

Vesicle-Mediated Solute Transport between the Vacuole and the Plasma Membrane¹

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The traditional view of the plant cell vacuole as a storage compartment has been replaced in recent years to that of a multipurpose organelle by the realization that the vacuole carries out numerous metabolic functions (Wink, 1993). For instance, under certain physiological conditions, in specific cell types, or at determined developmental stages, the vacuole participates in the export of a variety of solutes ranging from simple sugars and organic acids to amino acids and mineral ions. Mobilization of vacuolar components is a prominent function of many cells especially those comprising the storage organs of seeds during germination and of tubers, hypocotyls, roots, rhizomes, and corms during resumption of vegetative and/or reproductive growth. In photosynthetic cells, the vacuole is an important exporter of Suc during dark periods and at times of low photosynthetic activity. The vacuole also appears to serve as an interim location in the process of sugar secretion in nectaries, ion secretion by salt glands, and for the final protein-processing steps of several secreted enzymes in cell suspension cultures (e.g. α -mannosidase, class I chitinase, and class I β -1,3-glucanase; Wink, 1994; Kunze et al., 1998). Finally, metabolite efflux from the vacuole is intimately involved in the rapid volume changes associated with stomatal closure and with the loss of turgor in the pulvinar motor cells that drive leaf movements in *Mimosa pudica* (MacRobbie, 1999).

The basic question regarding how solutes are mobilized from the vacuole and secreted outward across the plasmalemma has been infrequently addressed and therefore, has yet to receive a definitive answer. However, by analogy to other intracellular transport systems, solute trafficking from the vacuole involves one or both of two fundamentally distinct pathways: carrier- or vesicle-mediated transport. Carrier-mediated transport refers to the movement of individual solute particles across biological membranes whether assisted by a membrane-bound carrier, by a pump, or simply by diffusion through specific channels. In contrast, vesicle-mediated transport refers to the collective movement of numerous solute particles enclosed within small membrane vesicles across the

cytosol. In most cases of vesicles-mediated transport, it is believed that the membrane of the secretion vesicle fuses and becomes incorporated with the plasmalemma as the secreted material is deposited in the extracytoplasmic space (Kronstedt-Robards and Robards, 1992; Battey et al., 1999). These two processes of secretion are not exclusionary, and both may occur in parallel in specific secretory systems.

The present review describes existing evidence supporting the presence of a vesicle-mediated pathway for solute movement from the vacuole to the apoplast in plant cells. It should be noted that the bulk of evidence in support of a vesicle-mediated vacuolar export system in plant cells is derived primarily from cytological and anatomical studies, many of which contain little or no physiological data. Although efforts were made to cover the most recent developments in this area, most of the direct data come from studies conducted around the 1970s when the relationship between structure and function received significant attention. References to more recent reports that indirectly support the existence of vesicle-mediated intracellular transport from the vacuole are also included, even though the primary intention of such studies was unrelated to vacuole exporting mechanisms. Finally, opposing evidence and possible alternatives are presented. The reader is referred to reviews by Battey et al. (1999) and Fahh (1988) for information on granulocrine secretions (exocytosis) by plant cells and secreting organs, respectively, mostly originating directly from Golgi and endoplasmic reticulum (ER).

Involvement of the vacuole in the secretion process of certain secretory cells has been inferred from compartmental analyses that indicate a higher concentration of secreted salts (Berry, 1970) and sugars in the exudate than in the secreting cells or in the translocation sap. In view of the vacuole as the location where sugar and salts accumulate, implication of the vacuole in these exporting systems is warranted. Finally, vesicle transport is also inferred from the inability of membrane-bound carrier transport systems to explain ion accumulation and excretion in cells such as those of *Nitella translucens* and in the fast moving guard and pulvinar cells (MacRobbie, 1999).

In organizing the available data, it became evident that any structure of classification would be fundamentally artificial. The peculiarities inherent in each

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system invariably make each case a unique process. Therefore, after much thought, I decided on a classification based on the content of secreted solutes. In the first group, secreted metabolites are those commonly found in plant vacuoles and presumably transported directly to the exterior of the cell. The second group consists of systems in which the secretions also contain metabolites known to originate from ER and/or the dictyosome, implying a coordinated endomembrane network of vesicle trafficking.

DIRECT TRANSPORT OF VACUOLAR SOLUTES

Vesicle Transport in Salt-Secreting Trichomes

The existence of a vesicle-mediated system for metabolite transport from the vacuole to the cell membrane was initially advanced by Ziegler and Lutge (1967) in their studies of the secretory salt glands of *Limonium vulgare*. They observed that salt-secreting cells contained numerous and well-defined mitochondria and were rich in ER, but lacked a conspicuous large central vacuole. Instead, the cells contained a series of smaller "vacuole-like" membrane vesicles, many of which were seen in close proximity to the cell membrane (Fig. 1). More important, the tonoplast of the "vacuole-like" vesicles often appeared to fuse with the plasmalemma (Fig. 1) in agreement with a vesicle-mediated secretion process. Comparable observations were concurrently made by Thomson and Liu (1967) in their studies of the salt-secreting gland cells of *Tamarix aphylla* (Fig. 2) and later by Cardale and Field (1971) and Shimony et al. (1973) for salt glands of the mangrove species *Aegiceras corniculatum* and *Avicenia marina*, respectively. During salt secretion in *T. aphylla*, numerous

