The Plant Cell, Vol. 11, 643–659, April 1999, www.plantcell.org © 1999 American Society of Plant Physiologists

Exocytosis and Endocytosis

Nicholas H. Battey,[a,1] Nicola C. James,[a] Andrew J. Greenland,[b] and Colin Brownlee[c]

[a]Department of Horticulture, School of Plant Sciences, University of Reading, Whiteknights, Reading RG6 6AS, United Kingdom
[b]ZENECA Agrochemicals, Jealott's Hill Research Station, Bracknell RG42 6ET, United Kingdom
[c]Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, United Kingdom

INTRODUCTION

Exocytosis is a general term used to denote vesicle fusion at the plasma membrane, and it is the final step in the secretory pathway that typically begins in the endoplasmic reticulum (ER), passes through the Golgi apparatus, and ends at the outside of the cell. Endocytosis refers to the recovery of vesicles from the plasma membrane. Exocytotic vesicle fusion involves the coalescence of vesicle and plasma membranes and allows the so-called fusion pore to form. The fusion pore is a channel that passes through the vesicle and plasma membranes and allows delivery of the vesicle contents to the extracellular compartment. Docking is the process by which the exocytotic vesicle is fixed beneath the plasma membrane before fusion. It is generally believed to involve molecular recognition between vesicle and plasma membrane and is therefore one aspect of vesicle targeting. Another kind of targeting can be provided by the cytoskeletal proteins that move vesicles around the cell. Sorting is a term that can be applied to vesicles, in which case it simply describes the consequences of targeting. Sorting has a more useful and distinct meaning when applied to vesicle contents: these contents vary according to the destination of the vesicle and the state of differentiation of the cell. Sorting of contents can occur in the ER or Golgi or post-Golgi compartments, as can processing, in which polysaccharides or proteins are modified enzymatically into their mature form, ready for delivery.

The problem in discussing exocytosis and endocytosis is deciding where to start and where to finish: it is difficult to consider vesicle fusion without docking, docking without targeting, and targeting without sorting. Similarly, to stop at the point of vesicle retrieval (perhaps only microseconds after its fusion) is arbitrary, if only because the subsequent journey can involve recycling (see, e.g., Murthy and Stevens, 1998), so that the same vesicle may visit the plasma membrane many times. Furthermore, for a full explanation of exocytosis and endocytosis, we must consider the myriad uses to which these processes are put in the full diversity of plant cell types; vesicle trafficking pathways are likely to vary according to the needs of the cell and its stage of development. Figure 1 illustrates the range of secretory vesicle/plasma membrane dynamics that require mechanistic explanations.

In this review, we follow the secretory vesicle on its journey from the Golgi apparatus to the plasma membrane and discuss the factors that control its docking, fusion, and recycling there. We emphasize the relatively new results from plant cells but place these against a background of data from animal cells and yeast, in which more is known. One interesting feature of exocytosis in plants is its Ca^{2+} sensitivity, and we discuss the significance of this in relation to the known importance of Ca^{2+} as an intracellular regulator. Finally, we consider key areas for future study that will lead to a more complete understanding of the role that the regulation of exocytosis and endocytosis plays in plant development.

EXOCYTOSIS

From the Golgi Apparatus to the Plasma Membrane Docking Zone

A recent review indicated that there are at least three parallel pathways from the Golgi apparatus to the cell surface in mammals and that these pathways operate to varying extents in different cell types (Keller and Simons, 1997). There is no reason to suppose that in plants the situation is any less complex. Indeed, the older, morphological literature abounds with possible pathways and vesicle types (see Battey and Blackbourn, 1993). It cannot be determined by inspection whether vesicles are exocytotic, endocytotic, or bound for destinations other than the plasma membrane. In many cases, however, particularly in green algae (Domozych, 1991), it seems clear that there is more than one type of vesicle delivered to the plasma membrane. Electrophysiological data similarly indicate at least two vesicle populations in

[1]To whom correspondence should be addressed. E-mail n.h.battey@reading.ac.uk; fax 44-118-9750630.

