Inclusion Bodies and Tubular Structures in Chenopodium amaranticolor Plants Infected with Strawberry Latent Ringspot Virus

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SUMMARY

The first change observed in cells of leaves systemically infected with strawberry latent ringspot virus was the formation of inclusion bodies near the nucleus. The inclusions were largely composed of endoplasmic reticulum, complex membranous structures and ribosomes. Three days later their outer parts contained unbranched, double-walled, slightly flexuous tubules about 50 nm. wide and up to at least 2.5 μm. long. Each tubule, or occasionally two or three tubules, was enclosed in a membranous sheath 80 to 120 nm. in diameter, joined to the endoplasmic reticulum. The tubules contained a single row of up to 100 or more darkly stained virus-like particles. Some tubules ended within the inclusion and some at plasmodesmata, in which virus-like particles occurred. The central, predominantly membranous, regions of the inclusions contained masses of faintly stained, apparently hollow structures resembling empty shells of virus coat protein.

INTRODUCTION

Electron microscopy of ultrathin sections of infected cells has provided much evidence that different plant viruses can accumulate in different parts of cells, form different kinds of arrays of particles and induce the formation of different characteristic structures, such as inclusion bodies. Some viruses with other properties in common, however, have similar effects on cells. For example, many of the viruses grouped with potato virus Y (Brandes & Bercks, 1965) induce the formation of pinwheel-like structures (Edwardson, 1966).

Little work of this kind is reported with nepoviruses. However Gerola, Bassi & Betto (1964) found that the virus-like particles in cells of Chenopodium amaranticolor leaves infected with arabis mosaic virus were aggregated as though in thin layers on the surface of a sphere or ellipsoid. Davison (1969), working with root tips of Phaseolus vulgaris infected with tobacco ringspot virus, found that plasmodesmata contained single rows of virus-like particles. The rows continued, on one side of the cell wall, within a tubule projecting into the cytoplasm. Walkey & Webb (1968) also found tubules, containing a single row of up to 150 virus-like particles, in preparations obtained by squashing meristematic tips of shoots infected with cherry leaf roll or strawberry latent ringspot viruses in drops of potassium phosphotungstate or ammonium molybdate. In this paper we describe the structure of these tubules, as seen in sections of leaf cells infected with strawberry latent ringspot virus, and show that the tubules occur in large numbers in characteristic inclusion bodies, which may be sites of virus synthesis or assembly.
METHODS

Infected plants. The T39 isolate of strawberry latent ringspot virus [cryptogram */*/: */*: S/S: S/Ne] (Lister, 1964) was transmitted by inoculation of sap to C. amaranticolor plants growing in a glasshouse at about 20°. After 5 days, mottling appeared in the uninoculated tip leaves. The mottling later became less obvious, but the newly expanded tip leaves were stunted and distorted. Tip leaves showing symptoms (20 to 25 mm. long) were sampled 6 days after the plants were inoculated, and again 3 days later, when they had expanded slightly.

Preparation of tissue for electron microscopy. Slivers of tissue, about 4 mm. × 1 mm., were cut from the mottled parts of the leaves. They were then fixed, dehydrated and embedded by the ‘modified method’ of Harrison, Stefanac & Roberts (1970). Sections cut with an LKB Ultratome I were mounted on collodion-covered grids and post-stained with lead citrate. They were examined in a Siemens Elmiskop I at 80 Kv using a 50 μm. objective aperture.

Fig. 1. Inclusion body adjacent to nucleus (N) in a spongy mesophyll cell showing many membranous structures (M) 6 days after plants were inoculated with strawberry latent ringspot virus.
RESULTS

Inclusion bodies

Cells in tissue, sampled 6 days after the plants were inoculated, were well preserved and contained normal-looking chloroplasts, mitochondria and nuclei. Several cells, however, contained large regions of cytoplasm that bulged into the vacuoles and were similar in size to the nuclei. These inclusion bodies (Fig. 1) contained ribosomes, endoplasmic reticulum, membranous material often arranged concentrically, Golgi material and fat globules.

They were found in many palisade and spongy mesophyll cells but seldom in the epidermis. Only one inclusion body occurred in any one cell, and in almost every instance it was close to, or in contact with, the nucleus. By inspecting serial sections of palisade and spongy mesophyll cells, it was found that not every nucleus had an inclusion body associated with it, suggesting that not all such cells contain inclusions.

Tissue sampled 9 days after the plants were inoculated again contained normal-looking nuclei, mitochondria and chloroplasts, which contained large starch grains. The appearance
of the inclusion bodies was however now different. They consisted of two zones, an outer one containing large numbers of tubules, each with its row of virus-like particles, and an inner zone largely composed of membranes, vesicles and granular material (Fig. 2).

In a few areas of the 9-day tissue, the inclusion bodies had yet another kind of appearance, which may represent a third stage of development. These inclusions contained many ribosomes in clumps but little endoplasmic reticulum and few vesicles or tubules. They seemed to be disintegrating.

No structures resembling inclusion bodies were found in uninoculated control plants of *C. amaranticolor*.

