Role of Mitochondria in the Formation of X-bodies in Cells of 
*Nicotiana clevelandii* infected by Tobacco Rattle Viruses

By B. D. HARRISON, ZLATA STEFANAC* AND I. M. ROBERTS
Scottish Horticultural Research Institute, Invergowrie, Dundee

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SUMMARY

Non-crystalline inclusions, referred to as X-bodies, developed in leaf-hair cells of *Nicotiana clevelandii* infected with a RNA-producing defective isolate (CAM/DF) of tobacco rattle virus but not in those infected with the nucleoprotein particle-producing strain (CAM) from which CAM/DF was derived. The X-bodies appeared 3 days after inoculation; some persisted for at least 10 weeks. They were digested by pronase and were rich in RNA, which was protected by proteinaceous material from digestion by pancreatic ribonuclease; they did not contain virus coat-protein detectable by fluorescent antibody tests. Electron microscopy of thin sections showed that mitochondria in infected cells developed two abnormalities—peripheral membranous sacs and membrane bounded vacuoles—and then aggregated, together with ribosomes and material containing small darkly staining granules, to form small X-bodies. These fused to form larger X-bodies, in which the mitochondria were further modified and eventually became barely recognizable. Finally, the contents of the X-bodies became increasingly amorphous, and the bodies disintegrated.

X-bodies also developed in leaf-hair cells infected with the virus particle-producing strain PRN, but were less common and mostly persisted for only a few days. These X-bodies were formed in the same way as those produced by CAM/DF, but contained in addition small aggregates of virus particles resembling the aggregates found in the cytoplasm in other parts of the same cells. The frequency of formation, and persistence, of X-bodies induced by these tobacco rattle viruses paralleled the severity and persistence of their macroscopic effects on *N. clevelandii*. The possibility that mitochondria are sites of synthesis of tobacco rattle virus RNA is discussed.

INTRODUCTION

Although it is well known that many plant viruses can induce the formation in infected cells of microscopically visible, non-crystalline inclusion bodies, in no instance is their composition and role in infection fully understood. These cytoplasmic bodies, called X-bodies, are usually spherical, ovoid or lobed in shape, and have a granular or amorphous substructure. The X-bodies induced by tobacco mosaic virus are the best studied. They seem largely composed of tubules, many of which are grouped in threes; ribosomes, endoplasmic reticulum and a few pockets containing virus particles (Kolehmainen, Zech & Wettstein, 1965; Esau & Cronshaw, 1967). Esau & Cronshaw (1967) speculate that the tubules may be composed of excess virus coat-protein, and Kolehmainen et al. (1965) suggest that the regions in which they occur are the sites of

* Present address: Botanical Institute, University of Zagreb, Yugoslavia.
virus protein synthesis. We were therefore interested to find that a defective variant of tobacco rattle virus, that was of the kind apparently unable to synthesize virus coat-protein (Sänger & Brandenburg, 1961; Cadman, 1962), nevertheless induced X-bodies to form, whereas the strain from which it was derived, and which produced the typical tubular nucleoprotein virus particles, did not. In this paper we describe studies on the composition, structure and mode of formation of these X-bodies, and of those induced by another strain of tobacco rattle virus. We have used evidence obtained by electron microscopy of thin sections and by light microscopy.

METHODS

Viruses and plants. Two strains of tobacco rattle virus, CAM and PRN, were used; both produce tubular nucleoprotein virus particles of two predominant lengths (Harrison & Nixon, 1959; Harrison & Woods, 1966). A defective variant of strain CAM, referred to as CAM/DF, was provided by Mr J. I. Cooper. This variant was derived from CAM by culturing from a local lesion produced in an inoculated leaf of Chenopodium amaranthicolor Coste & Reyn. by greatly diluted inoculum. It did not produce tubular nucleoprotein particles detectable by electron microscopy of leaf extracts, and suspensions obtained by grinding leaves in water had little infectivity. CAM/DF did, however, produce virus nucleic acid, and was subcultured using inocula prepared with the aid of water-saturated phenol (Cadman, 1962). Inocula of CAM and PRN were made by grinding infected leaves in water.

