ANNULATE LAMELLAE IN PHLOEM CELLS OF VIRUS-INFECTED *SONCHUS* PLANTS

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ABSTRACT

The occurrence of annulate lamellae (AL) in differentiating phloem of *Sonchus oleraceus* (Compositae) singly infected with sowthistle yellow vein virus (SYVV) and doubly infected with a combination of SYVV and beet yellow stunt virus is documented by electron microscopy. Cell types in which AL were found were immature sieve elements and phloem parenchyma cells. AL were found only in cells that also contained SYVV particles although a direct association between the virus and AL was not apparent. The substructure of the AL and the relationships between the AL and the nuclear envelope and endoplasmic reticulum are similar to those reported in other descriptions of this organelle in the literature. This report appears to be the first one concerning the association of AL with a plant virus disease.

Annulate lamellae (AL) are subcellular organelles that are structurally similar to both the nuclear envelope and the endoplasmic reticulum (ER). AL cisternae, containing definitive pore complexes similar to those of the nuclear envelope, are found either singly or in parallel stacks, usually in the perinuclear cytoplasm but sometimes elsewhere in the cytoplasm or within the nucleus itself. AL have been reported from a wide variety of animal cell types including germ cells and embryonic cells, adult somatic cells, and some tumor and cancer cells, and have been reviewed extensively (10, 33) and more briefly (4, 11). An increase in the amount of AL in virus-infected animal cells, both in vivo and in vitro, has been documented (1, 12, 13, 20, 21, 22, 24). AL have also been induced to form in cells treated with a variety of chemical stimuli (2, 15, 16).

Only a few reports of AL in plant cells have been published. Kessel (10), in the addendum of his review, states that Skvarla saw AL in developing pollen of *Canna*, but these observations were not published. The earliest documented report of AL in plant cells appears to be that of Gianordoli (8), who described their structure, origin, and formation in the peripheral cytoplasm of the central cell of the gymnosperm *Sciadopitys verticillata* (Taxodiaceae). Sheridan and Barrnett (30) reported but did not illustrate the presence of cytoplasmic AL near meiotic nuclei in *Lilium* microsporocytes. Sen (29) also identified structures in *Lilium* microsporocytes as AL, but, as pointed out by Franke et al. (6), these appear to be stacked ER, not AL. Single cisternal sheets of both intranuclear and cytoplasmic AL were found in cultured stalk cells of the composite *Haplopappus gracilis* (6). More extensively developed stacks of cytoplasmic AL were found in developing pollen and pollen mother cells of *Canna generalis* (Cannaceae) (28). Two reports of AL in the Rhodophyta have described the occurrence of intranuclear AL in post-meiotic tetraspore mother cells of *Corallina officinalis* (25), and the occurrence of both single cisternae of intranuclear AL and more

elaborate stacks of cytoplasmic AL in postfertilization development of *Polysiphonia novae-angliae* (32). Recently, cytoplasmic AL also have been seen in developing pollen of *Beta vulgaris* (Chenopodiaceae) (L. L. Hoefert. Unpublished observations). This report documents for the first time the occurrence of typical cytoplasmic AL in the phloem of a virus-infected plant, *Sonchus oleraceus* L. (Compositae). These observations were made during a broader study of single and double virus infections of sowthistle plants.

MATERIALS AND METHODS

Leaves of greenhouse-grown healthy sowthistle (*Sonchus oleraceus* L.) and sowthistle infected singly with either beet yellow stunt virus (BYSV) or sowthistle yellow vein virus (SYVV), or with a combination of both viruses, were collected and fixed at 12–14 days after inoculation when symptoms in leaves first became apparent. The tissue was fixed in full- or half-strength paraformaldehyde-glutaraldehyde (9) at room temperature in 0.05 M phosphate buffer (pH 6.8) for 2 h. The tissue was then washed in buffer and postfixed in 2% OsO_4 overnight at 4°C. Dehydration in acetone was followed by embedding in Epon. Thin sections, cut paradermally, were poststained with uranyl acetate and lead citrate.