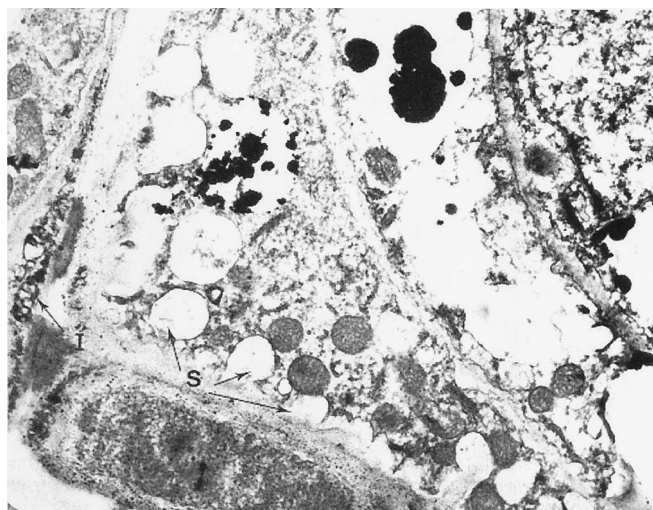


Figure 1. Electromicrograph view of a secretory cell of the *L. vulgare* salt gland during secretion. Note the apparent fusion of small vesicles with the plasma membrane (arrows). Reproduced with kind permission from Dr. A.E. Hill (Hill and Hill, 1976) and Academic Press (New York).

"microvacuoles" (similar to those of *L. vulgare*) appear at the periphery of the secreting cells and often in close association with wall projections (Fig. 2A; Thomson and Liu, 1967). At higher magnification, direct contact between the tonoplast and the plasmalemma was also noted (Fig. 2, B and C). From these observations Thomson et al. (1969) concluded that, as the salt accumulates in the glands cells, it becomes compartmentalized within the microvacuoles and is secreted when the microvacuoles fuse with the plasmalemma.

Some indirect anatomical and physiological considerations support the direct involvement of the vacuole and subsequent tonoplast vesicle-mediated system of intracellular transport to the plasmalemma. For example, secretory "gland cell complexes" are completely enclosed by an impermeable cuticular envelope opening sporadically to the underlying cells to allow symplastic connections. Across these "transfusion areas" (Thomson and Liu, 1967) the secretory cells make contact with the surrounding mesophyll through abundant plasmodesmata (Thomson and Platt-Aloia, 1985). Compartmental analysis confirmed symplastic movement of ions from the mesophyll and into the gland cells (Hill, 1970). As the concentration of secreted salts reach exceedingly high levels (1 M Cl^- in *Aegitalis annulata*; Atkinson et al., 1967) a concentrating mechanism must occur within a compartment inside the salt-secreting cells or at the plasmalemma if extrusion were to occur by an active pump.

Two independent observations in *A. marina* indicated that the active salt-concentrating step occurs at the tonoplast. First, salt continues to be secreted during the night hours when transpiration rates in this mangrove species have virtually ceased. This observation led Fitzgerald et al. (1982) to suggest that salt accumulated in the vacuoles during the day hours is later extruded at night. Subsequent x-ray maps of secreting cells confirmed this observation (Fitzgerald et al., 1982; Robards and Oates, 1986). Second, using ion selective electrodes, Shimony et al. (1973) noted a much lower salt concentration in the cytosol of secreting cells than in the xylem sap, suggesting accumulation of salts at some intracellular compartment. The experiments of Thomson and Liu (1969), however, offer the most convincing evidence for the vacuole as the site for ion accumulation in secreting cells. When *T. aphylla* plants were grown in a solution containing rubidium, electron dense accumulations appeared in their "microvacuoles" (Fig. 2B). The darkly stained vesicles were visible in micrographs, and in many instances their membranes appear fusing with the plasmalemma (Fig. 2C). Taken together these data indicated that salts initially accumulate within the vacuoles prior to secretion by vesicle fusion. More recent evidence has demonstrated the vacuole as the site of salt accumulation and the exist-