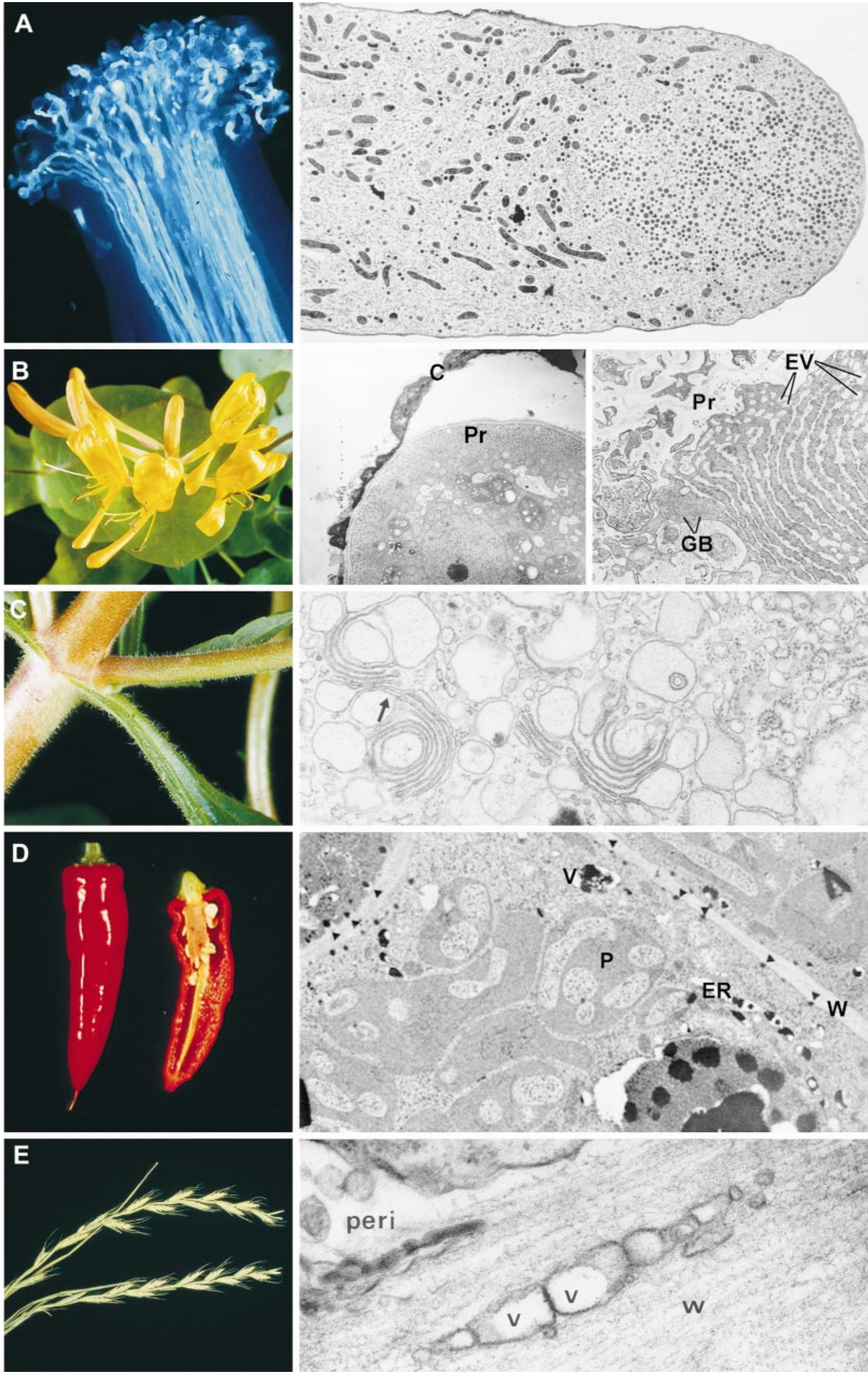

Figure 1. The Exocytotic Pathway in Plants.

barley aleurone protoplasts (Homann and Tester, 1997). We must nevertheless await future classification of the factors that sort vesicle contents and regulate the delivery of different vesicle types to the plasma membrane. A major technical obstacle is the lack of good assays of the kind that have been used so widely and effectively in mammalian and yeast cells for analysis of post-Golgi transport (reviewed in Battey et al., 1996).

The commonly held view is that secretory vesicles are delivered to their target membrane by the cytoskeleton. In animal cells, this delivery role is effected by microtubules (Cole and Lippincott-Schwartz, 1995), and vesicles are distributed from a centrally located Golgi apparatus to the plasma membrane. In plants, there are many Golgi apparatuses in each cell, and microfilaments play the major role in vesicle delivery, as is evidenced in highly polarized cells such as the pollen tube, where microfilaments must transport vesicles considerable distances (Figure 1A; Taylor and Hepler, 1997; see also Franklin-Tong, 1999, in this issue). Microtubules are also present but appear to be more concerned with nuclear migration and cytoplasmic zonation than vesicle transport (Cai et al., 1997). Even in cells from the coleoptile and root, which exhibit less polarized growth, current evidence suggests a mainly microfilament-based vesicle transport mechanism (see Battey and Blackbourn, 1993).

Although the cytoskeletal proteins provide a mechanism for vesicle delivery, it cannot be assumed that they determine where vesicles dock and fuse; that information, according to a hypothesis with strong support in animals and yeast, resides on the vesicle and target membranes themselves (Rothman, 1994). This view is strikingly illustrated by the behavior of secretory vesicles visualized with green fluorescent protein in mammalian Vero cells, in which vesicle movement appears random, suggesting that microtubules maintain vesicles in motion so as to increase the chances of vesicle–plasma membrane contact (Wacker et al., 1997). On the other hand, a key role for the actin cytoskeleton in localizing secretion is indicated by cytoskeletal mutants of yeast (*Saccharomyces cerevisiae*) in which polarized growth and vesicle distribution are disrupted concomitantly (Winsor and Schiebel, 1997). Such data may relate directly to cell plate formation in plant cells, in which there is evidence for both microfilament- and microtubule-based transport of secretory vesicles (Schopfer and Hepler, 1991; Samuels et al., 1995). It also may be relevant that prospore membrane formation in yeast is similar to cell plate formation in that a

Figure 1. (continued).

(A) The pollen tube. At left, aniline-blue staining of pollen tubes growing from the stigmatic surface down the style of *Capsicum*. Photograph courtesy of Dr. Naci Onus (Department of Agricultural Botany, University of Reading). At right, directional exocytosis maintains polar growth in the lily pollen tube. Golgi vesicles accumulate at the tip, where they undergo exocytosis to supply membrane and wall material for growth. ×3240. Reprinted from Lancelle et al. (1997), by permission of the authors and Springer-Verlag, Vienna.

(B) The honeysuckle nectary. At left, honeysuckle flowers. The floral tube is lined with hairs, which secrete sugary nectar. Photograph by Dave Maw (Jealott's Hill Research Station). Middle, the one-celled hairs develop elaborate wall protuberances (Pr) and a ribbed cuticle (C), which detaches from the wall early in development. The wall structure is believed to result from fusion of Golgi-derived vesicles. ×1310. At right, later, when the flower opens, sugar secretion is at a maximum and is associated with massive swelling of the ER. The relatively insignificant Golgi bodies (GB), coupled with the elaborate ER and associated vesicles (EV), suggest that the ER plays a major role in secretion by direct trafficking to the plasma membrane. ×7840. Reprinted from Fahn and Rachmilevitz (1970), by permission of the authors and Academic Press, London; and from Fahn (1988), by permission of the author and the New Phytologist Trust, Lancaster, UK.

(C) Secretion of polysaccharide slime by *Mimulus*. At left, leaf, branch, and stem of *Mimulus*, showing a thick covering of secretory epidermal hairs. The hairs actively secrete polysaccharide slime from their head cells. Photograph by Dave Maw (Jealott's Hill Research Station). At right, the head cells have Golgi apparatuses with hypertrophied cisternae, which give rise to very large vesicles (arrow), often directly as a result of maturation of the *trans*-most cisterna. ×30,150. Reprinted from Schnepf and Busch (1976), by permission of the authors and Gustav Fischer Verlag, Jena.

(D) Capsaicin secretion from the placenta of chili pepper. At left, chili peppers. The placenta of hot pepper has a glandular epidermis that synthesizes the capsaicinoids responsible for the "hot" characteristic. Photograph courtesy of Dr. Barbara Pickersgill (Department of Agricultural Botany, University of Reading). At right, placental epidermal cells of chili pepper have enlarged ER that contain and give rise to vesicles (V) containing electron-dense capsaicinoid droplets that are deposited outside the plasma membrane (arrowheads) by a currently undefined exocytotic mechanism. P, plastid; W, cell wall. ×9600. Reprinted from Zamski et al. (1987), by permission of the authors and the University of Chicago Press.

