Incorporation of Thymidine-^{3}H into Carnation Etched Ring Virus

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ABSTRACT

Characteristic X-bodies occurred in leaves of *Dianthus* spp. infected systemically with carnation etched ring virus. Most X-bodies were round and smaller than the nuclei. The X-bodies consisted of an amorphous matrix, vacuolelike spaces, and spherical particles about 40-45 nanometers in diameter. These general profiles of X-bodies associated with carnation etched ring virus infection resembled those of viruses belonging to the cauliflower mosaic virus group. According to purification and sucrose density-gradient centrifugation of extract from infected leaves labeled with thymidine-^{3}H, the radioactivity was in one fraction. Since this fraction consisted of spherical particles about 47 nanometers in diameter, it was concluded that carnation etched ring virus contained deoxyribonucleic acid. Carnation etched ring virus is a possible member of the cauliflower mosaic virus group. Phytopathology 61:681-684.

Additional key words: carnation, virus particle.

Cauliflower mosaic virus (CaMV) is the first higher plant virus reported to contain DNA (13, 14). According to Gibbs (4), the CaMV group consists of CaMV and dahlia mosaic virus (DMV). The viruses of the CaMV group are spherical particles 40-50 nm in diam (2), and are restricted in or on the characteristic intracellular X-bodies (3, 9, 10). Since CaMV is related serologically to DMV (2), it is likely that DMV also contains DNA. Carnation etched ring virus (CERV) was described first by Hollings & Stone (6). The particle size (5) and X-bodies (12) of CERV resemble those of CaMV group. CERV is related serologically to CaMV and DMV (5). Thus CERV may contain DNA as well as CaMV.

This paper deals with the characteristic intracellular X-bodies associated with CERV infection and thymidine-^{3}H uptake of CERV.

MATERIALS AND METHODS.—*Viruses and plants.*—Original inocula of CERV used were collected in Madrid (Spain). They always contained not only CERV but also carnation mottle virus (CMoV). About 1 month after mechanical inoculation, *Dianthus caryophyllus* L. sometimes showed slight mottling, but frequently had no visible symptoms. Conspicuous necrotic blotches of irregular shape appeared on all inoculated leaves of *D. chinensis* L. Etched rings appeared on some of upper leaves. The inocula was not infective to *Brassica perviridis* Bailey and *Zinnia elegans* Jacq., susceptible to CaMV and DMV, respectively. CaMV and *B. perviridis* used in the study were those described previously (3, 8).

Light microscopy.—Epidermal strips of systemically infected leaves of *D. caryophyllus* and *D. chinensis* were stained with phloxine and examined under a light microscope as described previously (3).

Thin-sectioning.—Small pieces of systemically infected leaves of *D. caryophyllus* were examined by thin-sectioning described previously (3).

Isotopes.—Thymidine-6-^{3}H (5.0 c/mmole) and uridine-5-^{3}H (6.0 c/mmole) were used.

Purification.—Young leaves of *D. caryophyllus,* a systemic host of CERV and CMoV, were inoculated by rubbing with crude virus solution containing Carborundum. About 1 month after inoculation, the infected plants were harvested. Two hundred g of leaves were frozen and were homogenized in 300 ml of cold 0.5 M phosphate buffer, pH 7.5, containing 0.01 M Na_2SO_3. The sap of homogenate was stirred at 4 C for 1 hr after addition of 8 ml of *n*-butanol/100 ml of the sap. Denatured host protein and cell debris were removed by low-speed centrifugation. The viruses were sedimented by centrifugation at 78,000 *g* in the No. 30 rotor of a Spinco Model L ultracentrifuge. The pellet was suspended in 0.01 M phosphate buffer, pH 7.5. One hundred g of leaves of systemically infected *B. perviridis* were used for purification of CaMV.

Isotope uptake.—Systemically infected leaves of *D. caryophyllus* were floated for 4 days on a uridine-^{3}H or thymidine-^{3}H solution under continuous illumination at 27 C. They were purified in the same way. Thymidine-^{3}H uptake of CaMV-infected *B. perviridis,* uridine-^{3}H or thymidine-^{3}H uptake of healthy *D. caryophyllus,* and their purifications were carried out in the same way.