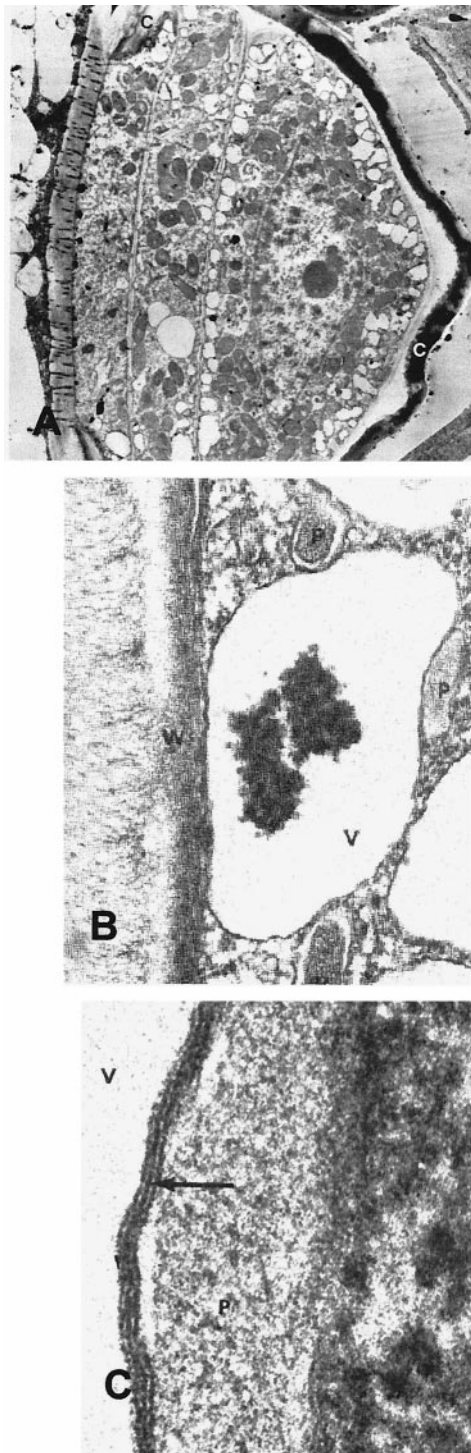


Figure 2. A, Longitudinal section through a salt gland of *T. aphylla*. The transfusion area can be seen at the left base wall. Note the proliferation of small vacuoles around the periphery of the secretory cell and their apparent association with the plasmalemma at the wall extension areas. B, Close association between a small vacuole and the plasmalemma in a secreting cell. Rubidium dense areas are clearly visible. C, High magnification of the contact point between the tonoplast and the plasmalemma. c, Cuticle; V, vacuole; W, cell wall; P, cell wall protrusions. A, Reproduced with kind permission from Dr. W.W. Thomson (Thomson et al., 1967) and the journal

tence of ion antiports, pumps, and channels at the tonoplast of plant cells (Blumwald and Gelli, 1997).

Vesicle Involvement in Organic Acid and Suc Secretion

Two studies involving the salt and organic acid-secreting trichomes of chickpeas (*Cicer arietinum*; Lazzaro and Thomson, 1992a, 1992b, 1996) and the mobilizing parenchyma cells of red beet (*Beta vulgaris*) hypocotyls (Echeverría and Achor, 1999) offer additional evidence in support of a direct vesicular transport of solutes from the vacuole to the plasmalemma. In the multicellular trichome of chickpea, the highly acidic secretion contains a variety of solutes including malic, oxalic, hydrochloric acids, and calcium ions. With a pH of 0.5 to 1.0, the secreted solution must be compartmentalized, otherwise it could cause irreparable damage to normal cytosolic operations. Using x-ray microanalysis, fluorescent dye lucifer yellow-CH, non-toxic levels of lanthanum, antimonate-calcium precipitation, and confocal and Nomarski-differential interference contrast microscopy, Lazzaro and Thomson (1996) identified the intracellular location of secreted solutes to be the vacuole. The vacuole of chickpea trichome cells, however, consists of an unusual network of tubular compartments that extend along the entire multicellular structure. This vacuolar continuum traverses through plasmodesmata and terminates in the secreting head cells. Calcium deposits were also localized in the numerous smaller "vesicles" located at the periphery of secretory head cells, some of which fuse with the plasmalemma. Figure 3A demonstrates the proliferation of small "vacuole-like" vesicles proximal to the secreting cell wall, calcium deposits within the small vacuoles (Fig. 3B), and the fusion of a vesicle with the plasmalemma (Fig. 3C). It is pertinent to note at this point that tubular vacuoles are common in filamentous fungi and are intimately involved in intracellular transport (Cole et al., 1998).

Further evidence for direct vesicle-mediated solute transport from the vacuole comes from the mobilizing parenchyma cells of red beet hypocotyls during resumption of growth (Echeverría and Achor, 1999). After a dormant over-wintering period, hypocotyl cells begin a process of reserve mobilization to support the growth of leaves, roots, and reproductive organs. Unlike previous examples of cell secretions, mobilized solutes from storage parenchyma are not secreted to the plant surface but rather are deposited in either the apoplast or symplast for long distance transport. To supply their own metabolic demands, storage cells possess a tonoplast-bound ATP-dependent Suc carrier (Echeverría and Gonzalez,