(E) Secretion from the ligule of darnel grass (*Lolium temulentum*). At left, darnel. Photograph courtesy of Dr. Bob Froud-Williams (Department of Agricultural Botany, University of Reading). In darnel, as well as in other plants such as *Selaginella* (Bilderback and Sloane, 1987), polysaccharide is secreted from the ligule (Chaffey, 1985). The adaxial epidermal cells have hypertrophied Golgi bodies, consistent with a function in polysaccharide production. Because the primary function of exocytosis is secretion, not growth, plasma membrane recycling is presumably very active. At right, the presence of vesicular "paramural bodies" (V) in the cell wall (W), outside the plasma membrane and periplasmic space (peri), may reflect passage of vesicles through the plasma membrane (as with mucilage secretion in *Plantago* [Hyde, 1970]) or extrusions derived from the plasma membrane. It is possible that the vesicular membrane is a functionally important element of the secreted material. ×51,770. Reprinted from Chaffey (1995), by permission of the author and Academic Press, London.

branch of the secretory pathway operates to direct new plasma membrane growth during sporulation (Neiman, 1998).

Maturation and Processing in Secretory Vesicles

It is known that polysaccharides and secreted proteins reach the cell wall after their production or modification in the Golgi apparatus (Driouich et al., 1993). Therefore, it is assumed that secretory vesicles in plants contain cell wall polysaccharide precursors and proteins, and both biochemical (e.g., Van der Woude et al., 1971) and electron microscopic (e.g., Moore et al., 1991; Lynch and Staehelin, 1992, 1995) analyses of the polysaccharide content of secretory vesicles have been undertaken (for a more detailed discussion, see Battey et al., 1996). Apart from these studies, the composition of the cargo and membrane of secretory vesicles is largely unknown, and the extent of processing and maturation that takes place within vesicles is therefore unclear. Our ignorance of these issues is partly due to the difficulties associated with isolating and identifying the vesicles (Hohl et al., 1996).

Protein Processing

The identification in plants of subtilases, a superfamily of serine proteases that predominantly cleave dibasic residues (Siezen and Leunissen, 1997), is circumstantial evidence that cleavage of plant proproteins may occur in post-Golgi vesicles. In prokaryotes and lower eukaryotes, these enzymes have low specificity and are secreted to function extracellularly in growth or defense (Siezen and Leunissen, 1997). In yeast, however, the subtilase Kex2 is responsible for the maturation of the α-mating factor, and in mammals there are at least four classes of subtilase, including prohormone convertases and furin (Barr, 1991). These enzymes play an important role in post-Golgi processing of proteins that are released by exocytosis. In plants, at least six putative subtilases have been identified (Siezen and Leunissen, 1997), although only the evidence from a study of a tobacco subtilase related to Kex2 of yeast directly argues for a processing role for subtilases in plant secretory vesicles. Specifically, this Kex2p-like activity in tobacco processes the preprotoxin KP6 of *Ustilago maydis* (Kinal et al., 1995). Presumably, this occurs late in the secretory pathway, because no active toxin can be detected intracellularly.

Other plant subtilases, such as AG12 from *Alnus glutinosa* (Ribeiro et al., 1995) and the systemin binding protein SBP50 from tomato (Schaller and Ryan, 1994), may be involved in processing foreign proteins and signaling peptides from other parts of the plant. SBP50, located in the tomato plasma membrane, binds to the defense signaling peptide systemin at the recognition site required by furin and has furin-like proteolytic activity (Schaller and Ryan, 1994). Studies of the biosynthesis of plant signaling peptides, including ENOD40 and systemin, indicate that they are produced cytosolically and therefore would not undergo processing in the secretory pathway (Schaller and Ryan, 1995; Cohn et al., 1998).

Proteins that have a putative protective function often exemplify post-Golgi processing and maturation (Broekaert et al., 1997). In many cases, these proteins accumulate within vacuoles and presumably are released by holocrine secretion, a crude type of exocytosis inasmuch as it is characterized by the release of vacuolar contents coupled with cell degeneration. For example, a proteinase inhibitor that is believed to act against insects (Heath et al., 1997) is expressed in stigmatic cells of *Nicotiana alata* as a pentameric protein that is thought to undergo conversion to its active form within the vacuole (Atkinson et al., 1993). On the assumption that the protein precursor is inactive, it has been suggested that such post-Golgi activation may provide a self-protection mechanism (Heath et al., 1995). Proteins with putative antimicrobial activity, such as 2S albumins (Terras et al., 1992), thionins (Bohlmann and Apel, 1991), and chitin binding proteins (Raikhel et al., 1993), also accumulate within vacuoles, and their release may simply involve nonspecific cell breakdown. However, the release of vacuolar contents in response to invasion and other triggering processes may be more regulated. Fusion of the mammalian lysosome with the plasma membrane, for example, is regulated by Ca^{2+} in a manner that suggests an analogy with regulated exocytosis of vesicles (Rodriguez et al., 1997).

Polysaccharide Processing

A model system for the study of polysaccharide biosynthesis is the hypersecretory cells of the maize root cap (Rougier, 1981; Battey and Blackbourn, 1993). All root cap cells secrete primitive cell wall polymers, but those in the hypersecretory region additionally secrete a fibrillar polysaccharide mucilage (Rougier, 1981). In maize, the mucilage polymers in secretory vesicles undergo a morphological transition from a granular, Golgi-like appearance to a fibrillar appearance. The mechanisms governing this maturation process, which is termed fibrillogenesis, are unknown but seem to coincide with vesicle membrane maturation (Volkman, 1981). A similar pattern has been observed in pea (Vian and Roland, 1974).

Perhaps the most impressive examples of post-Golgi maturation of polysaccharides are found in the Prasinophycean algae. Figure 2 shows that in these plants, the scales that ultimately coat the plasma and flagellar membranes are synthesized in the Golgi apparatus as macromolecular structures and then delivered to the cell surface by exocytosis. In *Pyramimonas*, the scale reservoir is a post-Golgi compartment that contains a range of different scale types (Figure 2) and appears to sort and layer the scales in preparation for their release at the plasma membrane. The

scale reticulum in *Scherffelia* is believed to play a similar role, sorting the pentagonal and rod scales that will coat the flagellar surface into separate vesicles for exocytosis (McFadden and Melkonian, 1986; Melkonian et al., 1986). In contrast, the wall scales of *Scherffelia* are not sorted in this way; instead, whole, mature Golgi cisternae fuse directly with the plasma membrane to release the scales (McFadden et al., 1986).