All the viruses were cultured in Nicotiana clevelandii Gray plants kept in glasshouses maintained at 15 to 20°. To study leaf-hair cells, narrow pieces of tissue bearing hairs were cut from the edges of leaves. Four to six such pieces were used for any one treatment. A few of the leaf hairs were much larger than the others. We mainly studied the more numerous smaller hairs, but less detailed work with the larger type suggested that the sequence of events we describe below applies to hairs of all sizes.

Fixation for light microscopy. Unless otherwise stated the leaf pieces were fixed for 5 min. in cold acetone, but in some experiments they were fixed for 10 to 15 min. in Carnoy's fluid. The pieces were then transferred to 60% (v/v) ethanol until use. Fixation with glutaraldehyde and osmium tetroxide, as described below for material to be examined with the electron microscope, was also tested.

Staining for light microscopy. Leaf pieces were washed free from ethanol, before staining for 5 min. with acridine orange R (0·02%) in citrate/phosphate buffer (0·01M-citric acid + 0·2M-Na₂HPO₄, pH 7). After staining, the leaf pieces were washed for 5 min. in each of three changes of citrate/phosphate buffer, and were then mounted in buffer for microscopy. Feulgen staining was applied to leaf pieces fixed in Carnoy's fluid. These were heated in N-HCl at 60° for 5 min. before treatment with Schiff's reagent. Naphthol yellow S (0·02% in 1% acetic acid) was used to stain fixed leaf pieces as described by Himes & Moriber (1956). For staining in Sudan black B, fresh leaf pieces were placed in 50% (v/v) ethanol for 2 min., then for 5 min. in a saturated solution of the stain in 70% (v/v) ethanol before they were differentiated for 1 min. in 50% ethanol and mounted in glycerol diluted with water.

Enzyme digestion tests. For these tests the tips of the hairs on fixed leaf pieces were cut off using a razor blade, and the leaf pieces transferred for the specified periods to solutions of pronase (B grade, Calbiochem, Los Angeles) or pancreatic ribonuclease.
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(Koch–Light and Co. Ltd., Colnbrook, five times crystallized, salt and protease free) held in a water bath at 37°. They were then washed free of enzyme.

Fluorescent antibody tests. For these tests also, the tips of the hairs on acetone-fixed leaf pieces were cut off. The leaf pieces were then washed in buffer (0.01 M-phosphate, pH 7.3, containing 0.15M-NaCl) for at least 2 hr, incubated in antiserum to virus for 45 min. at 37°, again washed for at least 2 hr, incubated for 30 min. at 37° in fluorescein-conjugated anti-rabbit globulin obtained from sheep (Progressive Laboratories Inc., Baltimore), washed for at least 2 hr and mounted in 50% (v/v) glycerol in buffer. The antisera to virus strains CAM and PRN were obtained from rabbits and both had precipitation end-points of 1/256 to 1/512 against their homologous viruses in tests using mixed liquids in tubes. Virus antisera and fluorescein-conjugated antiserum were all used diluted 1/5 with the phosphate buffered saline.

Fluorescence microscopy. Preparations of leaf pieces were examined with a Zeiss Large Fluorescent microscope equipped with a HBO 200 ultraviolet light source; the exciting light was passed through filters BG 38 and BG 3. For acridine orange-treated material, two barrier filters excluding light of less than 530 and 410 nm. wavelength respectively were used, and for material treated with fluorescent antibody, barrier filters were used that excluded light of less than 470 and 440 nm. wavelength respectively.

Preparation of sections for electron microscopy. The leaf tissue used in the first experiments was fixed, embedded, stained and sectioned as described by Harrison & Roberts (1968). Later, the method was modified to the following. Pieces less than 1 mm. thick, cut from leaf edges, were fixed for 2 hr in 5% (v/v) glutaraldehyde in buffer (0.04M-sodium cacodylate, pH 7.4 + 1% sucrose), then washed 16 hr in buffer, then treated for 5 hr in 0.2% osmium tetroxide in buffer; these stages were done at 4°. The leaf pieces were then washed with water, dehydrated with increasing concentrations of ethanol, stained for 16 hr with 2% (w/v) uranyl acetate in ethanol, infiltrated with increasing concentrations of methyl methacrylate + styrene (6:1) and polymerized, first for 48 hr at 40° and then for 24 hr at 60°. These polymerization conditions gave good preservation of intracellular structures without compression of the cells. Finally the sections were cut as before and post-stained with uranyl acetate and lead citrate, or lead citrate alone.