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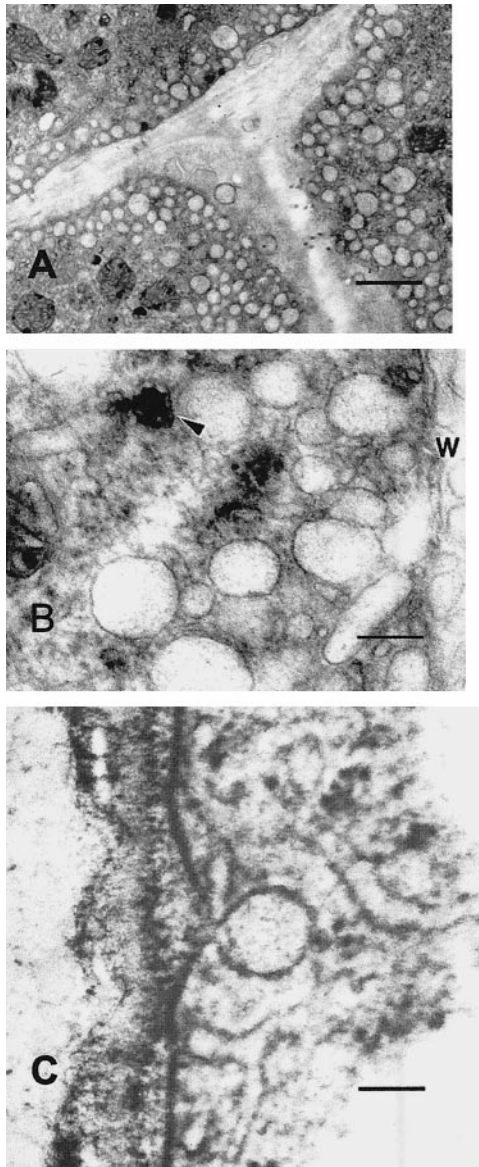


Figure 3. A, Electronmicrograph of the head cell of *C. arietinum*. The salt and organic acid-secreting trichome contains numerous small vacuoles. B, Observe the lanthanum deposits inside the small vacuoles at the tip of the secreting cell (arrow). C, Fusion of a small vacuole with the plasmalemma. Bar = 1.0 μm . A, Reproduced with kind permission from Dr. M. Lazzaro (Lazzaro and Thomson, 1992a) and the Botanical Society of America. B and C, Reproduced with permission from Dr. M. Lazzaro (Lazzaro and Thomson, 1992b) and the *Canadian Journal of Botany*.

2000) for the direct transport of Suc from the vacuole to the cytosol.

Electron and light microscopic observations of mobilizing red beet hypocotyl parenchyma cells established the presence of an array of single membrane vesicles located scattered throughout the cytoplasm. In contrast to mobilizing cells, dormant cells possess a clear and homogeneous cytoplasm with no apparent vesiculation. Most vesicles in mobilizing cells ranged in size from one to 10 μm , although larger

vesicles are frequently observed. When protoplasts from the same tissues were examined under light microscopy, the vesicles appeared red in color, revealing their content of the soluble pigment betacyanin, and more importantly, identifying them as vacuolar in origin (Fig. 4A). As the tonoplast vesicles become smaller, the intensity of the red color subsides appearing light pink in the smallest visible vesicles. The decrease in red intensity in the smaller vesicles is likely due to two factors: a decrease in depth area resulting from reduced volumes and/or changes in the content composition as the internal solution is modified prior to secretion. In electron micrographs, vesicles are seen fusing with the plasmalemma (Fig. 4B), and both membranes (tonoplast and plasmalemma) appear contiguous. Vesicle movement of vacuolar solutes is advantageous in that large amounts of metabolites can be transported while being protected from enzymatic attack by cytosolic enzymes.

Vesicle Involvement in the Movement of Solutes from Guard and Motor Cells

In a recent analysis on the mechanisms of ion uptake into vacuoles of *N. translucens*, MacRobbie (1999) carefully described kinetic irregularities that could not be reconciled by a process of single ion transport at the plasmalemma and tonoplast. The anomalies in ion transport are more consistent with a transport process of salt-filled vesicles to and from the vacuole than with a static system of protein carriers mediating single ion molecules. MacRobbie (1999) speculated that the same mechanism may be responsible for the rapid loss of solutes from the vacuole of guard cells and the motor cells responsible for the slow sleep movements of leaf pulvini in *Samanea* sp. and the fast leaflet closures of *M. pudica* (the sensitive plant). In *M. pudica*, leaf movement follows a mechanical disturbance of the sensitive leaflets and the ensuing propagation of the stimulus to the pulvinus. Upon its arrival to the pulvinus, the action potential initiates a cascade of events starting with a massive loss of solutes from the vacuole and the cell, followed by structural collapse, and finally leaf closure. Although initially thought to involve ion fluxes alone, work by Fromm and Eschrich (1988) showed that stimulation of the action potential in motor cells also involves release of Suc. Suc release seems to be involved with turgor lost from the stomatal guard cells as well. The simultaneous release of ions and Suc strongly suggest a common transport mechanism difficult to explain by movement through membrane channels alone. In all cases, motor cells cycle between a turgid state with one single vacuole (open stomata and open leaf blades) and a shrunken state of much reduced vacuolar volume with many small vacuoles (during stomatal closure and folded leaf blades). Microscopic observations revealing the presence of mi-