OBSERVATIONS AND DISCUSSION

AL were found in the cytoplasm of several cells of the differentiating phloem of young leaves singly infected with SYVV or doubly infected with BYSV and SYVV. No AL were seen in any cells from uninoculated or BYSV singly infected plants AL appeared to be infrequent and transitory structures that were found in very few cells from leaves about one-fourth to one-third the size of mature leaves, no AL were found in leaves younger or older than these. Cell types in which AL were located included immature sieve elements (Figs. 1, 2, and 6) and phloem parenchyma cells (Figs. 3 and 5). AL were observed only in cells that also contained particles of SYVV, but not all immature phloem cells having SYVV also contained AL. AL were not found in differentiating xylem or mesophyll cells, although these cell types were not examined as extensively as were the phloem cells.

AL in the form of one to four short, straight, or slightly curved cisternal sheets bearing characteristic pore complexes are located in the perinuclear cytoplasm. The AL are found in various relationships to the nuclear envelope, including orientations oblique (Figs. 1 and 2), perpendicular (Figs. 3 and 4) or parallel (Figs. 5 and 6) to the nuclear envelope. AL sometimes appear in close proximity to the nuclear envelope with some pores of the AL and nuclear envelope appearing to be in alignment (Fig. 5). Extensive stacks of AL as seen in *Canna* (28) and *Polysiphonia* (32), and circular configurations shown in *Sciadopitys* (8) and *Polysiphonia* (32), were not observed in *Sonchus*.

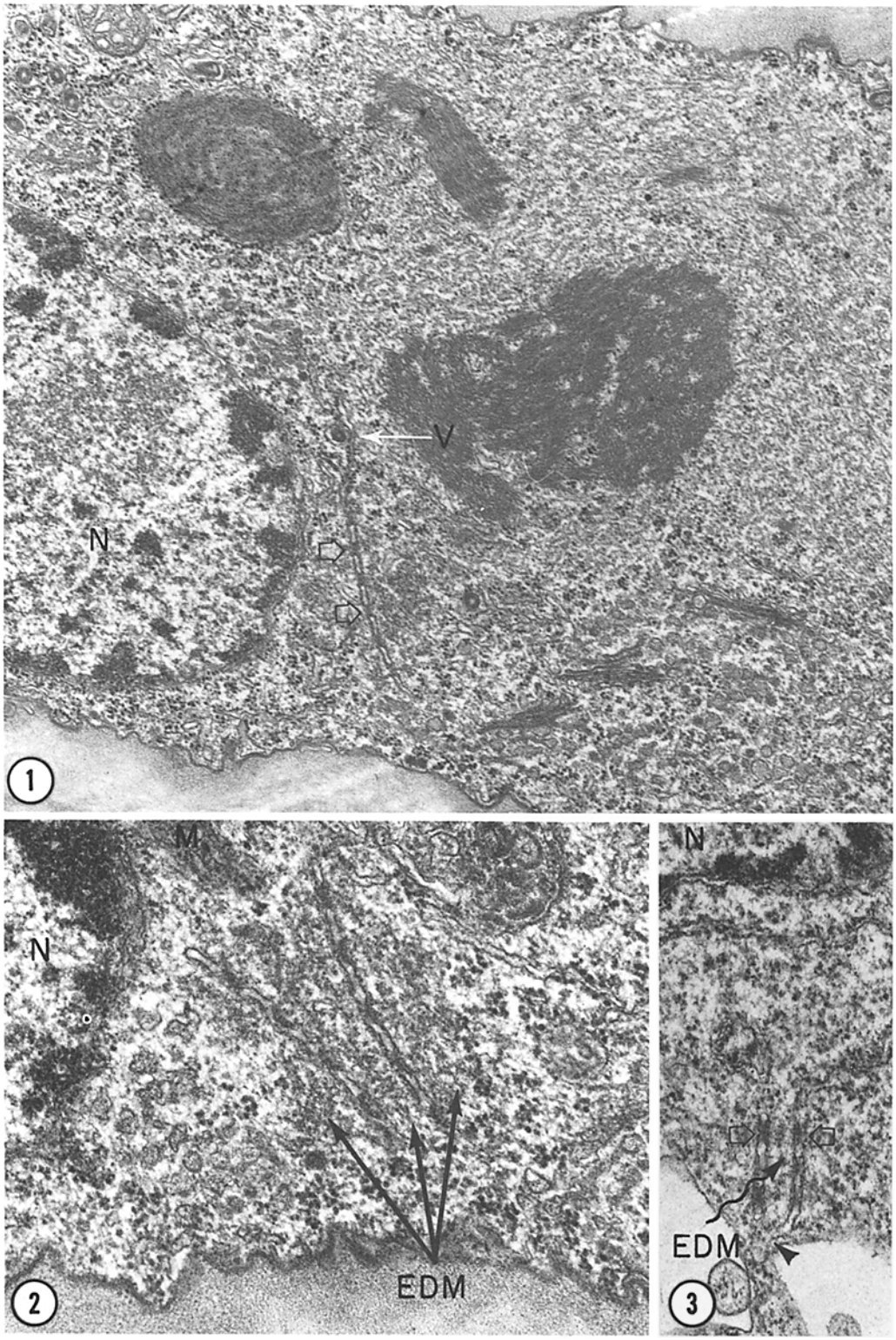
The substructure of the AL pores is similar to that of the nuclear envelope pores (Fig. 4), but pore complexes appear to be more densely concentrated in the membranes of the AL than in the nuclear envelope. This observation is in agreement with other reports on the concentration of AL pores in both plant and animal cells (4, 11, 18, 28). In favorable surface or tangential sections, pores are seen to consist of a central electron-dense granule that is surrounded by an electron-dense, somewhat granular ring (Fig. 4). In cross section, electron-dense, granular to somewhat fibrillar material with either a relatively diffuse or compact configuration is frequently associated with the AL (Figs. 2, 3, and 6). This material appears both to the exterior of the AL and in the intercisternal spaces (Fig. 2) and sometimes appears to be more concentrated in the region between juxtaposed pores of adjacent lamellae (Fig. 3). Observations similar to these have been made in other studies of AL in plants (28) and in virus-infected animal cells (24). Electron-dense material