Arrival: Docking at the Plasma Membrane

As schematized in Figure 3, the distinction between the docking and fusion of vesicles is clear: when a vesicle docks at the plasma membrane, it is tethered there, probably reversibly (Steyer et al., 1997). Fusion is a separate, later event that involves the coalescence of vesicle and plasma membrane and formation of the fusion pore, resulting in the release of vesicle contents into the extracellular matrix (see Thiel and Battey, 1998). Much less clear is whether some proteins are specifically concerned with docking and other proteins are needed for fusion or whether both docking and fusion events are mediated by a common set of proteins. Current models, based on work in animals and yeast, propose that the correct docking of vesicles is mediated by complexes formed between v-SNARE (soluble NSF-attachment protein receptor) proteins located on the external surface of vesicles, and t-SNAREs, partner proteins located on the cytosolic face of the target membrane (the plasma membrane in the case of exocytosis) (Hay and Scheller, 1997). Specific v- and t-SNAREs are associated with exocytosis; different combinations of SNAREs are characteristic of other docking events (see Sanderfoot and Raikhel, 1999, this issue). Whereas the base model for docking predicts that a dimer formed between a t- and a v-SNARE is sufficient, it is more likely that multimeric complexes are formed with one or more SNAREs contributed from each of the two fusing membranes (Hay and Scheller, 1997). In presynaptic nerve terminals, for example, the core complex is trimeric, comprised of syntaxin and SNAP-25 (both t-SNAREs; in this instance, SNAP stands for synaptosome-associated protein of 25 kD) and synaptobrevin (a v-SNARE) (Söllner et al., 1993; Hayashi et al., 1994).

Work on the product of the Arabidopsis *KNOLLE* gene provides evidence in plants that docking at the plasma membrane also is dependent on the formation of SNARE complexes. Mutations in *KNOLLE* affect early development of the Arabidopsis seedling so that the radial patterning of tissue layers is disrupted (Lukowitz et al., 1996). In *knolle* embryos, cytokinesis is impaired, cross walls fail to form, and the enlarged cells that result contain polyploid nuclei. Cloning of *KNOLLE* has revealed that the protein it encodes is related to the syntaxin group of t-SNAREs (Lukowitz et al., 1996), and like other proteins in this class, it contains a conserved C-terminal domain, a variable N-terminal domain, and a putative membrane anchor. It is thought that KNOLLE functions specifically in vesicle docking during cell plate formation, a conclusion supported by the observations that vesicle trafficking to the cell plate is not impaired in *knolle* embryos (Lauber et al., 1997) and that other exocytotic events are not affected.

Although the work with KNOLLE indicates that SNARE function in exocytosis is conserved during cell plate formation in

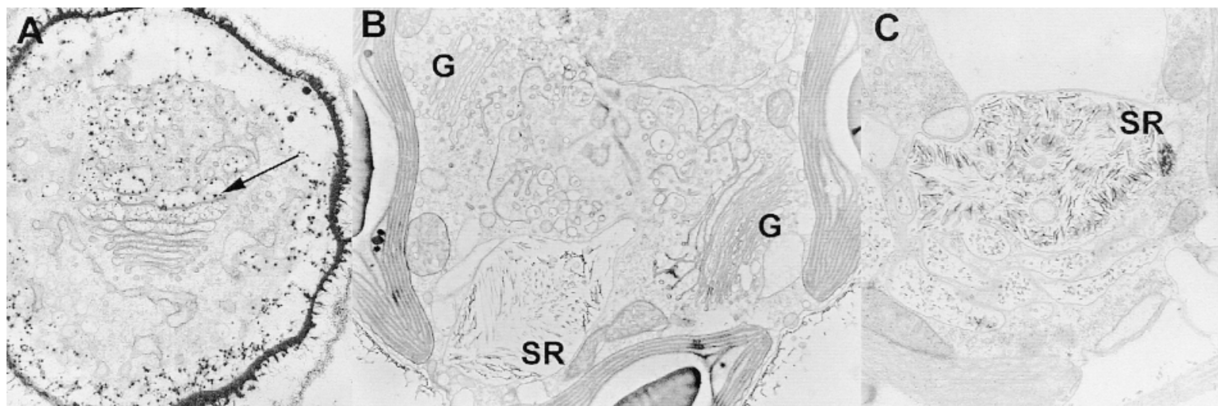

Figure 2. Post-Golgi Maturation in Algae.

(A) The scaly green alga *Tetraselmis*. Cell wall precursors assemble in the Golgi and condense at the *trans* face into scale precursors (arrow). These are exocytosed at the cell surface, where they self-assemble into the rigid wall, or theca. ×13,300.

(B) The prasinophyte alga *Pyramimonas*. Wall scales are synthesized in the Golgi apparatus (G) and arranged in the scale reservoir (SR) before exocytosis. ×11,800.

(C) *Pyramimonas*. The scale reservoir is multichambered and contains a range of different scale types. ×10,910.

Original negatives kindly supplied by Dr. David Domozych (Department of Biology, Skidmore College, Saratoga Springs, NY).

