Mitochondrial Aggregates in Datura Leaf Cells Infected with Henbane Mosaic Virus

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SUMMARY

Thin sections of Datura stramonium L. leaf tissues infected either by the Rothamsted culture (HMV-r) or an Italian isolate ('alkekengi' strain = HMV-A) of henbane mosaic virus, were examined in the electron microscope.

The cytoplasm of most cells contained lamellar inclusions, which appeared as parallel lines, pinwheels, rings or arcs in the sections, typical of infection with viruses of the potato virus Y group, and long crystalline structures, apparently made up of elongated elements 8 to 10 nm. in diameter, arranged in a lattice. Flexuous threads, about 10 nm. wide, of moderate electron density, probably representing HMV particles in situ, were seen scattered in the cytoplasm or lying parallel to the tonoplast or the lamellar inclusions.

The most remarkable observation, however, was the presence of aggregates of numerous (up to 40) long, cylindrical mitochondria, tightly packed in certain areas of the cytoplasm. Between adjacent mitochondria there was a layer of elongated, virus-like particles, running parallel and longitudinally to the mitochondrial surface.

INTRODUCTION

Henbane (Hyoscyamus niger L.) mosaic virus (HMV), first described by Hamilton (1932) was included in the potato virus Y group because of its morphological and serological properties (Brandes, 1959; Bartels, 1963/64). Brandes's (1959) initial estimate of the normal length of HMV particles, around 725 nm., was not confirmed in later measurements of the Rothamsted culture (Lovisolo & Bartels, 1970; Watson, Plumb & Woods, 1971) and of an Italian strain, isolated from Physalis alkekengi L. (Lovisolo & Bartels, 1970), which gave values around 830 to 850 nm. Atropa mild mosaic virus, a strain of HMV (Govier & Woods, 1971) has a modal length of about 925 nm. (Harrison & Roberts, 1971). Govier & Woods (1971) observed that some viruses, including HMV, could appear either straight and long or flexuous and relatively short, depending on the presence of magnesium ions.

Intracellular changes induced by the Rothamsted culture of HMV and Atropa mild mosaic virus were studied respectively by Plumb & Vince (1971) and Harrison & Roberts (1971), both reporting the presence of cytoplasmic lamellar inclusions, typical for infection with viruses of the PVY-group (Edwardson, Purcifull & Christie, 1968); the cytoplasm also contained crystalline inclusions and flexuous rods, probably the virus particles.

In this paper, we report the results of a comparative study of the intracellular aspects of leaf tissues of Datura stramonium L. systemically infected with either the Rothamsted culture or the 'alkekengi' strain of HMV. Besides previously observed structures, a complex aggregate of mitochondria and virus-like particles is described.
Fig. 1. Lamellar inclusions (L) with varied configurations in the cytoplasm of a Datura mesophyll cell, infected with HMV-R. Some elongated particles cut transversely can be seen running parallel to the tonoplast (arrows). C, Crystalline inclusions; M, mitochondrion.

Fig. 2. Detail of Datura leaf cell infected with HMV-A, showing lamellar inclusions (L) and elongated, virus-like particles (arrows) scattered in the cytoplasm or associated with lamellar inclusions. M, Mitochondrion.

METHODS

Virus strains. The Rothamsted culture of HMV (HMV-R) was provided by Dr R. Bartels (Institut für Vирусологии, Braunschweig, West Germany), who obtained it from the Rothamsted Experimental Station, Harpenden, England, in 1961. The ‘alkekengi’ strain of HMV (HMV-A) was obtained from P. alkekengi growing wild near Turin, Italy. Both strains were those used by Lovisolo & Bartels (1970); they were maintained in Nicotiana tabacum L. cvs. ‘White Burley’ and ‘Samsun’ or in D. stramonium, to which they were transmitted mechanically.