RESULTS

Formation and disintegration of X-bodies

In the conditions of our glasshouses, strain CAM multiplied rapidly in inoculated leaves of Nicotiana clevelandii, usually without producing lesions; in 4 days the virus concentration in leaf sap was close to its maximum. The virus was readily detected in uninoculated tip leaves about 5 days after the plants were inoculated. Systemic symptoms consisted of faint etch patterns in the first systemically infected leaves, and slight stunting of the plant (Fig. 1a). X-Bodies were never found in these plants. By contrast they were easily found in plants infected with CAM/DF. This isolate produced more or less necrotic lesions in inoculated leaves, plus systemic mottling and necrosis. The systemic symptoms appeared about a week after the plants were inoculated, and were often first seen not in the tip leaves but in those already half expanded. Later they developed in the tip leaves, which were killed, or became misshapen, partly necrotic or mottled (Fig. 1b). The X-bodies were first found in inoculated leaves at 3–4 days after inoculation, and in non-inoculated leaves at 6–8 days. At this time the X-bodies
occurred in only a few large or small leaf hairs, they were small and numbered up to ten per cell. In living material they moved around the cell, often rapidly, or were stationary for some hours. They had a uniform finely granular appearance.

Fig. 1. (a) *Nicotiana clevelandii* plant systemically infected with CAM. (b) *N. clevelandii* plant systemically infected with CAM/DF. (c–f) Cells of leaf hairs of *N. clevelandii* plants infected with CAM/DF. The X-bodies (X) are often associated with the nuclei (N). (c) Fresh unfixed. (d) Fixed in glutaraldehyde and osmium tetroxide. (e) Fixed in Carnoy’s fluid and treated with acridine orange R; illuminated with white light. (f) As (e), but illuminated with ultraviolet light.
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At 6 to 10 days after inoculation (inoculated leaves) or 14 to 20 days (non-inoculated leaves) there were usually only one or two larger X-bodies per cell, and many of these were in contact with the nucleus (Fig. 1 c-f), although some moved around the cell. They tended to be round or oblong in outline, with well defined margins and a clearly granular substructure. In many hairs they occurred in every cell (except perhaps the globular tip cell which was too dense to allow observations), but in others they occurred only in the basal cells or only in non-basal cells. This suggests that X-bodies do not develop in all hair cells through which virus RNA has passed. Nine days after inoculation, measurements of 50 X-bodies induced by CAM/DF in hair cells of non-inoculated leaves gave sizes ranging from 5 μm. in diameter to 210 μm. long x 21 μm. wide. The commonest size was about 17 μm. in diameter.

Most of the X-bodies disappeared by 6 weeks after inoculation but some persisted for at least 10 weeks. These were then mostly small, had a more or less amorphous structure and had irregular shapes with indistinct margins. They seemed in process of dissolution.

Although no detailed observations of other types of cell were made, X-bodies were also seen in some epidermal cells.

Strain PRN produced symptoms in N. clevelandii leaves that were intermediate in severity between those of CAM and CAM/DF. X-bodies were found in some PRN-infected plants but they were less numerous, and disappeared sooner, than those in plants infected with CAM/DF. They appeared in hair cells of inoculated leaves after 3 days and were most numerous in these and in non-inoculated leaves after about 9 days. They were similar in appearance and intracellular location to those in cells of CAM/DF-infected plants. Three days after inoculation, 50 X-bodies in inoculated leaves were measured; their sizes ranged from 2 μm. in diameter to 45 μm. long x 10 μm. wide, but the commonest diameters were 7 to 10 μm.