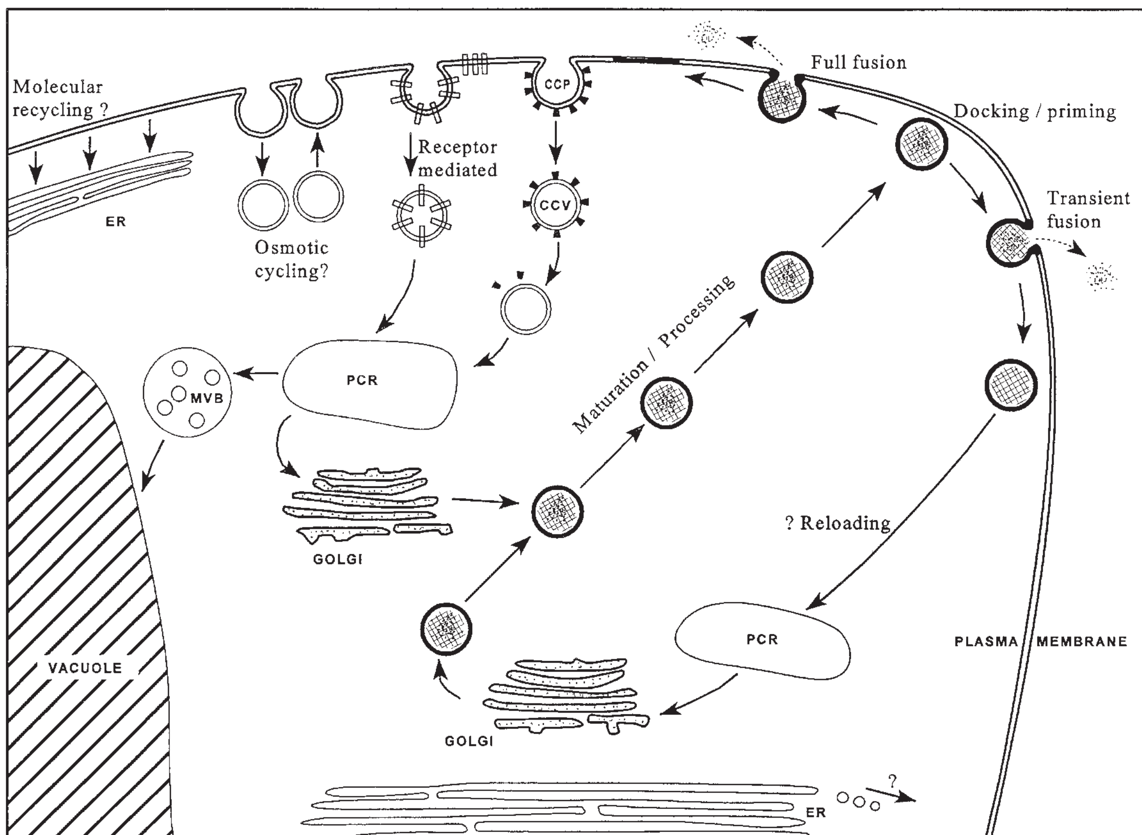

Figure 3. Sequence of Exocytosis and Endocytosis: Transport, Docking, Fusion, Content Release, and Recycling.

Shown is a speculative diagram that incorporates the suggested pathways to and from the plasma membrane in plants. CCP, clathrin-coated pit; CCV, clathrin-coated vesicle; ER, endoplasmic reticulum; MVB, multivesicular body; PCR, partially coated reticulum.

plants, a number of questions remain. A pressing issue is to identify the v-SNAREs (and potentially other t-SNAREs) that are required for vesicle docking at the cell plate so as to confirm that the animal and yeast docking models apply in this process. Based on a mutant phenotype, the product of the *KEULE* gene has been proposed as a candidate for a v-SNARE (Lauber et al., 1997), but KEULE easily could be another component of the docking machinery. It is also necessary to establish the extent of SNARE involvement in other types of exocytosis in plants. Interestingly, cell expansion and tip growth in root hairs and pollen tubes, developmental processes that are all dependent on trafficking of membrane and wall materials to the cell surface, are unaffected in *knolle* mutants (Lukowitz et al., 1996). This suggests either that a completely different docking machinery is involved or more likely, and in common with other organisms, that different sets of t-SNAREs (and v-SNARES) may be employed. Table 1 shows that candidate plant genes for both SNAP-25 and synaptobrevin can be found in the databases; in time, their involvement in exocytosis may be unraveled.

Two other types of protein, NSF (*N*-ethylmaleimide–sensitive factor) and SNAPs (soluble NSF-attachment proteins), are important in docking and fusion of vesicles in animal and yeast cells (Goda and Südhof, 1997), although their exact roles are less clear than is the case for the SNAREs. Originally proposed as a regulator of the final fusion event (Söllner et al., 1993), the function of NSF is now a subject of controversy: it may act to prime SNAREs before docking (Ungermann et al., 1998) or to recycle SNAREs after fusion (Hay and Scheller, 1997). Other recent data are consistent with a role immediately after docking but before fusion (Schweizer et al., 1998). As with the SNAREs, genes encoding proteins related to NSF and SNAP have been isolated from plants (Table 1). Although there has been no confirmation of the biological role of these homologs in plant cells, it may be significant that in *Capsicum*, a 72-kD protein with some similarity to NSF is involved in plastid membrane biogenesis (Hugueney et al., 1995).

GTPases from the Ras superfamily, termed Ypt in yeast and Rab in mammals, are proteins that play a crucial regula-

tory role in the docking/fusion process. Sec4p is the Ypt member that regulates vesicle fusion at the plasma membrane of *S. cerevisiae* (Goud et al., 1988). Its homolog in *Schizosaccharomyces pombe*, Ypt2p, is also required for transport between the Golgi apparatus and plasma membrane (Craighead et al., 1993), and the mammalian homolog, Rab8, plays a role in basolateral plasma membrane trafficking (Huber et al., 1993). Plant homologs are known and are more similar to mammalian Rab8 and *S. pombe* Ypt2p than to Sec4p of *S. cerevisiae*. This indirect evidence suggests the plant Rab8 homologs may function at the ultimate, exocytotic step in the secretory pathway. The way in which Ypt/Rab GTPases regulate exocytosis is unclear, but data for Rab5, a GTPase that controls vesicle trafficking through the endocytotic pathway, indicate that GTP hydrolysis is not directly coupled to membrane fusion; rather, the complete cycle of GDP/GTP binding and release, together with GTP hydrolysis, regulates the kinetics of docking, possibly by affecting the activation or stability of SNAREs on vesicle and target membranes (Rybin et al., 1996).

Rho-type GTPases are another group in the Ras superfamily. They have functions in a range of cell biological processes, but of particular relevance are the roles of the Rho-related Cdc42p and the Ras-related Bud1p in polarized growth in *S. cerevisiae* (Chant, 1996). Rop1, a plant Rho GTPase, is localized at the apical cortex of pollen tubes (Lin et al., 1996) such that microinjected antibodies to Rop1 inhibit pollen tube growth (Lin and Yang, 1997). These data have led to the proposal that Rop1 regulates polarized exocytosis at the pollen tube tip (Lin and Yang, 1997). It is unclear whether this GTPase controls docking or some other aspect of vesicle targeting.