**The tubules**

The tubules have a well-defined structure (Fig. 3). Each tubule was 45 to 55 nm. in diameter and enclosed a single row of densely stained, isometric particles 25 to 29 nm. in diameter, resembling the particles found in purified preparations of strawberry latent ringspot virus (Lister, 1964). These particles were in close contact with one another. The tubules were surrounded by a sheath 80 to 120 nm. in diameter. There was only one tubule in most sheaths but, in some instances, two or three were found (Fig. 3(c)). The tubules never branched, and some could be traced through serial sections for more than 2.5 μm. and contained more than 100 virus-like particles. Other tubules were much shorter (Fig. 4) and some contained as few as ten particles. As observed by Walkey & Webb (1968) in tissue suspensions, the wall of the tubule has two layers but, unlike those in tissue suspensions, the tubules in the sections were somewhat flexuous, with some tubules bending through an angle of 90° in a distance of 500 to 750 nm. The outer sheath was a single membrane continuous in some places with the endoplasmic reticulum (Fig. 3(c)). At the
ends of the tubules, the outer sheath seemed to join the outer layer of the tubule wall and the inner layer of the tubule wall came to an end (Figs. 4 and 5). One or two virus-like particles lay close to the apparently open ends of some of the tubules, but it is not certain whether both ends of any one tubule were open.

Some tubules ended at a plasmodesma and the row of virus-like particles continued through the cell wall (Fig. 6). In the adjacent cell the row continued for only three or four particles and the tubule wall apparently did not continue for more than a very short distance into the cytoplasm, possibly as the inner layer only. These observations are very similar to those of Davison (1969) on tobacco ringspot virus. On reaching the plasmalemma, the outer sheath appeared to join the outer layer of the tubule wall.

No tubules or virus-like particles in plasmodesmata were seen in cells of virus-free C. amaranticolor.

**Structures resembling shells of virus coat protein**

In the figures published by Walkey & Webb (1968), none of the virus-like particles in the tubules found in tissue extracts took up the phosphotungstate stain. Also, in our work, all the particles stained darkly in tubules in sections treated with uranyl acetate plus lead citrate. Hence the particles behaved as though they all contained nucleic acid. By contrast, a purified preparation of strawberry latent ringspot virus (Fig. 7) made some months
previously and kindly provided by Dr A. F. Murant, like preparations of other nepoviruses

treated with phosphotungstate (Harrison & Nixon, 1960), contained at least two kinds of
particles with the same diameter, one apparently hollow and the other not. By analogy with
other nepoviruses (Stace-Smith, Reichmann & Wright, 1965; Stace-Smith, 1966), these
components are probably respectively RNA-free and RNA-containing protein shells.

Fig. 6. Two serial sections, showing a tubule passing through a plasmadesma linking two spongy
mesophyll cells, and continuing into the cytoplasm of the cell at the right. P, plasmalemma;
W, cell wall.

Fig. 7. Particles of Strawberry latent ringspot virus from a purified preparation. Mixed with
2% sodium phosphotungstate. Some particles are penetrated by the stain and others are not.

Close examination of material in the vacuoles of some cells however revealed many
densely stained particles together with others of the same diameter, with unstained centres
and lightly stained walls. Both kinds of particle probably also occurred in some areas of
cytoplasm but could not be identified as confidently. More detailed inspection of the ‘granu-
lar areas’ in the second-stage inclusion bodies showed that these were largely composed of
great numbers of faintly stained, apparently hollow particles (Fig. 8). These particles may
be RNA-free virus protein shells.

Fig. 8. Clumps of lightly stained, apparently hollow particles (H) in the ‘granular areas’ of an
inclusion body. The hollow particles are probably similar to those penetrated by phosphotungstate
in Fig. 7.

**DISCUSSION**

Our observations show that the tubules produced by strawberry latent ringspot virus
occur in expanding leaves in addition to the shoot apices where they were found previously
by Walkey & Webb (1968). Virus-containing tubules were also found in cells infected with
various other plant and animal viruses, for example, maize rough dwarf (Gerola et al.
1966), clover wound tumour (Shikata & Maramorosch, 1966), rice dwarf (Shikata, 1966),
polyoma (Mattern, Takemoto & Daniel, 1966) and simian virus 40 (Levinthal, Wicker &
Cerottini, 1967) but the tubules in strawberry latent ringspot virus-infected cells differ from
all these in having an outer sheath.

The function of the tubules is not clear, but their association with plasmodesmata
suggests they may be involved in movement of virus from cell to cell. However, particles were
found in the plasmodesmata 6 days after the plants were inoculated and before any tubules
were seen. In general, the tubules were numerous only after the leaves developed symptoms and hence after virus replication had started. This might suggest that their formation is a consequence of virus replication and that they are not involved in the synthesis or assembly of virus material. However, failure to detect the tubules earlier in infection may simply reflect the small volume of cell that is examined in ultrathin sections and does not prove they were not present in small numbers. The fact that the particles within the tubules all seem to contain nucleic acid, whereas those packed in the 'granular areas' of the inclusions all seem to be devoid of nucleic acid, could indicate that the virus nucleic acid is synthesized or assembled into virus particles in some parts of the cell, possibly in or near the tubules, and virus coat protein is made in different parts, possibly in or near the 'granular areas'.

It would be premature to say whether nepoviruses share types of intracellular behaviour not shown by other plant viruses, but inclusion bodies resembling the early stages of those induced by strawberry latent ringspot virus are also produced by two more nepoviruses, arabis mosaic and cherry leaf roll (I. M. Roberts, unpublished results). Also, of the five plant viruses whose particles have been seen within plasmodesmata, three—tobacco ringspot (Davison, 1969), tomato ringspot (de Zoeten & Gaard, 1969) and strawberry latent ringspot—are nepoviruses.

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REFERENCES


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