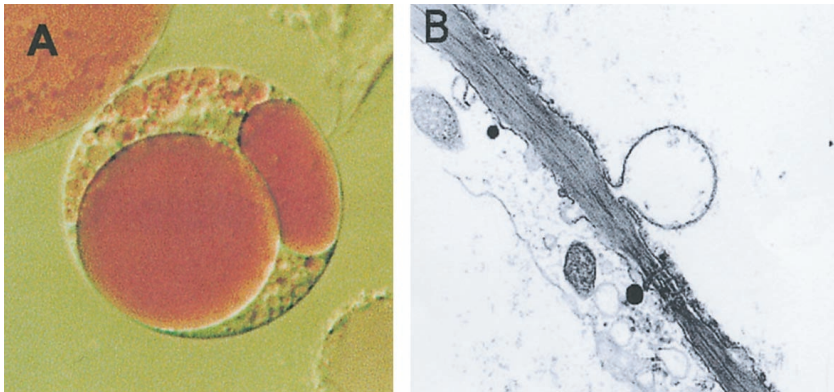


Figure 4. Light and electron micrographs of a protoplast and fixed storage cells, respectively, of mobilizing red beet hypocotyl. A, Mobilizing parenchyma protoplast showing multiple vacuoles and smaller tonoplast vesicles as evidenced by their betacyanin content. B, Electron micrograph of fixed tissue from similar mobilizing cells demonstrating a fusing vesicle with the plasmalemma. The vesicle is approximately 2 μm in diameter.

crovacuoles during the dramatic shrinkage of *M. pudica* pulvini associated with excitation (Weintraub, 1951) argues in favor of a vesicle-mediated solute transport from the vacuole.

TRANSPORT OF SOLUTES OF COMBINED VACUOLAR AND NON-VACUOLAR ORIGIN

Vesicle Transport in Nectar Secretion

Evidence for a direct vesicle-mediated transport system between the vacuole and the plasmalemma is less apparent from cytological studies in sugar-secreting nectary cells, although in a few instances claims of such have appeared (Findlay and Mercer, 1971). Instead of a direct transport route of tonoplast vesicles from the vacuole to the plasmalemma, a more complex vesicle-mediated system is envisioned when one integrates the extensive cytological and anatomical observations of secreting nectaries with our current knowledge of Suc accumulation into intracellular compartments (Leigh, 1997). Nectaries are multicellular surface glands specialized in secreting sugar solutions often containing different amounts of other hydrophilic substances such as oligosaccharides, amino acids, organic acids, ions, vitamins, etc. (Fahn, 1979; Caldwell and Gerhardt, 1986). The high concentration of the secreted sugar and the differences in concentration between the nectar and the phloem sap (Pate et al., 1985) indicate that at some point selective transport has occurred against a concentration gradient. As in salt-secreting glands, prenectar enters the secretory cells by the symplast (Sawidis, 1991). During nectar secretion, the cellular architecture of the nectar-secreting cells is overwhelmingly dominated by an extensive network of ER (Fig. 5). This observation has been reported in nearly all species examined including *Tropaeolum majus* (Rachmilevitz and Fahn, 1975), *Achillea millefolium* (Figueroa and Pais, 1994), and *Cucurbita pepo* (Nepi et al., 1996). Vesicles apparently derived from the edges of the ER cisternae are believed to be associated with the secretion process as they frequently fuse with the plasmalemma (Fig. 5). Autoradiographic evidence showing accumulation of supplied

radiolabeled sugars in the ER (Fahn and Rachmilevitz, 1975), ion accumulation in the "secretory reticulum" (Robards and Oates, 1986), and the conspicuous swelling of ER cisternae during secretion (Christ and Schnepf, 1985; Fahn, 1988) support the contention that the ER is the site of sugar location prior to secretion (Sawidis, 1987).

In accordance with our present understanding of intracellular compartmentation, the vacuole constitutes the site for sugar accumulation within the cell (Leigh, 1997). However, vacuoles are not prominent in nectar-secreting cells and only sparsely present in micrographs. If we assume sugars are to be concentrated in the small vacuoles and the secretion later modified in the ER, a sequential multi-step vesicle transport system must occur in which different nectar components are sequentially added as solutes pass from one organelle to another and finally excreted by ER or dictyosomes-derived vesicles. Although not yet reported for higher plants, retrograde transport from the vacuole to the endosomal com-

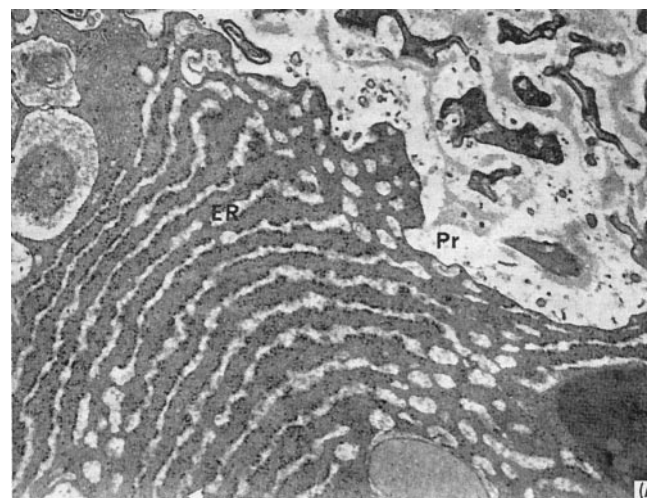


Figure 5. Secretory cells of *Lonicera japonica* during nectar secretion stage. Note the abundance of ER and ER-derived vesicles often in close association with the plasmalemma. Pr, Wall protuberances. Reproduced with kind permission from Dr. A. Fahn (1979) and the Botanical Society of America.