Sucrose density-gradient centrifugation.—The mixture of labeled and nonlabeled virus solution (2-3 ml) obtained by purification was layered onto a 25-ml sucrose density-gradient (5-30%) made with 0.01 M phosphate buffer, pH 7.5. The tubes were centrifuged at 24,000 rpm for 2 hr at 4 C. After centrifugation, the gradient was divided into 38 fractions from the bottom of the gradient.

Radioactivity measurement.—One-half of a fractionated volume was transferred to 5 ml of Bray's solution (1), and the radioactivity was assayed in a liquid-scintillation counter.

Electron microscopy.—Radioactive fractions obtained by sucrose density-gradient centrifugation were dialyzed overnight against phosphate buffer and were stained with 2% potassium phosphotungstate solution.

RESULTS AND DISCUSSION.—Under a light microscope, the X-bodies, uniformly stained deep red with phloxine, were easily observed. Most X-bodies were round and smaller than the nuclei (Fig. 1). General

profiles of these X-bodies were similar to those associated with CaMV or DMV infection (3, 11).

Under an electron microscope, the X-bodies appeared as dense organelles in the cytoplasm (Fig. 2). The X-bodies consisted of an amorphous matrix, vacuolelike spaces, and spherical particles. No membranes were around either the X-bodies or the vacuolelike spaces within the X-bodies. The spherical particles were found within the matrix, vacuolelike spaces, and on the X-bodies. They formed no crystal arrays. The diameter of these spherical particles was 40-45 nm. The spherical particles were of two types: uniformly dense particles and doughnutlike particles consisting of an outer shell and empty inner core. These characteristics of the X-bodies associated with CERV infection were similar to those associated with CaMV or DMV infection (3, 9, 10). We did not observe CMoV particles within or on the X-bodies, and no X-bodies were found in healthy *D. caryophyllus*.

According to analysis by sucrose density-gradient centrifugation, the extract from CaMV-infected leaves of *B. perviridis* labeled with thymidine-^{3}H exhibited a single peak in the radioactivity as shown in Fig. 3. Electron microscopy of this radioactive fraction revealed spherical particles about 42 nm in diameter

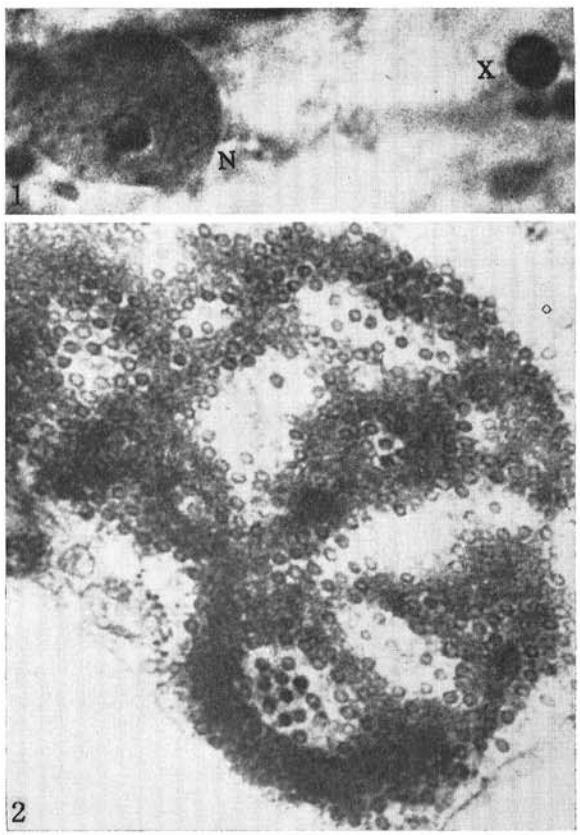

Fig. 1-2. **1)** Light micrograph of nucleus (N) and X-body (X) in an epidermal cell of *Dianthus caryophyllus* infected with carnation etched ring virus. (× ca. 3,500) **2)** Electron micrograph of X-body associated with carnation etched ring virus infection. (×38,000)