Cytochemical evidence of the composition of X-bodies

The appearance of the X-bodies from plants infected with CAM/DF was substantially unaltered by fixation with either acetone, or Carnoy's fluid, or glutaraldehyde plus osmium tetroxide, the last of which however made them dark grey (Fig. 1 d). Leaves collected 14 to 20 days after the plants were inoculated were used as sources of X-bodies for the tests described below.

Tests for protein. The X-bodies contain protein. They stained yellowish with naphthol yellow S and were completely digested by exposure to pronase (1 mg./ml.) for 1 hr.

Tests for nucleic acids. In cells from plants infected with CAM/DF and stained by the Feulgen method, the nuclei stained violet but the nucleoli and X-bodies did not stain. In cells treated with acridine orange R, the nuclei showed the greenish yellow fluorescence characteristic of double-stranded nucleic acid, and the nucleoli and cytoplasm lining the cell wall gave the red fluorescence typical of single-stranded nucleic acid. Most X-bodies fluoresced bright red (Fig. 1 e to f) but a few were greenish. In general the fluorescence was stronger in cells fixed with acetone than in those fixed with Carnoy's fluid. Only faint fluorescence occurred in cells not treated with acridine orange.

Tests were made to see whether the ability of the X-bodies to fluoresce red after treatment with acridine orange was abolished by pretreatment with pancreatic ribonuclease (previously heated to 100° for 2 to 3 min. to inactivate any traces of deoxy-
ribonuclease). Incubating cut leaf-hair cells in ribonuclease (1 mg./ml.) for 1 hr had little or no effect on the staining of the X-bodies or nuclei by acridine orange but it abolished the staining of the cytoplasm. Resistance of X-body material to digestion by ribonuclease was, however, much decreased by pretreatment with pronase (0.1 mg./ml.) for 30 min. After subsequent incubation with ribonuclease (1 mg./ml.) for 30 min. and treatment with acridine orange, the X-bodies showed little or no fluorescence; the nuclei stained as before. Fluorescence of X-bodies was abolished not only in the cut hair cells but also in many intact cells, showing that pronase treatment made the cells permeable to ribonuclease. Treatment with pronase alone did not affect the acridine-orange fluorescence of the X-bodies or nuclei. These results therefore indicate that most X-bodies are rich in single-stranded RNA, which is protected by proteinaceous material from digestion by ribonuclease.

**Test for lipids.** The X-bodies induced by CAM/DF stained dark blue or black with Sudan black B, indicating that they contain free fatty acids and/or phospholipids (Jensen, 1962).

### Table 1. Fluorescent antibody tests with leaf-hair cells infected with tobacco rattle viruses

<table>
<thead>
<tr>
<th>Leaves infected with</th>
<th>Virus antiserum</th>
<th>Fluorescein-conjugated anti-rabbit globulin</th>
<th>Appearance of treated cells in fluorescence microscope</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Not infected</td>
<td>CAM</td>
<td>+</td>
<td>Very weak cytoplasmic fluorescence</td>
</tr>
<tr>
<td>(2) CAM</td>
<td>CAM</td>
<td>+</td>
<td>Strong cytoplasmic fluorescence in granules and strands</td>
</tr>
<tr>
<td>(3) CAM</td>
<td>TNV*</td>
<td>+</td>
<td>No cytoplasmic fluorescence, weak green fluorescence of nuclei and X-bodies</td>
</tr>
<tr>
<td>(4) CAM/DF</td>
<td>None</td>
<td>-</td>
<td>As (1)</td>
</tr>
<tr>
<td>(5) CAM/DF</td>
<td>None</td>
<td>+</td>
<td>As (4)</td>
</tr>
<tr>
<td>(6) CAM/DF</td>
<td>TNV*</td>
<td>+</td>
<td>As (4)</td>
</tr>
<tr>
<td>(7) CAM/DF</td>
<td>CAM</td>
<td>+</td>
<td>As (4)</td>
</tr>
<tr>
<td>(8) Not infected</td>
<td>PRN</td>
<td>+</td>
<td>As (1)</td>
</tr>
<tr>
<td>(9) PRN</td>
<td>PRN</td>
<td>+</td>
<td>Strong cytoplasmic fluorescence, slightly more fluorescence of X-bodies than in 10</td>
</tr>
<tr>
<td>(10) PRN</td>
<td>TNV*</td>
<td>+</td>
<td>No cytoplasmic fluorescence, slight fluorescence of X-bodies</td>
</tr>
</tbody>
</table>

* Antiserum to tobacco necrosis virus, used as a control treatment.

