Virus Inclusions Formed by the PM₂ Mutant of TMV

(Accepted 18 October 1971)

Plants infected with the FLAVUM or the NI-118 strain of TMV contain electron-dense cytoplasmic X-bodies (Kolehmainen, Zech & Von Wettstein, 1965; Kassanis & Milne, 1971), whereas plants infected with the type strain do not. Strain NI-118 produces insoluble coat protein, especially in infected plants that are kept at temperatures above 30°C (Jockusch, 1966; Kassanis & Bastow, 1971), and it is possible that the X-bodies contain this protein. We thought that information about the nature of the X-bodies might be obtained by comparing the behaviour of the two variants of PM₂, only one of which forms insoluble coat protein. PM₂ was originally obtained by treating type TMV with nitrous acid and produces a defective protein unable to coat its RNA (Siegel, Zaitlin & Sehgal, 1962). The original PM₂ has a soluble protein, but a variant, isolated at Rothamsted from it, from a single local lesion, produces insoluble coat protein (Kassanis & Bastow, 1971). A new inoculum of the original isolate was obtained from Dr S. Sarker of the Max Plank Institute, Tubingen. For convenience, we shall call the original isolate the 'German PM₂', and the variant with the insoluble coat protein the 'English PM₂'.

In gel immuno-diffusion tests, using sap from infected leaves as antigen and an antiserum prepared against purified protein from the original PM₂, a line was formed with the German but not the English PM₂. The English PM₂ gave a specific line only when the green pellet from sap, centrifuged at 8000 g, was treated with sodium dodecyl sulphate to solubilize the virus protein (Hariharasubramanian & Zaitlin, 1968). The German but not the English PM₂ also showed open flexuous helical structures of the aggregated coat protein in negatively stained preparations of sap when examined in the electron microscope (Fig. 1). The structures were the same as described by Zaitlin & Ferris (1964) in purified preparations of PM₂ when the pH is lowered to 5.

Light microscopy showed differences between the behaviour of the two variants in epidermal strips obtained from the midrib of the lower leaf surface. Some hair cells, infected with the German PM₂, contained long fibres usually twisted in the form of a figure eight (Fig. 2), similar to the inclusions described by Bald (1964) with an isolate of PM₂. Inclusions in the form of a figure eight were described also with TMV strains that produce complete virus particles, e.g. the FLAVUM strain (Kassanis & Sheffield, 1941). As PM₂ does not produce complete virus particles it is reasonable to conclude that these inclusions consist of virus coat protein. Such inclusions were not produced by the English PM₂ and, by contrast, cells infected with this variant contained amorphous inclusions (X-bodies) similar to those found by Bald (1964) in plants infected with PM₁ which produces insoluble virus protein. In the electron microscope, these inclusions appear electron-dense (Fig. 3). Material for electron microscopy was prepared using the methods of Milne (1970). These X-bodies resemble those described in plants infected with the Ni-118 strain (Kassanis & Milne, 1971) and the FLAVUM strain (Kolehmainen et al. 1965). These two strains also have some insoluble coat protein, suggesting that the X-bodies may consist of the insoluble coat protein. Because of the affinity of X-bodies to osmium, Kolehmainen et al. (1965) suggested that they contain lipids, but the osmiophilic lipid droplets in chloroplasts stain darker than the X-bodies. Such X-bodies were not found in plants infected with the German PM₂ or the type strains of TMV.
Fig. 1. Aggregated protein of the German PM$_{25}$, × 160,000.
Fig. 2. Two hair cells containing fibrous inclusions produced by the German PM$_{25}$, × 500.
Fig. 3. Electron dense X-body in a cell infected with the English PM$_{25}$, × 80,000.
Kolehmainen et al. (1965) showed that it is the RNA of complete particles that stains with osmium, and if free RNA is coiled tightly in the same configuration as in the whole particles, it would have stained in the same way and be indistinguishable from complete virus particles in the electron micrographs. As PM₂ does not form complete virus particles, and as we have seen nothing resembling stained RNA particles, it is reasonable to suggest that either the free RNA is enclosed in some organelle or it is in a different configuration from that in complete virus particles.

Fig. 4. Filaments of the German PM₂. ×40,000. Inset shows cross-banding. ×140,000.
Fig. 5. A montage of two micrographs showing a large area of flexuous particles in a cell infected with the German PM₂. ×39,000.
With the strains of TMV so far studied areas in the cytoplasm have been reported that contain characteristic long electron-dense filaments (Shalla, 1964; Kolehmainen et al. 1965; Milne, 1966). In cross-section the filaments formed by the FLAVUM strain appear as groups of three, or less often as groups of two rings, each ring with diameter of 25 to 30 nm. In longitudinal section the individual particles of the filaments cannot be distinguished (Kolehmainen et al. 1965). Similar filaments occurred in plants infected with the German but not with the English PM2. Some micrographs containing longitudinal sections of the filaments of the German PM2 show the separate particles and also cross-banding along part of their length (Fig. 4). The filaments occur in areas of cytoplasm where the endoplasmic reticulum was much folded and there were many ribosomes. However, one preparation had a large area where flexuous particles were greatly concentrated, mostly oriented in one direction, and free from other cellular components (Fig. 5). In cross-section the particles were hollow. The nature of the filaments is unknown, but Shalla (1964) and Milne (1966) suggested that they were developmental forms of the virus. However, Kolehmainen et al. (1965) thought that they contained particles of the coat protein polymerized in a different way from that in complete particles and therefore able to stain and suggested that they may consist of excess X-protein, beyond that needed to coat the RNA. The fact that filaments were found in plants infected with German but not the English PM2 fits this suggestion, as does the width of the particles within the filaments which is 19 to 22 nm. The apparent discrepancy in width between PM2 and other strains of TMV might be because other workers had to measure the cross-section which might not have been a right angle to the axis of the particle or because the PM2 protein aggregated differently. It is possible that the large area of pure flexuous particles corresponds to the long fibres seen with the light microscope in plants infected with German PM2.

Rothamsted Experimental Station
Harpenden Hertfordshire

B. Kassanis

REFERENCES


(Received 8 October 1971)