The Ca^{2+} Connection: Controlling Exocytotic Membrane Fusion

In animal cells, Ca^{2+} seems to be a key regulator of exocytosis. This turns out to be true even in some cells that have been conventionally regarded as showing "constitutive"

Table 1. Plant Proteins with Sequence Similarity to Components of the Docking/Fusion Machinery in Other Organisms

Type	Protein Name	EMBL Accession Number	Species[a]	References	Related to[b]	EMBL Accession Number	Species[c]	Identity with Plant Sequence[d]	References
t-SNARE	KNOLLE	U39451	At	Lukowitz et al. (1996)	Syntaxin 2	D14582	Hs	46 (39/84)	Hirai (1993)
	SNAP25A	X92419	At	X. Gansel and L. Sticher (unpublished data)	SNAP-25	U85806	Hm	39 (25/64)	Bruns et al. (1997)
v-SNARE	SAR1	M90418	At	M. Schena and R.W. Davies (unpublished data)	Synaptobrevin	X96737	Mm	44 (93/211)	M. D'Esposito et al. (unpublished data)[e]
	Unnamed	023429[f]	At	M. Bevan et al. (unpublished data)[g]	Synaptobrevin	X92396	Hs	59 (20/39)	D'Esposito et al. (1996)
SNAP	Unnamed	AB001375	Vv	S. Matsumoto, I.B. Dry, and M. Thomas (unpublished data)	β-SNAP	P81126[h]	Bt	48 (122/256)	Whiteheart et al. (1993)
NSF	Unnamed	D86506	Nt	Sato et al., 1997	NSF	X15652	Cg	62 (190/302)	Wilson et al. (1989)
	Unnamed	D25240[i]	Os	H. Uchimiya (unpublished data)	NSF	AF006826	Dd	66 (71/106)	Weidenhaupt et al. (1998)

[a] Plant species: At, *Arabidopsis thaliana*; Vv, *Vitis vinifera*; Nt, *Nicotiana tabacum*; Os, *Oryza sativa*.
[b] The protein showing the highest BLAST score with the plant sequence.
[c] Animal/fungal species: Hs, *Homo sapiens*; Hm, *Hirudo medicinalis*; Mm, *Mus musculus*; Bt, *Bos taurus*; Cg, *Cricetulus griseus*; Dd, *Dictyostelium discoideum*.
[d] Percentage of identity and, in parentheses, the number of identical amino acids over the longest matching region.
[e] Full contributors are M. D'Esposito, M.R. Matarazzo, A. Ciccodicola, R. Mazzarella, N. Quaderi, M. Rocchi, N. Archidiacono, D. Schlessinger, and M. D'Urso.
[f] ORF from contig fragment 4, chromosome 4, EMBL accession number Z97339.
[g] Full contributors are M. Bevan, W. Stiekema, G. Murphy, R. Wambutt, T. Pohl, N. Terryn, M. Kreis, T. Kavanagh, K.D. Entian, M. Rieger, R. James, P. Puigdomenech, P. Hatzopoulos, B. Obermaier, A. Duesterhoft, J.D.G. Jones, K. Palme, W. Ansorge, M. Delseny, I. Bancroft, H.W. Mewes, C. Schueller, and N. Chalwatzis.
[h] SwissProt entry.
[i] Partial sequence.

secretion (Morimoto et al., 1995; Chavez et al., 1996; Coorssen et al., 1996). In plants, all cells studied so far show pronounced Ca^{2+} regulation of exocytosis (Zorec and Tester, 1992, 1993; Thiel et al., 1994; Homann and Tester, 1997; Carroll et al., 1998). Yeast, on the other hand, does not show Ca^{2+} regulation of exocytosis. Similarly, although there are reports of a role for Ca^{2+} at earlier stages in the secretory pathway (e.g., Beckers and Balch, 1989), in animal cells, Ca^{2+} dependence is most clearly associated with exocytosis and the subsequent retrieval of vesicles via the endosomal route (Gruenberg and Maxfield, 1995). This information suggests that many plant and animal cells may have a specific requirement for Ca^{2+} at the late stages of the secretory pathway. This requirement links exocytosis closely to the needs of the cell, because Ca^{2+} is a signaling element whose level is rapidly regulated in the local vicinity of the plasma membrane.

Because fusion of membranes can only occur once they have been brought into close proximity, the first potential role for Ca^{2+} is the regulation of the process that draws the docked vesicle membrane into immediate apposition with the plasma membrane. Although the mechanism that underlies such membrane juxtaposition is not fully understood, work on the mode of membrane fusion that promotes viral infection provides the clearest pointer. The influenza virus is a very efficient machine for cell invasion, an essential component of which is the ability to cross the plasma membrane. The viral hemagglutinin protein forms a scaffold that draws the virus and host cell membranes together and promotes their fusion (White, 1996). Membrane fusion involves transient domains that lead ultimately to formation of the fusion pore (Blumenthal et al., 1995).

Evidence from a variety of sources suggests that in animal cells, membrane fusion may involve a similar scaffold mechanism (Monck and Fernandez, 1996). Recent data suggest that synaptobrevin in association with syntaxin and SNAP-25 can cause membrane fusion when incorporated into separate populations of artificial liposomes, and it has been proposed that the v-/t-SNARE complex achieves this by a mechanism analagous to that used by the viral proteins (Weber et al., 1998). The Ca^{2+} binding proteins that might regulate this process in vivo have not been defined, but synaptotagmin has become a strong candidate in synapses (Goda and Südhof, 1997), where it exhibits Ca^{2+}-dependent binding to syntaxin, SNAP-25, and phospholipid. It has further been suggested that synaptotagmin binding could be involved in formation of the fusion pore (Südhof and Rizo, 1996). However, synaptotagmin homologs have not been detected in neutrophils, where analysis of exocytosis indicates that different vesicle populations have specific Ca^{2+} sensitivities and that sophisticated Ca^{2+} regulation is therefore at work (Nüsse et al., 1998).