**Fluorescent antibody tests.** Although CAM/DF was thought not to produce virus coat-protein, it seemed worth testing to see whether serologically active but functionally defective coat protein could be detected. Fluorescent antibody tests were therefore applied to cut hair cells from plants infected with CAM, CAM/DF or PRN, or from healthy plants. The results (Table 1) show that CAM- and PRN-infected cells gave strong cytoplasmic fluorescence after reaction with their homologous virus antisera, and that there was little fluorescence in any of the control treatments. There was no evidence of fluorescent antibody staining in cells containing X-bodies induced by CAM/DF. A few tests were also made with cells containing X-bodies induced by PRN. These X-bodies
Fig. 2. Electron micrograph of section of part of hair cell from plant infected with CAM/DF, showing X-body (X), nucleus (N), cytoplasmic process penetrating nucleus (R), chloroplast (C) and cell wall (W).
Fig. 3. (a) Section of part of a first stage X-body induced by CAM/DF, showing many mitochondria (M), darkly-staining granules (D) and lobe of nucleus (N); 11 days after plants were inoculated. (b) Part of leaf-hair cell from uninfected *N. clevelandii* plant; the mitochondria (M) contain numerous cristae. (c) Abnormal mitochondria (M) with peripheral sacs (P) in second stage X-body induced by CAM/DF.
fluoresced only slightly more strongly than those in untreated cells, when treated with homologous antiserum and fluorescent antibody. They seemed not to contain large amounts of virus antigen.

Electron microscope observations of leaf-hair cells

Cells from healthy plants. These cells contained a thin layer of cytoplasm lining the cell wall, and had large vacuoles. Mitochondria were distributed through the cytoplasm, which was rich in ribosomes. The mitochondria contained more numerous cristae (Fig. 3b) than those in mesophyll cells. Plastids were scattered through the cytoplasm, but did not appear to contain starch grains. A nucleolus or chromatinic material was evident in several of the nuclei, some of which were penetrated by cytoplasmic processes containing ribosomes.

Cells from plants infected with isolate CAM/DF. There was no evidence of infection in cells which did not contain X-bodies. Cells without X-bodies were examined in leaf-pieces collected when X-body formation was starting. The mitochondria seemed normal and the other cell components resembled those in cells of healthy leaves.

The X-bodies had a characteristic internal structure (Fig. 2), the appearance of which could be divided into three intergrading and seemingly consecutive types. X-Bodies sampled at an early stage of infection were enclosed by a membrane resembling the tonoplast and continuous with it; they were mostly adjacent to a nucleus. Their most remarkable feature, however, was that they seemed largely composed of mitochondria (Fig. 3a). Indeed there were few mitochondria elsewhere in cells which contained X-bodies. The mitochondria in the X-bodies were surrounded and separated by material containing ribosomes and/or darkly staining granules. The mitochondria showed two kinds of abnormalities: (1) small membranous sacs, perhaps better seen in mature X-bodies (Fig. 3c), occurred between the outer and inner mitochondrial membranes; and (2) central membrane-bounded regions containing weakly staining threadlike material (Fig. 4a) occurred in several mitochondria. These regions are thought to be vacuoles rather than invaginations, because invaginations of the mitochondrial surface might be expected to become filled with the material surrounding the mitochondria, and this was not observed.

