but may be explained in part by assuming the increase of pressure gradients (4) within the cell and the resulting tendency to equilibrate virus concentrations throughout the infected cell during virus multiplication.

Spilling of virus from nuclei (bulge towards the cytoplasm) as shown in Fig. 4a was seen rarely, but could constitute another way of virus translocation between the nucleus and the cytoplasm.

In light of the possibility of translocation of virus between nuclei and cytoplasm and the fact that SBMV is visualized in the cytoplasm of young cells before being seen in nuclei, it seems highly speculative to deduce sites of plant virus synthesis from observational electron microscopy only.

If translocation of virus between the cytoplasm and the nucleus in an infected cell is caused in part by diffusion pressure deficits in either the cytoplasm or the nuclei, it is clear that viruses that are present in low concentrations in the cytoplasm and exhibit natural tendencies to crystallize will not exert enough pressure to be found also in nuclei. This was the case with TomRV in Jimson weed.

The complete absence of paracrystalline or crystalline structures in the cytoplasm of infected bean leaf cells, especially since partially purified SBMV forms paracrystalline aggregates with great ease, is considered peculiar. It seems that a virus-host interaction is required to produce crystalline inclusions of these plant viruses. This is illustrated by the fact that no inclusions of TomRV were found in cucumber, although in Jimson weed small crystalline inclusions were present in the cytoplasm of infected plants.

The form in which virus is translocated has been a subject of considerable interest for some time (12). Only for TMV and a few other viruses has it been established (12) that virus spread from cell to cell may be mediated by transport of infectious nucleic acid.

Shalla (13), in 1959, calculated that plasmodesmata are large enough to allow passage of whole virus. The first visual proof that virus may be translocated in its particulate form was obtained by Esau and coworkers (4) for beet yellows, a flexuous rod.

In this work we have shown that icosahedral viruses two or three times as wide as beet yellows may move through plasmodesmata. Though pressure gradients could possibly be responsible for virus movement through plasmodesmata, if cytoplasmic streaming takes place between cells through these structures, then virus transport may be entirely passive.

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