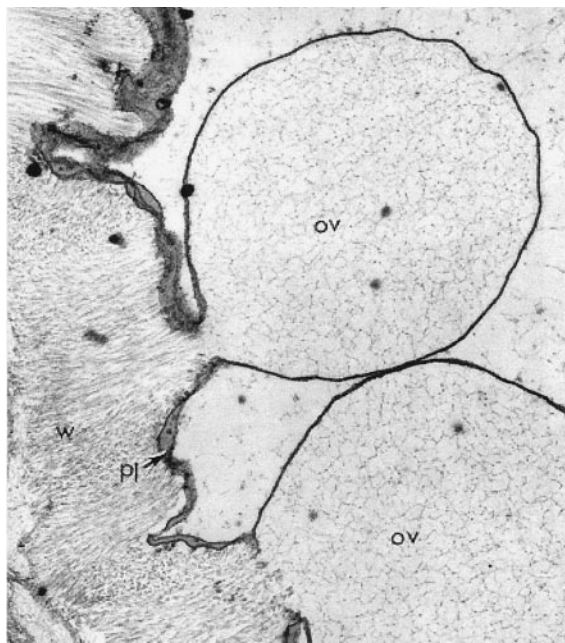


Figure 6. Electron micrograph of viscain tissue at the stage of cellulose microfibrils. Tissue stained for polysaccharide. Observe the fibrillar material within the open vacuoles (ov), which are contiguous with the plasmalemma (pl). W, Cell wall ($\times 22,500$). Reproduced with kind permission from Dr. J. Kuijt (Gedalovich and Kuijt, 1987) and Springer-Verlag (Berlin).

partment has been demonstrated in yeast and from the lysosome to ER in animal cells (Bryant et al., 1998). An alternative route for nectar secretion could occur if different components are secreted separately by vesicles arising from several sources and subsequently mixed after being secreted to the exterior (Bosabalidis and Thomson, 1984).

The difficulties encountered in analyzing a dynamic process such as nectar secretion by two-dimensional microscopy is reflected in the lack of a coherent view concerning the involvement of secretory vesicles and their exact origin. In this context, the observations of Verbelen and Tao (1998) should be given special consideration. Using confocal laser scanning microscopy, they demonstrated the presence of conspicuous ripples along the vacuole surface, which often gave the appearance of ER. The unusual ripples resemble tubules with a width of approximately $2 \mu\text{m}$ and lengths ranging from 5 to over $100 \mu\text{m}$. Their comparison of fixed material to that made under differential interference microscopy made apparent that the arrays of tubular/spherical structures observed *in vivo* were disrupted upon fixation and became profusely vesiculated. In considering these findings, it is possible that the structures identified as ER in the many nectaries may well be corrugated vacuoles. Alternatively, the ER may have the capacity to accumulate sugars in special circumstances such as in nectary cells, although this possibility has not yet been reported.

Vesicle Secretions of Sugars and Adhesive Polysaccharides by Mistletoe Seeds

Developing mistletoe (*Phthirusa pyrifolia*) seeds present a peculiar example of tonoplast vesicle involvement in intracellular transport. Mistletoe seeds are surrounded by viscain, an elastic, mucilaginous and sticky tissue that adheres the falling seeds to branches and stems. The characteristic elastic seed walls are synthesized from large amounts of polysaccharides secreted by the wall cells. In addition, the cells secrete adhesive polysaccharides and sugars that function as attractants of dispersal agents. Active viscain cells contain abundant ER cisternae and Golgi bodies. Both structures are seen at the periphery of the cells and in close proximity to the distinctively large vacuoles. The fibrillar character of the vacuolar contents and their positive staining with toluidine

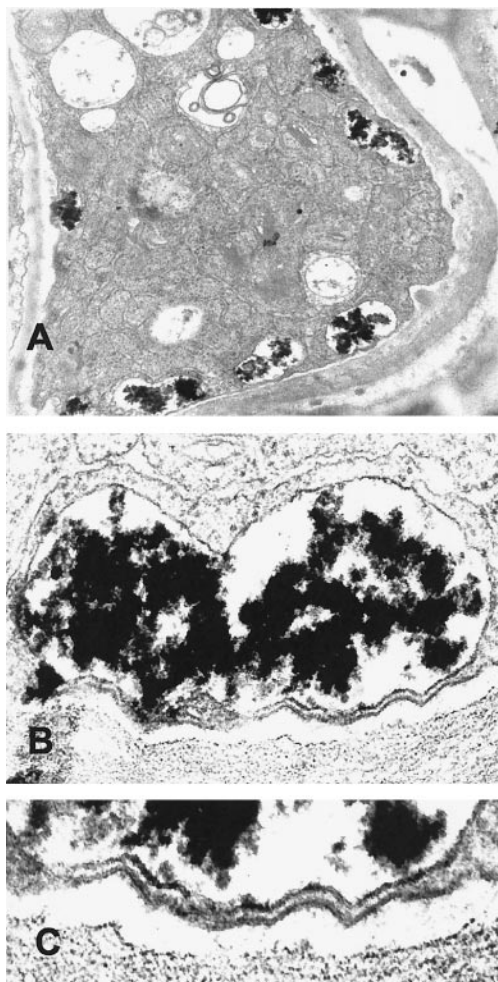


Figure 7. A, The secretory cell of *L. vulgare* salt gland containing many small peripheral vacuoles. Bar = $1 \mu\text{m}$. B, Vacuole in close contact with the plasmalemma. Bar = $0.25 \mu\text{m}$. C, Enlarged portion of contact between the tonoplast and the plasmalemma. Note the connecting threads between membranes (arrow). Bar = $0.1 \mu\text{m}$. Reproduced with kind permission from Dr. A.E. Vassilyev (Vassilyev and Stepanova, 1990) and the *Journal of Experimental Botany*.