Electron microscopy. Small pieces of systemically infected leaf tissue from D. stramonium were collected 53 days after the plants had been inoculated either with HMV-R or HMV-A, fixed in 3% glutaraldehyde in 0.05 M-phosphate buffer, pH 7.2, for 90 min. at 4°, and then postfixed in 1% OsO₄ in 0.15 M-phosphate buffer, pH 7.2, for 2 hr at 4°. Fixed tissues were dehydrated in increasing concentrations of ethanol, transferred to propylene oxide and finally embedded in Araldite. Blocks were cut on a Porter–Blum MT-1 ultramicrotome equipped with a diamond knife. The sections were stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop I.
RESULTS

The changes induced in leaf tissues of *D. stramonium* infected with either HMV-r or HMV-A were essentially similar. In both cases, mesophyll cells appeared somewhat shrunken, although no major alterations were noticed in the nuclei or plastids. The cytoplasm contained many lamellar inclusions, usually clustered in certain areas (Fig. 1, 2), similar to those previously described in cells infected with other viruses of the PVY-group (Edwardson *et al.* 1968) and they appeared in different configurations according to the angle of sectioning. A set of parallel lines, rings, pinwheels and arcs, but rarely dense bands, were the most common profiles exhibited by these lamellar inclusions.

Elongated, crystalline structures were also frequently observed in the cytoplasm (Fig. 3, 4). They had relatively small cross-sections (less than 1 μm. × 1 μm.) but were sometimes very long (up to 10 μm.), thus being more or less needle-shaped. At higher magnifications, longitudinal sections of these crystals revealed parallel dense lines with a periodicity of 8 to 12 nm. (Fig. 4) and transverse sections showed an ill-defined lattice of dots (Fig. 3). Thus the crystals seemed to be composed of elongated elements about 10 nm. wide, tightly packed in an ordered array.

Flexuous particles of various lengths, and about 10 nm. in diameter, were occasionally seen scattered in the cytoplasm (Fig. 2). They were more frequently observed running parallel
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to the tonoplast (Fig. 1) or to the surface of lamellar inclusions (Fig. 2). In cross-sections the particles could be seen as densely staining dots.

A striking feature observed in Datura leaf cells infected with HMV was the occurrence of aggregates of numerous, elongated, cylindrical mitochondria in certain areas of the cytoplasm (Fig. 5 to 8). These mitochondrial aggregates were more frequent in tissues infected with HMV-R than with HMV-A. The mitochondria did not show any special internal or external peculiarity, except in length (up to 5 \( \mu m \)). Sometimes more than 40 mitochondria were clustered in a restricted cytoplasmic region; they were arranged more or less regularly, with their long axes parallel. Between adjacent mitochondria there was a layer of elongated particles (Fig. 6 to 8). These particles, presumably virus, were apposed to the surface of the mitochondrion, lying mainly in the same direction as its long axis.

DISCUSSION

The finding of lamellar inclusions, crystalline structures and flexuous particles in the cytoplasm of leaf cells of D. stramonium infected with either HMV-R or HMV-A, confirms the previous observations made by Plumb & Vince (1971) in tissues infected with HMV-R and by Harrison & Roberts (1971) in tissues infected with Atropa mild mosaic virus, a strain of HMV.

Several viruses, longer than 800 nm., the length suggested by Brandes (1964) as the upper limit for virus in the PVY group, have been shown to induce the lamellar inclusions which were suggested by Edwardson et al. (1968) as being specific for viruses in this group. Besides HMV, Malva vein clearing (850 nm.) (Martelli, Russo & Castellano, 1969) and sweet potato mosaic (850 nm.) (Kitajima, Camargo & Costa, 1972) viruses were also found to induce lamellar inclusions. Although the formerly established serological relationship between HMV and other viruses of PVY group must be re-examined (Govier & Woods, 1971), HMV still resembles viruses of the PVY group in its ability to act as a helper virus in aphid transmission of potato aucuba mosaic virus (Kassanis & Govier, 1971). The inference, thus, is that the upper limit of the PVY group must be extended and the suggestion of Edwardson et al. (1968) is still valid.