Ca^{2+}-dependent activator protein for secretion (CAPS) is required for Ca^{2+}-triggered exocytosis from permeabilized animal neuroendocrine PC12 cells (Walent et al., 1992). CAPS may activate exocytosis by Ca^{2+}-dependent binding to phospholipids, in particular phosphatidylinositol (4,5)-bisphosphate, whose presence is a prerequisite for fusion (reviewed in Martin, 1997). It is interesting that the failure to identify CAPS homologs in yeast, where exocytosis is Ca^{2+}-independent, is taken to reflect a key role for CAPS in regulated (Ca^{2+}-dependent) exocytosis (Martin, 1997). Exactly the same argument could be applied to another group of Ca^{2+}-dependent phospholipid binding proteins, the annexins, which are also absent from yeast. Some members of this family aggregate vesicles and lead to membrane deformation (Swairjo et al., 1994; see Thiel and Battey, 1998). Recent work has further suggested a role for annexins in exocytosis from plant cells (Carroll et al., 1998). The question thus arises as to how annexins might be related to SNAREs. The latter may facilitate targeting and membrane specificity, whereas the former may promote membrane fusion and pore formation (see also Jahn and Hansen, 1998).

Recently, the sequence of a tomato gene (*CLB1*) was published (Kiyosue and Ryan, 1997) that contains a Ca^{2+}-dependent lipid binding domain similar to that found in the C2 domain proteins, such as synaptotagmin, that regulate membrane traffic in neuronal cells (Südhof and Rizo, 1996). An Arabidopsis gene (*CaLB*; accession number X96598; unpublished data) very similar to *CLB1* is present in the sequence databases. Also reported in the databases is an open reading frame (TREMBL accession number O23480), identified in the Arabidopsis genome sequencing project (Bevan et al., 1998), that shows similarity to frequenin, an EF-hand Ca^{2+} binding protein also thought to play a role in membrane trafficking (Pongs et al., 1993). Although the existence of these genes further suggests that exocytosis may be regulated in a Ca^{2+}-dependent manner by using proteins conserved between plants and animals, hard proof that these proteins are involved awaits biochemical and genetic tests.

Once the fusion pore has formed, vesicular contents can be released to the extracellular matrix. In animal cells, fusion pore opening can be transient, with content release followed very rapidly by pore closure (Figure 3; Alvarez de Toledo et al., 1993). Alternatively, pore formation is followed by full pore opening with concomitant incorporation of the vesicle membrane into the plasma membrane (Spruce et al., 1990). Both transient and permanent fusion can be observed at different times in the same animal (Oberhauser et al., 1992) and, indeed, plant cell (Thiel and Battey, 1998; Thiel et al., 1998). The frequency of transient relative to permanent fusion is a very important issue because it determines whether the vesicle matrix (to which the vesicle cargo is bound) is lost or recycled and whether vesicle membrane becomes incorporated into the plasma membrane (Figure 3). Figure 4 indicates the potential significance of regulation of transient versus permanent vesicle fusion for the physiology of the plant cell. Clearly, in nonexpanding cells, a mechanism for cargo discharge without membrane incorporation would be useful, especially in view of the energy costs of generating endocytotic vesicles in the presence of high turgor pressure (Cram, 1980; Gradmann and Robinson, 1989; see below).

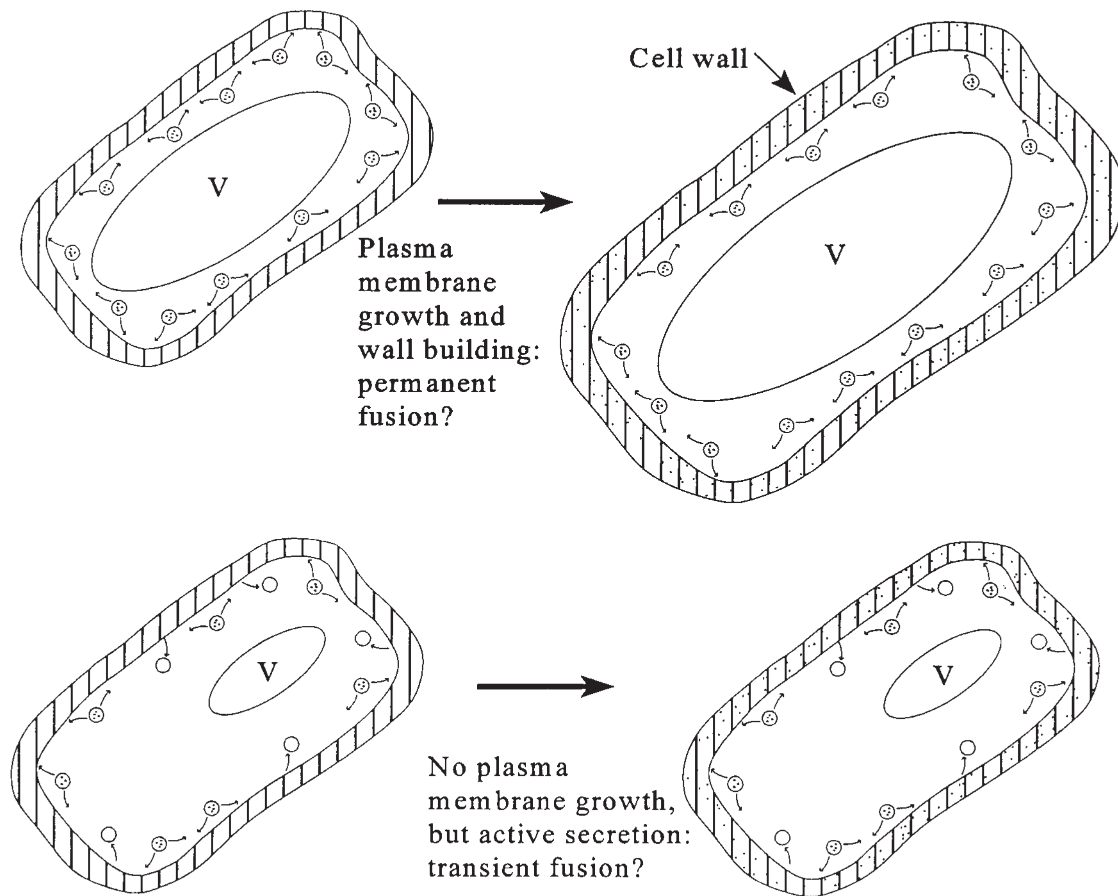

Figure 4. Transient or Permanent Fusion: Consequences for the Plant Cell?

The diagram depicts the potential effects of transient and permanent vesicle fusion on plant cell growth. Although events believed to correspond to transient and permanent vesicle fusion have been detected in plants (Thiel and Battey, 1998; Thiel et al., 1998), the extent to which either or both predominate or are regulated at all remains unknown. V, vacuole.