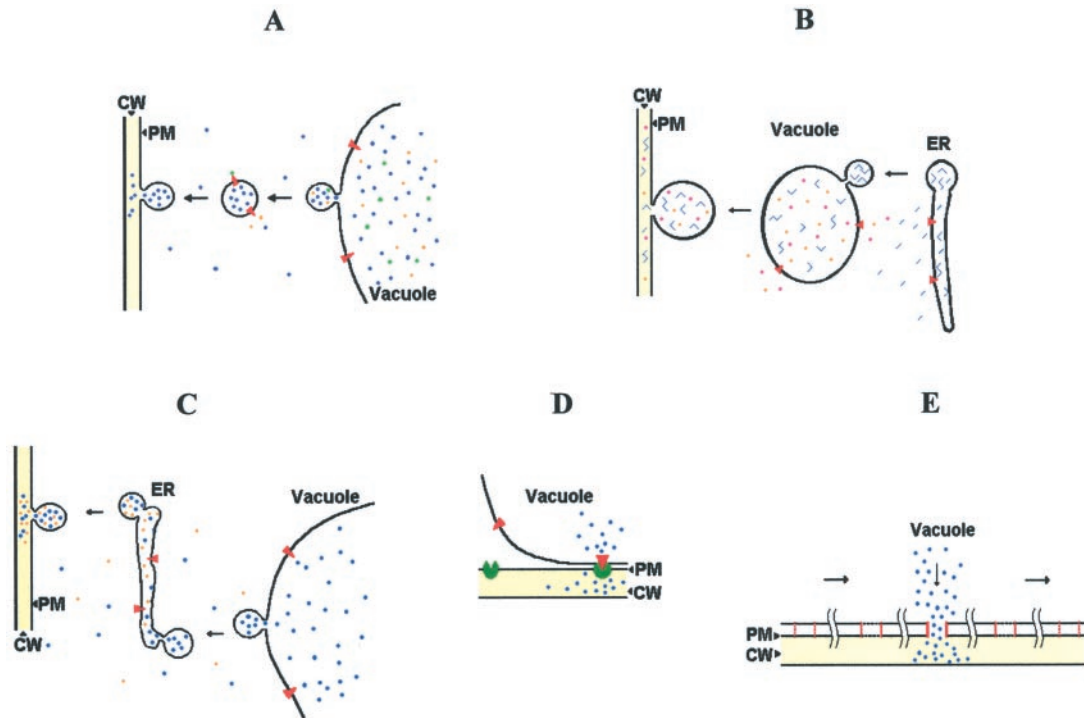


Figure 8. Diagrammatic representation of vesicle-mediated transport systems from the vacuole to the plasmalemma. A, Direct transport of vacuolar solutes by means of tonoplast vesicles. Red arrowheads represent tonoplast-bound carriers, and the direction indicates the flow of metabolites. The transported solutes are presumably modified prior to secretion into the apoplast. B, Vacuolar solutes modified by ER prior to secretion as for nectary glands. C, Transport of ER derived solutes to the vacuole and subsequent release to the apoplast. D, Juxtaposition of membrane-bound carriers located at the tonoplast and the plasmalemma. These "junctional complexes" allow the direct transfer of metabolites to the apoplast. E, Transient formation of sphincters connecting the vacuolar lumen to the apoplast. Solutes are expelled to the apoplast by hydrostatic pressure.

blue reveal that at least some of the vacuolar secretions are pectin in nature. The pectic nature of the vacuolar contents suggests the inclusion of secretions originating from the Golgi and ER and the possible coalescing of their vesicles with the larger vacuoles. Figure 6 shows two vacuoles (approximately 5 μm in diameter) merging with the plasmalemma and releasing their contents to the apoplastic space.

OPPOSING VIEWS

Despite all the cytological and anatomical evidence in support of a vesicle-mediated system for solute transport between the vacuole and the plasmalemma and the apparent feasibility of such system, strong objections to this process have been raised (Hill and Hill, 1976; Kronstedt-Robards and Robards, 1992). These objections are based primarily on the incompatibility between the rates of membrane turnover and the volumes secreted by nectaries and gland cells. For example, the rates of vesicle fusion necessary to sustain the volumes of nectar secreted by *Abutilon striatum* trichomes range from 750,000 vesicles s^{-1} (for 50-nm vesicles) to 750 vesicles s^{-1} (for 500-nm vesicles; Kronstedt-Robards and Robards, 1992). These values are many orders of magnitude

higher than reported values of 650 to 850 vesicles s^{-1} for oat coleoptile and of 50 to 85 vesicles s^{-1} for *Tradescantia virginiana*-germinating pollen tubes (Picton and Steer, 1981). In *A. striatum* trichome cells, the estimated rates of vesicle fusion necessary to accommodate the levels of secreted nectar would result in the turnover of the entire plasmalemma every minute. This rate is 10-fold higher than rates determined for nonexpanding secretory cells of one cell surface turnover every 10 min (Steer, 1988). In *Strelitzia reginae* flowers (bird of paradise), however, rates of vesicle fusion with the plasmalemma during nectar secretion are much lower, ranging from 3,400 to 3.4 vesicles s^{-1} per cell for 50- and 500-nm vesicles, respectively (Kronstedt and Robards, 1987).