FIGURES 1–3 Annulate lamellae (AL) in immature sieve elements of a plant singly infected with SYVV (Figs. 1 and 2) and in phloem parenchyma cell of a plant doubly infected with SYVV and BYSV (Fig. 3).

FIGURE 1 Single sheet of AL with several pores (arrows) near nucleus (*N*); AL continuous with rough ER adjoining a transversely cut virus particle enclosed in ER (*V*). × 28,000.

FIGURE 2 Relatively diffuse electron-dense material (*EDM*) outside and between two sheets of AL; a portion of a mitochondrion (*M*) and nucleus (*N*). × 54,000.

FIGURE 3 Pores (arrows) of AL appear in alignment; relatively compact electron-dense material (*EDM*) appears between AL, which may terminate in an ER cisterna (arrowhead). × 37,500.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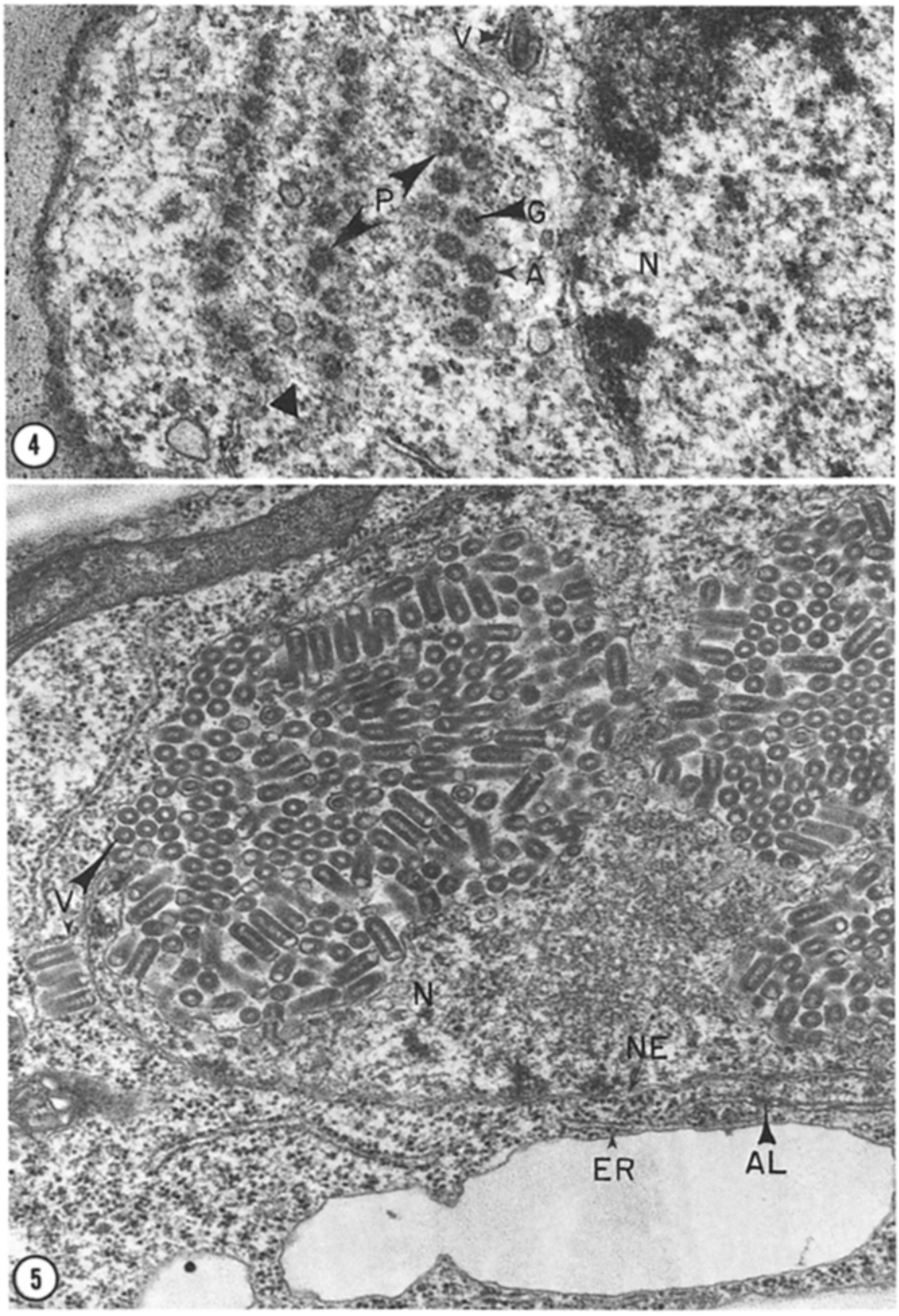
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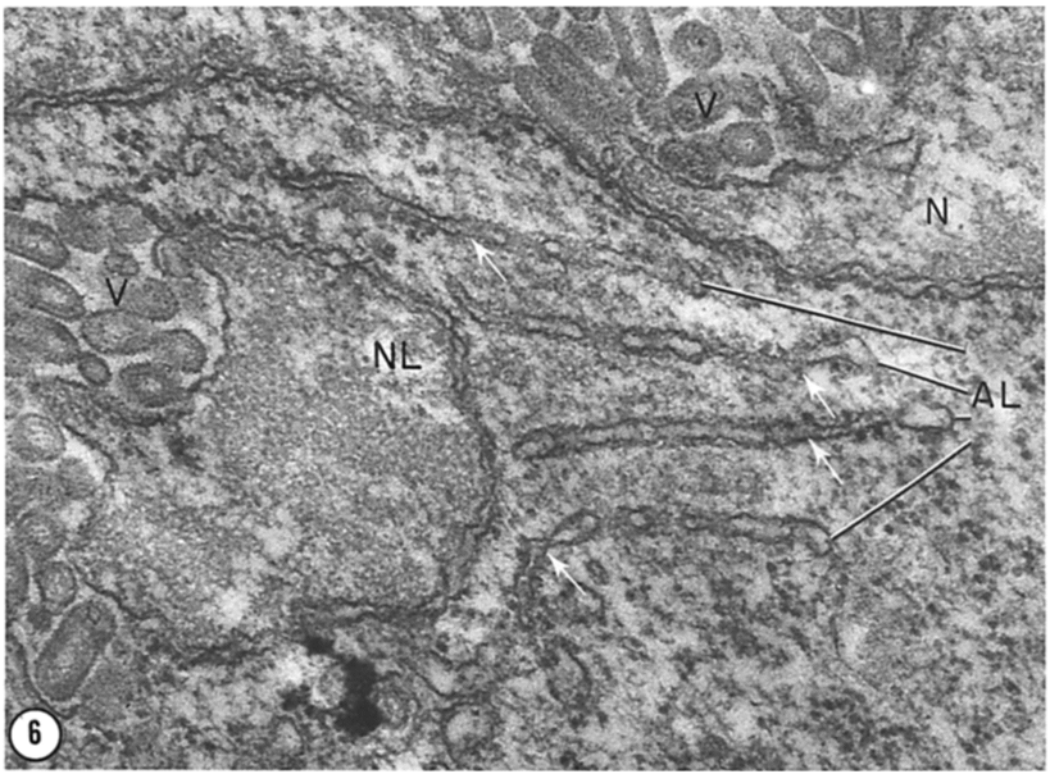