At the second stage (in non-inoculated leaves 14-20 days after the plants were inoculated), the mitochondria were almost all in the X-bodies (Fig. 2). They were now totally abnormal in appearance and recognizable mainly by their more or less oval outlines; the marginal sacs were prominent but their cristae were barely discernible (Fig. 4c). In some instances the marginal sacs seemed to be arranged in characteristic rosette patterns (Fig. 4b), and in other mitochondria too the sacs appeared to be invaginations of the outer membrane. However, there was no unequivocal evidence of a connexion between the sacs and the outer membrane. At this stage the X-body was more compact than before, but ribosomes were clearly discernible between the mitochondrial structures. Nearly all the X-bodies were in contact with nuclei, which resembled those in healthy plants.

At the third stage (in non-inoculated leaves, more than 30 days after the plants were inoculated) the X-bodies were irregular in outline and contained empty vacuoles. The other contents of the X-bodies were more or less amorphous and outlines of the mitochondria were barely detectable (Fig. 4d). Parts of the X-bodies nevertheless still contained the darkly staining granules first found in the early stage of development.
Fig. 4. (a) Abnormal mitochondrion with central vacuole (VC) in X-body induced by PRN; 9 days after inoculation. CAM/DF induces similar abnormalities. (b) Rosette membrane structure projecting into mitochondrion in X-body induced by CAM/DF. (c) Part of second stage X-body induced by CAM/DF, adjacent to nucleus (N), showing closely packed abnormal mitochondria. (d) Part of third-stage X-body, 49 days after inoculation with CAM/DF; components are scarcely identifiable.
Fig. 5. (a) Part of X-body induced by PRN, 9 days after inoculation. Note the many abnormal mitochondria (M) and bundles of virus particles (V). (b) Bundle of PRN particles (V) in cytoplasm lining cell wall, 9 days after inoculation. (c) Three abnormal mitochondria in cytoplasm lining wall of cell infected with PRN, 9 days after inoculation. Note peripheral sacs (P) and vacuole (VC).
Cells from plants infected with strain PRN. The formation and structure of the X-bodies found in plants infected with strain PRN closely resembled those described for isolate CAM/DF, but PRN X-bodies contained in addition small aggregates of virus particles (Fig. 5a). The particles seemed all to be of the characteristic long length (c. 185 nm.) and were aggregated side-to-side. The aggregates were randomly orientated within the X-bodies and the particles were not positioned end-on to the mitochondria, by contrast with those in palisade cells infected with strain CAM (Harrison & Roberts, 1968). Small aggregates of PRN particles also occurred in the cytoplasm outside the X-bodies (Fig. 5b). At the early stage of X-body formation, the few mitochondria remaining in the cytoplasm were found to have the two main kinds of abnormality seen in mitochondria within X-bodies, namely, sacs between the mitochondrial membranes and central vacuoles (Fig. 5c). This suggests that the mitochondria become abnormal before they are incorporated into X-bodies.

DISCUSSION

Despite their superficial similarity in the light microscope, it is increasingly clear that the X-bodies induced by different viruses can have different structures and be formed in different ways. Those produced by cauliflower mosaic virus, for example, appear amorphous in the light microscope (Mamula & Milicic, 1968), and electron microscopy of thin sections shows they are rich in virus particles (Kitajima, Oliveira & Costa, 1965; Fujisawa, Rubio-Huertos, Matsui & Yamaguchi, 1967). They may be virus factories (Kamei, Rubio-Huertos & Matsui, 1969). By contrast the X-bodies induced by both tobacco mosaic and tobacco rattle viruses appear granular in the light microscope and contain relatively few virus particles. However, our work shows that this similarity too is only superficial, for whereas mitochondria are seldom found in tobacco mosaic virus X-bodies (Kolehmainen et al., 1965; Esau & Cronshaw, 1967), they are a major constituent of tobacco rattle virus X-bodies. Further examples of differences between X-bodies caused by different viruses could be cited (Matsui & Yamaguchi, 1966). The term ‘X-body’ is therefore an indication of the gross morphology of inclusions and implies nothing about their structure or function.