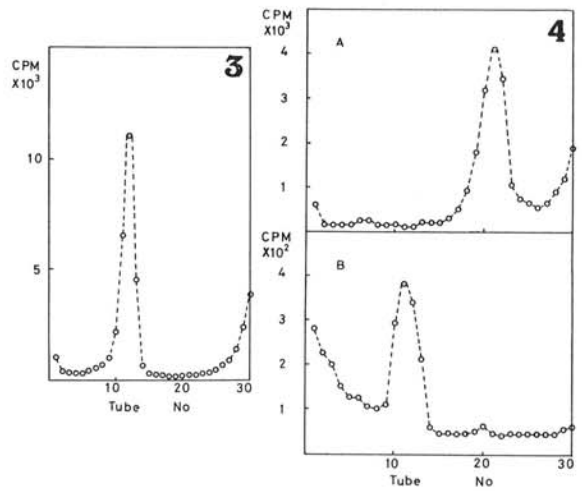

Fig. 3-4. **3)** Radioactivity profile of a centrifuged sucrose density-gradient containing thymidine-^{3}H labeled cauliflower mosaic virus. Sedimentation from right to left with the smaller numbers representing tubes from the bottom of the gradient. **4)** Radioactivity profiles of centrifuged sucrose density-gradients containing uridine-^{3}H or thymidine-^{3}H labeled leaf extracts of systemically infected *Dianthus caryophyllus*. **A)** Leaves were floated on water containing uridine-^{3}H. **B)** Leaves were floated on water containing thymidine-^{3}H.

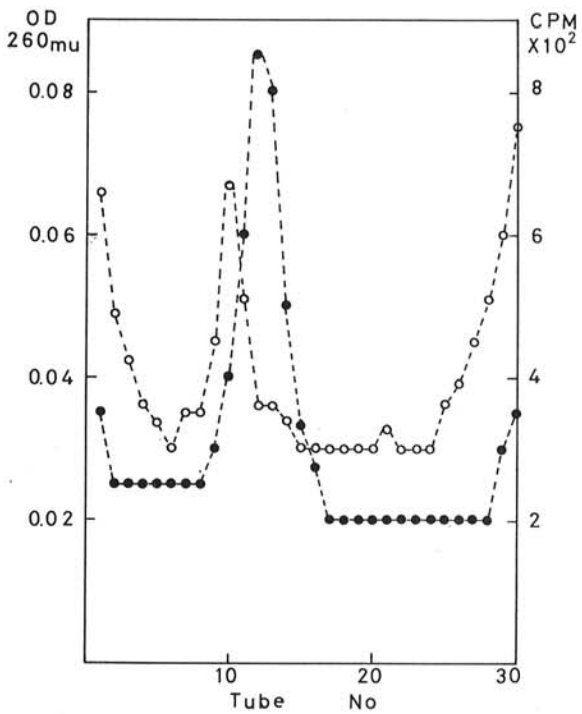

Fig. 5. Optical density profile of a centrifuged sucrose density-gradient containing completely purified cauliflower mosaic virus (●-----●) and radioactivity profile of a centrifuged sucrose density-gradient containing thymidine-^{3}H labeled carnation etched ring virus (○-----○).