FIGURE 6 Annulate lamellae (*AL*) in immature sieve element of a plant singly infected with SYVV. Four sheets of AL located near nucleus (*N*) and a lobe of the nucleus (*NL*) containing particles of SYVV (*V*). Margins of some AL continuous with rough ER (white arrows). Relatively diffuse electron-dense material outside and between AL cisternae. × 78,000.

also may be found between the nuclear envelope and AL (Fig. 5). Franke (4) described electron-dense material between juxtaposed pores of the nuclear envelope and AL.

The margins of AL cisternae sometimes appear to be continuous with cisternae of the ER (Figs. 1, 4, and 5). In *Sonchus* the ER membranes bear ribosomes, while membranes of the AL are smooth. The association of ribosomes with AL has, however, been noted in some other studies (6, 10, 11, 28). The ER associated with the AL in *Sonchus* is not generally inflated as it is in some AL-ER connections (28, 32, 33).

The association of AL with other cell organelles has been described by Scheer and Franke (28) with dictyosomes in *Canna* pollen mother cells, by Maul (18) with dictyosomes in melanoma cells, and by Wetherbee et al. (32) with developing

FIGURES 4–5 Annulate lamellae (*AL*) in phloem cell of a plant doubly infected with SYVV and BYSV (Fig. 4) and in phloem parenchyma cell of a plant singly infected with SYVV (Fig. 5).

FIGURE 4 Tangential and surface views of AL showing dense concentration of pores; nucleus (*N*). AL pores (*P*) show dense central granule (*G*) and annulus (*A*); margin of AL seems to be connected with rough ER (arrowhead). Virus particle (*V*) enclosed in ER membrane near nucleus. × 51,000.

FIGURE 5 *AL* in close proximity to the nuclear envelope (*NE*); some pores of the *AL* and *NE* appear to be aligned. Small amounts of electron-dense material located between the *AL* and NE; rough *ER* may be in continuity with the *AL* at each margin of the *AL* cisterna. Some SYVV particles (*V*) are found within the membranes of the *NE*, × 38,000.

mitochondria in *Polysiphonia*. Dictyosome activity in immature sieve elements and young phloem parenchyma cells of *Sonchus* appears to be intense, as judged by the large numbers of dictyosomes and dictyosome-derived vesicles apparent in the micrographs of these cells. Some dictyosome vesicles are found in the vicinity of AL (Figs. 1 and 2), but there is not sufficient evidence at this time to suggest a functional association between these organelles. Normal immature sieve elements often contain large numbers of dictyosomes and dictyosome-derived vesicles, which are believed to function in the deposition of the nacreous wall characteristic of this cell type (26). Mitochondria also may appear in close proximity to the AL in *Sonchus* (Figs. 2, 3, and 8), but again a functional relationship is not suggested.

Recently, DeBrabander and Borgers (2) reported the accumulation of AL in several lines of cultured cells treated with a variety of antitubulins that caused the disruption and disappearance of microtubules. They hypothesized that the AL might serve as a nucleation site for tubulin synthesis or microtubule polymerization in cells that had lost their microtubules; as such, the AL were viewed as a reactive hyperproduction of such sites. No similar function could be envisioned in *Sonchus*, although in Fig. 4 microtubules appear in close proximity of AL.

