Electron Microscopy of the Formation of Carnation Etched Ring Virus Intracellular Inclusion Bodies

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In two previous papers, Rubio-Huertos et al. (1968a), Fujisawa, Rubio-Huertos & Matsui (1971), inclusion bodies induced by carnation etched ring virus (CERV) were studied by light and electron microscopy. The inclusion bodies were composed of a dense matrix and virus particles. They were not limited by membranes and their general appearance, both in the light and electron microscopes, was very similar to that described for cauliflower mosaic virus (CAMV) Rubio (1956), Rubio-Huertos et al. (1968a) to which CEMV is serologically related, Hollings & Stone (1969).

In this paper, we describe the formation of these intracellular inclusions, and the presence of CERV virus particles in the nuclei of Dianthus barbatus infected cells.

Several small plants of Dianthus barbatus were inoculated mechanically with crude sap of Dianthus caryophyllus L. infected with CERV. Samples were taken at intervals from the infected plants and fixed and embedded in Durcupan, as described previously, Rubio-Huertos et al. (1968b). The sections, which were made with a LKB ultramicrotome, were stained with lead citrate and observed with a Siemens Elmiskop I.

In samples taken from plants infected for 20 to 30 days, spherical particles, some of them whole and most of them with a clear centre and diameter of 42 to 46 nm. were found in the nucleoplasm of several nuclei (Fig. 1–3) sometimes near the nucleolus. CERV particles were also found scattered freely in the cytoplasm and in association with a dark matrix. No complete inclusion bodies were observed at this stage.

Samples collected from plants infected for more than a month had already formed inclusion bodies which had the characteristics described previously. These, however, were found in very few cells, and spherical particles of CERV were observed within the nuclei.

Before complete formation of inclusion bodies, virus particles were observed mixed with ribosomes, with small irregular vesicles bound by a unit membrane and with a dark matrix distributed in small quantities in different areas. The small vesicles could be morphologically related with dictyosomes (Fig. 4).

The complete inclusion bodies of CERV have more virus particles and less dark matrix than those formed by CAMV. In fact, the dense areas of the inclusions are formed by very close, packed particles and very little dark matrix (Fig. 6, 7).

Some free CERV particles were seen to be present in plasmodesmata (Fig. 5).

Although we have been unable to observe the passage of virus particles from the nuclei to the cytoplasm or vice versa, the presence of virus particles inside nuclei before the formation of inclusion bodies seems to indicate that CERV particles form in the nuclei of the infected cells from whence they pass to the cytoplasm. In any case, the particles do not seem to form within the dark matrix or viroplasm. This dark substance, which in the case of CAMV is a protein (Martelli & Castellano, 1971), may be involved in the formation of inclusion bodies.
Fig. 1. Part of an infected cell of *Dianthus barbatus* showing CERV virus particles within the nucleus and others in the cytoplasm.

Fig. 2. Part of a nucleus showing CERV virus particles in the nucleoplasm and near the nucleolus.

Fig. 3. A nucleolus and nucleoplasm showing CERV virus particles in the nucleoplasm and some of them in the nucleolus.
Fig. 4. Part of a group of CERV virus particles, vesicles, ribosomes and dark matrix, prior to inclusion body, formation.

Fig. 5. CERV virus particles scattered in the cytoplasm. One of them seems to be in a plasmodesm (arrow).

Fig. 6. Part of an inclusion body of CAMV showing the granular dark matrix and few virus particles.

Fig. 7. Part of a CERV inclusion body showing the dark areas formed mainly by virus particles.
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REFERENCES


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