The flexuous threads seen occasionally scattered in the cytoplasm or apposed to the surface of the tonoplast or the lamellar inclusions probably represent HMV particles in situ. Plumb & Vince (1971) found no particles in HMV-infected tissues that were double-fixed with glutaraldehyde and OsO\(_4\) and attributed this to the deleterious effect of such fixation on the virus particle structure. We also had some difficulty in identifying the filamentous, virus-like particles in double-fixed tissues, but no definite conclusion can be drawn, because we did not fix any preparation with OsO\(_4\) alone.

The regular arrangement of virus-like particles parallel to the tonoplast is interpreted by Martelli et al. (1969) as accidental trapping. Weintraub & Ragel (1970) found the arrangement difficult to understand if the components were synthesized in the nucleus or in the

Fig. 5. Mitochondrial aggregate (M) in Datura leaf cell infected with HMV-R. L, Lamellar inclusion; P, chloroplast.

Fig. 6. Detail of Fig. 5, showing elongated particles (arrows) lying in between the mitochondria (M).

Fig. 7. Datura leaf cell infected with HMV-R. Similar to Fig. 6, in which the elongated particles (arrows) were cut obliquely.

Fig. 8. Datura leaf cell infected with HMV-R. Mitochondrial aggregate sectioned parallel to their longer axis. Elongated particles (arrows) can be clearly seen running parallel to the mitochondrial surface.
main body of cytoplasm. We suggest that the phenomenon may be connected with a defence reaction of the cell: the cytoplasm tends to eject the particles towards vacuoles so that most of the particles which are not attached to cell organelles reach the tonoplast and become arranged along it. A similar active mechanism has been discussed by Martelli et al. (1969).

The significance of the aggregates of mitochondria interspersed with virus-like particles is not clear. Abnormalities of the mitochondria have been reported in cells infected with some other plant viruses, as discussed by Weintraub & Ragetli (1971). A relationship between virus particles and mitochondria was described with a Brazilian strain of tobacco rattle virus (Kitajima, 1967; Harrison & Roberts, 1968; Kitajima & Costa, 1969), and it was suggested that the mitochondria may play a r61e in the synthesis of this virus. An alternative explanation for this virus/mitochondrion association, that the virus particles become adsorbed to mitochondria after assembly elsewhere, was also pointed out by Harrison & Roberts (1968). However, there are differences in the pattern of virus particle/mitochondrion association between HMV and tobacco rattle virus. HMV particles are attached to mitochondria by their sides while those of tobacco rattle, by their ends. In the case of HMV, the association was not as exclusive as with tobacco rattle virus, because particles were also seen scattered in the cytoplasm or apposed to lamellar inclusions and tonoplast. An association similar to that here described with HMV was already noticed by Borges & David-Ferreira (1968) in *Datura metel* L. leaf tissues infected with PVY, but with HMV a much greater proportion of the mitochondria in a cell seem to concentrate in a restricted area of the cytoplasm.

Such mitochondrial aggregates were not observed by Plumb & Vince (1971) in ‘White Burley’ tobacco plants infected with HMV nor by Harrison & Roberts (1971) in ‘Xanthi-nc’ tobacco infected with *Atropa* mild mosaic virus. We also could not find them in ‘Samsun’ tobacco, 30 days after inoculation with HMV-A or in ‘White Burley’ tobacco, 60 days after inoculation with HMV-R. Possibly aggregates of mitochondria are specific for the HMV/ *Datura* combination, or perhaps they appear only at a certain stage of infection. Obviously, electron microscopy alone cannot explain why particles and mitochondria aggregate. It seems, however, that there is a specific interaction between HMV particles and the mitochondria, perhaps involving specific receptor sites on the mitochondria for the virus coat protein; thus virus particles may be attached at opposite sides to two mitochondria and so cause them to aggregate.

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