A tentative sequence of events can now be proposed for the formation of tobacco rattle virus X-bodies. An early effect of infection is the induction of mitochondrial abnormalities. Either these abnormalities, or other changes in the cytoplasm, cause the mitochondria to aggregate in small groups as they move around the cell. Possibly the material containing the darkly staining granules may play a role in the formation of the aggregates. Other mitochondria are progressively added to these small aggregates, which in turn fuse to form larger ones, so that ultimately there are only one or two X-bodies in any cell. The reason for the frequent association of the X-bodies and nuclei at this stage is not clear. Although the mitochondria in such middle-aged X-bodies retain their abundance of peripheral membranous sacs they seem gradually to lose their cristae. Later, some of the membrane systems found bear little resemblance to mitochondria, and it is impossible to be sure they all originated from mitochondria. In old X-bodies, the mitochondria degenerate further and become difficult to distinguish from other X-body material, which appears increasingly amorphous. Vacuoles form within the X-body, and the tonoplast surrounding it becomes indistinct. Finally the X-bodies seem to disintegrate.
Role of mitochondria in the formation of X-bodies

Although the part in infection played by tobacco rattle virus X-bodies needs further study, some comments seem worth while. The X-bodies cannot merely be factories for virus coat-protein, because they were most commonly induced by isolate CAM/DF, which does not produce nucleoprotein virus particles, and no virus antigen could be detected in such X-bodies by fluorescent antibody tests using antiserum prepared against strain CAM. Nor is there any evidence that the X-bodies are sites of virus assembly for those isolates that produce nucleoprotein virus particles. Strain CAM never induced X-bodies and strain PRN induced them only sporadically. Furthermore the small aggregates of virus particles found in X-bodies induced by strain PRN resembled those occurring elsewhere in the cytoplasm. A possibility exists that the X-bodies are sites of synthesis of virus nucleic acid. Their large RNA content, as revealed by acridine orange staining before and after treatment with pronase and ribonuclease, is compatible with this suggestion. This RNA, moreover, seems protected from ribonuclease by proteinaceous material, perhaps because it is closely associated with the mitochondria within the X-bodies. Mitochondria possess their own DNA and protein-synthesizing system (discussed by Roodyn & Wilkie, 1968, and by Wagner, 1969), so that it is not impossible that virus RNA is synthesized in them. Indeed, Cadman (1962) found that much of the infective RNA, produced by another defective isolate of tobacco rattle virus, was partially protected in leaf extracts from inactivation by ribonuclease, and was associated with material that sedimented during low-speed centrifugation.

How the mitochondrial abnormalities are produced is not clear, but the cell’s metabolism must be deranged by the synthesis of virus RNA, and by the synthesis and activity of virus-coded proteins other than coat protein. In Nicotiana clevelandii, as in tobacco (Frost & Harrison, 1967), leaves infected with strain CAM attain their maximum virus content a few days after infection, and most of the virus RNA is presumably by then synthesized and enclosed in virus coat-protein. Thus the virus RNA is largely removed from the metabolic pool. With isolate CAM/DF, by contrast, no such removal occurs and considerable degradation and synthesis can be expected to continue. With strain PRN there could be a temporary lag of coat-protein synthesis and hence a somewhat prolonged turnover of virus RNA. Alternatively the persistence of uncoated virus RNA may have other effects, including synthesis on a larger scale of the hypothetical virus-coded proteins other than coat proteins, one or more of which may have adverse effects on the cell components. Which, if any, of these explanations is correct, it is worth noting that the frequency and persistence of effects at the cellular level, namely mitochondrial abnormalities and the induction of X-bodies, parallel the severity and persistence of the macroscopic symptoms produced in N. clevelandii by these isolates of tobacco rattle virus. These mitochondrial abnormalities in virus-infected cells seem to represent the early stages in a sequence of events which, unless arrested, can soon kill the cells.

The association with mitochondria of nucleoprotein particles of strain CAM (Harrison & Roberts, 1968) and of other similar isolates (Kitajima & Costa, 1969), together with the effects on mitochondria of infection with CAM/DF and PRN, suggest that these organelles may be specifically involved in the replication of tobacco rattle viruses. Whether mitochondria are of more general importance in the replication of viruses is unknown, but it may be significant that Ralph & Wojcik (1969) recovered most double-stranded RNA of tobacco mosaic virus from a cell fraction rich in mitochondria.
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