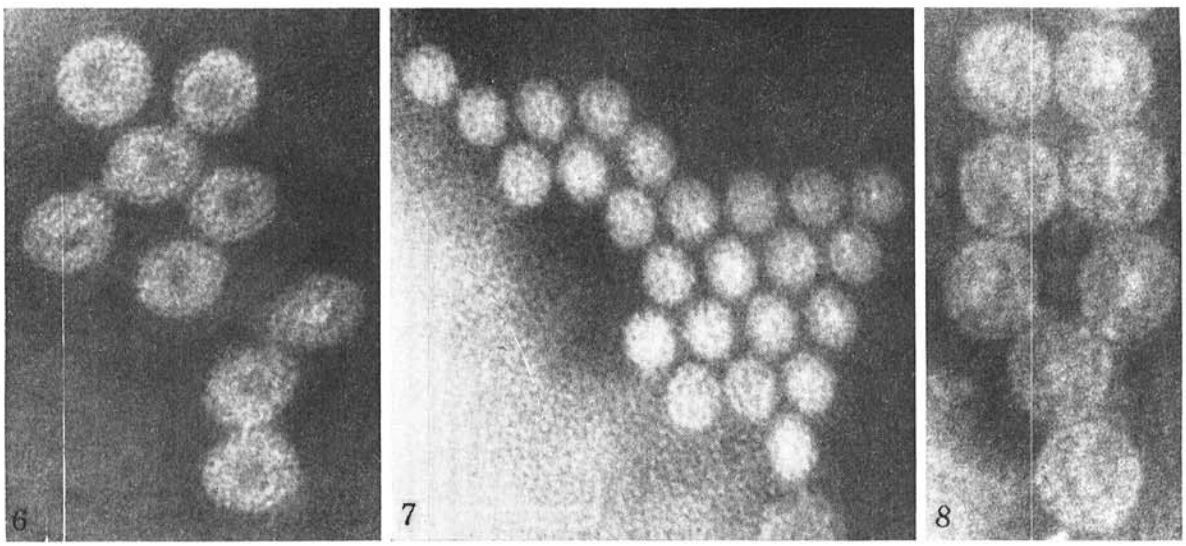

Fig. 6-8. Electron micrographs of negative stained virus particles. **6)** Cauliflower mosaic virus. (×260,000) **7)** Carnation mottle virus. (×300,000) **8)** Carnation etched ring virus. (×260,000)

(Fig. 6). These uniform particles corresponded to CaMV, and had infectivity to *B. perviridis*. Since it is common for thymidine-^{3}H to be incorporated into DNA, Fig. 3 and 6 showed that CaMV contained DNA.

The radioactivity profiles of extracts from systemically infected leaves of *D. caryophyllus* labeled with uridine-^{3}H or thymidine-^{3}H prepared by sucrose density-gradient centrifugation were in Fig. 4-A and 4-B. In uridine-^{3}H incubation, the radioactive peak appeared at tube No. 21 (Fig. 4-A). Electron microscopy of this radioactive fraction revealed spherical particles about 23 nm in diameter (Fig. 7). The fraction was infective to *D. caryophyllus, D. chinensis, Chenopodium amaranticolor, Beta vulgaris,* and *Gomphrena globosa.* These spherical particles corresponded to CMoV. Since uridine-^{3}H uptake occurred in CMoV, it is clear that the virus contained RNA. In thymidine-^{3}H incubation, on the other hand, the radioactive peak appeared at tube No. 11 (Fig. 4-B). Electron microscopy of this radioactive fraction revealed spherical particles about 47 nm in diameter (Fig. 8). Their size was uniform and similar to those encountered within the X-bodies (Fig. 2) and those reported previously (6). Since these spherical particles were infective to *D. caryophyllus* and formed X-bodies, it was considered that these particles corresponded to CERV. Furthermore, since thymidine-^{3}H uptake occurred in this fraction and the fraction consisted of CERV particles, it is clear that CERV contained DNA. The amount of CERV extracted from the infected leaves was so small that no extraction of infectious nucleic acid from the virus was carried out. In the extracts from leaves of healthy *D. caryophyllus,* neither uridine-^{3}H uptake nor thymidine-^{3}H uptake occurred at tube No. 21 or 11, respectively.

In the centrifuged sucrose density-gradient containing thymidine-^{3}H labeled CERV and completely purified nonlabeled CaMV, CERV occupied a slightly lower position than did CaMV (Fig. 5). In the present study, CaMV was about 42 nm and CERV was about 47 nm in diameter. The sedimentation constant of completely purified CaMV used in the study was 206 S (7), whereas there was no information on the sedimentation constant of CERV. So far as the present study was concerned, it was not clear whether the different sedimentation between CaMV and CERV resulted from their different sedimentation constant or not. It is likely that the difference in their sedimentation is due to the purity of viruses used in the study. Hollings (*personal communication*) pointed out that the purification of CERV was more difficult than those of CaMV and DMV.

We conclude, therefore, that particle morphology of CERV and their intracellular appearance closely resembles those of CaMV and DMV, though their host ranges were completely different. Both CaMV and CERV contain DNA; thus, CERV is a possible member of the CaMV group.

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