The origin of AL in *Sonchus* could not be determined with certainty in our limited observations of these organelles. When found, the AL appeared to be well formed or differentiated. Possibilities that the AL were formed by delamination from the nuclear envelope or by transformations of the ER are perhaps the best suggestions at this time. Close proximity or continuity of the AL with the nuclear envelope is suggestive of origin from the nuclear envelope in a manner similar to that proposed by Merkow et al. (22). In *Sonchus*, connections of the AL with the ER were observed; similar connections have been taken in other studies as evidence for AL formation from the ER (4, 24). AL-ER-nuclear envelope associations have been noted in some studies of virus-infected cells (20, 21). No evidence of nuclear blebbing or fusion of cytoplasmic vesicles to form AL-like cisternae was apparent in our micrographs. Nuclear envelope blebbing and subsequent coalescence of rough or smooth vesicles have been implicated in the formation of AL in many animal cells (10, 11, 33), but in the plant cell systems so far studied (6, 28, 32) and some animal cell systems (5) nuclear blebbing was not found, and instead there was evidence of transformation of smooth cytoplasmic cisternae (31) or of rough ER (6, 28).

The functions of AL are unknown at this time. The AL are relatively rare and transitory structures, and have, therefore, been difficult to study experimentally. They appear in a wide variety of cell types in association with a variety of other cell membranes and organelles, and in response to a variety of normal and abnormal stimuli. They appear most frequently in rapidly growing or differentiating cell systems under normal and abnormal circumstances, but they have been reported occasionally in association with degenerative cell changes (13). AL have been suggested to be important in nucleocytoplasmic enchanges (10), in protein synthesis (19, 33), and in synthesis of other cell products (10), and microtubules (2). Recently, evidence of their function as sites of ribonucleoprotein binding or storage (4, 5, 28) or dispersal (11) has been discussed.

The function of AL in virus-infected cells also is unknown, as is the mechanism by which a virus might induce the formation or increase the numbers of AL in an infected cell. In the virus-infected cell systems that have been studied thus far, a direct replicative association between the virus and AL has been suggested only in the study of rubella virus in RK-13 cells (24). Here, according to Patrizi and Middlekamp, particles that might represent a form of the virus are associated with membranous complexes of "radial cisternae" and AL. It was reported that no AL were observed in uninfected cells either in this study or in a similar study of rubella virus in LLC-MK2 cells (12); on the basis of these observations, Patrizi and Middlekamp suggested that the AL were formed under the influence of the viral genome (24). However, Merkow et al. (22) found AL in a few uninfected LLC-MK2 cells in connection with their studies of SV-30 infection, but also suggested that increased numbers of AL in infected cells were formed as a result of virus-host genome interaction. Viral toxicity also has been suggested to induce the appearance of AL (13, 22). Noting other studies that imply an association of AL with protein synthesis, several authors have suggested that AL in virus-infected cells may be associated with the synthesis of viral protein (12, 22).

The relationship between SYVV infection and the formation of AL in *Sonchus* also is unknown. It is tempting to suggest that SYVV induces the formation of AL since no AL were seen in unin-

fected controls or in BYSV single-infected plants; furthermore, AL have never been reported in any other of the many studies of the phloem, phloem-transmitted viruses, or plant rhabdoviruses (3, 7, 27, 31). Considering the work of Merkow et al. (22), however, the association of virus with AL may not be a direct cause and effect relationship due to the virus genome. Most of the cells having AL appear to be relatively heavily infected with SYVV, that is, many virus particles are seen within these cells, in both nucleus and cytoplasm. AL were not found in every SYVV-infected cell, even when the cells appeared to have about the same degree of maturity and virus infection as cells in which AL were found. AL have not been reported in other studies of the cytological effects of SYVV in *Sonchus* (14, 17, 23), although they have been reported in other metabolically active plant cell systems (6, 8, 25, 28, 32). No virus particles were found in contact with the membranes or within the lumina of the AL cisternae although they were regularly seen within the luminal spaces of the ER (Figs. 1 and 4) and within the membranes of the nuclear envelope (Fig. 5), where replication of SYVV is thought to occur (14, 17, 23). It does not appear, therefore, that AL are involved directly in SYVV replication. The presence of the virus may induce the host cell to form AL in response to the presumed increase in synthesis of material such as ribonucleoprotein, perhaps coded for by the virus genome, and the AL could function as a storage site (4) or dispersal mechanism (11) for this material.

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