The above calculations are based on the assumption that the concentration of secreted metabolites (mostly sugars) are identical in the vesicles as in the secreted nectar, implying that the volume of nectar is entirely secreted by vesicle fusion. This may not be the case if secreted solutes are more concentrated and movement of water follows osmotically. Water movement following the highly concentrated salt and sugar secretion could reduce the requirement for high rates of vesicle fusion necessary to reach the high volumes of exudate (Hill and Hill, 1976).

POSSIBLE ALTERNATIVES

Although there is compelling anatomical and cytological evidence supporting the existence of a vesicle-mediated transport system between the vacuole and the plasmalemma, in view of the lack of adequate physiological and biochemical data we cannot ignore the likelihood that some calculated rates of secretion are impossibly high to be accounted for by vesicle transport to the plasmalemma. Furthermore, vesicle-mediated transport to the plasmalemma would involve a constant recycling of membrane to accommodate the increasing amount of vesicle fusion. Some alternative mechanisms of vesicle-mediated secretion delivery to the plasmalemma may account for the inconsistencies with the abnormally high rates of membrane fusion. The proposed routes for vacuolar nectar secretion in *A. striatum* (Robards and Stark, 1988) and for the salt glands of *L. vulgare* (Vassilyev and Stepanova, 1990) are appealing in that the need for membrane recycling is substantially reduced or totally eliminated. In *A. striatum*, rapid freezing of the secretory trichomes followed by freeze-fracture replication revealed an extensive "secretory reticulum" within all the trichome cells. The membranous "secretory reticulum" appeared closely associated with the plasmalemma. This and other observations from similar freeze-substituted cells led Robards and Stark (1988) to propose a mechanism in which the pre-nectar solutes are actively transported into the "secretory reticulum" of all trichome cells. The ensuing increase in hydrostatic pressure induces the opening of "sphincters" that connect the cysternal space of the reticulum with the plasmalemma. Following the opening of the sphincter, the contents are released in a process reminiscent of the "water expulsion vacuoles" in zoospores of *Oomycetes* sp. (Mitchell and Hardham, 1999). Opening and resealing of vesicle pores after content discharge has been reported for mouse mast cells (Alvarez de Toledo et al., 1993) and for protoplasts from maize coleoptile (Thiel et al., 1998).

The model presented by Vassilyev and Stepanova (1990) is based on the lack of visible membrane fusion between secretory "vacuolar vesicles" and the plasmalemma in secreting glands of *L. vulgare*. In their proposal, "secretory vesicles" dock onto the plasmalemma without fusion (Fig. 7, A and B). The two membranes (tonoplast and plasmalemma) form "junctional complexes" where channels on both membranes connect allowing passage of solutes from the vesicle to the exterior. In the proposed system, active accumulation of solutes occurs across the tonoplast and into the "micro-vacuoles." From the vacuoles to the periplasmic space, transport occurs by diffusion through channels in the contacting area of the tonoplast and plasmalemma (Fig. 7C). Very similar secretory systems have been demonstrated for muscle cells engaged in Ca^{+2} release in which the empty vesicle undergoes a process of recycling sev-

eral times (Murthy and Stevens, 1998). For nonexpanding plant cells, a mechanism for cargo discharge without membrane incorporation (such as those described above) would be useful in view of the energy costs involved in generating endocytotic recovery vesicles in the presence of high turgor pressure (Gradman and Robinson, 1989).

CONCLUSIONS AND FUTURE PERSPECTIVES

Based on our present knowledge, it is difficult to draw firm conclusions regarding the transport of solutes from the vacuole to the plasmalemma. However, from the available information, it is possible to visualize the likelihood of a vesicle-mediated system for solute transport from the vacuole to the plasmalemma independent of vesicle fusion (Fig. 8). This system would facilitate the bulk movement of solutes to the exterior of the cell whether to be transported to distant organs, excreted to the plant surface, or simply temporarily deposited in the apoplast. It would seem that mobilized reserves are delivered more efficiently to the apoplast in protected compartments. Protection of transported solutes at high concentrations would be advantageous since deleterious effects are avoided and labile solutes are protected from enzymatic attack. Transport of specific solutes from the vacuole most likely involves the selective transformation of the milieu, assuming the vacuole contents are homogeneously distributed. In this respect, the tonoplast pumps, carrier and channel proteins may play a dominant role. Alternatively, cells may contain different vacuoles with specific organ-related functions as already demonstrated (Swanson et al., 1998). The use of modern techniques of biochemistry, microscopy, and cell biology and the availability of plants with mutations in the transport process should add substantial information needed to create a definite picture of intracellular transport systems originating from the vacuole.

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