More detailed discussion of this question can be found in Thiel and Battey (1998).

ENDOCYTOSIS

Recovering Excess Membrane

Evidence from several plant systems indicates that the rate at which new membrane is incorporated into the plasma membrane during cell elongation and growth far exceeds the net increase in cell surface area (Samuels and Bisalputra, 1990; Low and Chandra, 1994). This finding bespeaks the operation of significant membrane recycling. In nongrowing secretory cells, delivery of vesicles to the plasma membrane must be balanced by the equivalent recycling of membrane from the cell surface. In animal and *S. cerevisiae* cells, another major function of endocytosis is internalization and degradation of membrane proteins such as receptor/ligand complexes (Figure 3). Furthermore, plant cells often undergo volume fluctuations in response to changes in the external osmotic environment. Because any fixed amount of plasma membrane is essentially inexpandable (Wolfe and Steponkus, 1983), significant cell volume increase must be accompanied by increased exocytosis. Similarly, osmoregulatory volume decrease after hypoosmotic treatment is likely to be accompanied by increased recycling of plasma membrane (Taylor et al., 1996; see also Thiel and Battey, 1998).

Despite the evidence that membrane recycling occurs in plants, there has been considerable doubt until recently whether the underlying mechanisms involve vesicle endocytosis. These uncertainties arise mainly from consideration of

the energetics of vesicle recycling in the turgid cell (e.g., Cram, 1980), whereby two factors must be considered: the metabolic energy needed to sustain endocytosis against positive hydrostatic pressure and the free energy barrier to vesicle formation. It is likely that the total metabolic energy for endocytosis is well within the cell's metabolic energy budget (Raven, 1987). The free energy requirements for vesicle formation are lower for smaller vesicles (Saxton and Breidenbach, 1988) and crucially depend on the widely variable value of turgor (Gradmann and Robinson, 1989). Therefore, it can be argued that the energetics of endocytosis are likely to be favorable for a range of conditions and vesicle types. In addition, there may be a role for molecular recycling of membrane lipids (Craig and Staehelin, 1988), for which some evidence has been obtained using fluorescently labeled lipids to measure trafficking from the plasma membrane to the ER (Grabski et al., 1993).

Evidence for Endocytosis in Plants

Direct visualization of endocytosis in plant cells comes from observations of internalization of membrane-impermeant molecules. Although earlier interpretation of Lucifer Yellow uptake into plant cells has become problematic as a result of the appreciation that this dye can be transported across membranes (e.g., O'Driscoll et al., 1991), internalization of a range of other impermeant molecules has now been reported, including heavy metals (Hubner et al., 1985; Lazzaro and Thomson, 1992), biotinylated molecules (e.g., Horn et al., 1990), gold-conjugated proteins and lectin (Hillmer et al., 1986; Villanueva et al., 1993), and cationic ferritin (Tanchak et al., 1984). The use of lipophilic styryl (FM) dyes such as FM1-43, which fluorescently label the plasma membrane of intact cells, is beginning to provide additional evidence for membrane recycling (e.g., Smith and Betz, 1996). Figure 5 shows how fluorescence internalization thus can be readily visualized with confocal microscopy, giving a direct indication of membrane recycling. The related dye FM4-64 has been used to visualize endocytosis and vacuolar membrane dynamics in yeast (Vida and Emr, 1995), and more recently, the use of FM1-43 has revealed significant membrane internalization over a time scale of minutes in secretory maize root cap cells (Carroll et al., 1998). In zygotes of the marine alga *Fucus*, FM1-43 and FM4-64 internalization occurs predominantly at the growing rhizoid apex and at the developing cell plate (Belanger and Quatrano, 1998; Brownlee et al., 1998).

In animal cells and yeast, endocytosis occurs via clathrin-coated vesicles (CCVs) that act in plasma membrane recovery and in cycling of vesicles in the endomembrane system. CCVs are characterized by the presence of protrusions of clathrin on the cytoplasmic surface (Low and Chandra, 1994; Robinson, 1996). Clathrin-coated pits in the plasma membrane and CCVs have been described widely in plant cells (e.g., Fowke et al., 1991; Robinson, 1996) and are especially abundant in actively growing cells (e.g., Samuels and Bisalputra, 1990). Other structures known to be involved in endocytosis in animal cells that are also observed in plants and algae include Golgi bodies, multivesicular bodies, and partially coated reticulum (Record and Griffing, 1988; Domozych, 1991; Low and Chandra, 1994; see also Figure 3). Multivesicular bodies are involved in transport to vacuoles and appear to contain degradative enzymes. Partially coated reticulum may be either equivalent to the animal *trans*-Golgi network or an early endosomal compartment (Robinson, 1996).

Coated pits and CCVs have a well-conserved complex of polypeptides specific to their cytoplasmic surface. Clathrin polypeptides form the outer (cytosolic-facing) layer of the coat and comprise three heavy chains and three light chains that assemble to form triskelions (reviewed in Robinson, 1996). Plant clathrin heavy chains have a number of well-conserved regions in common with animal and yeast cells (Blackbourn and Jackson, 1996). Less information is available for clathrin light chains in plants, although these have been well characterized in animals (33- and 36-kD polypeptides) and yeast (38-kD polypeptide) and possess Ca^{2+}-calmodulin binding properties (Acton and Brodsky, 1990; Nathke et al., 1992). CCVs can be either Golgi- or plasma membrane–derived (Blackbourn and Jackson, 1996; Robinson, 1996). Recent work has concentrated mainly on CCVs in trafficking in the *trans*-Golgi network, although the study by Blackbourn and Jackson (1996) clearly implicates CCVs in plasma membrane recycling during pollen tube elongation, because assembled clathrin is present at the pollen tube tip, corresponding to the site of uptake of fluorescein isothiocyanate–dextran (O'Driscoll et al., 1991). Blackbourn and Jackson (1996) further present evidence for selective membrane cycling in the pollen tube tip via an early endosomal compartment and suggest that two previously described vesicle populations with diameters of ~300 and 50 nm could represent secretory vesicles and re-internalized membrane, respectively.

Another essential element of the inner region of the vesicle coat in CCVs is the adaptins. In animals and yeast, these heterotetrameric adaptor proteins are involved in attaching to the membrane and can interact with membrane-spanning receptors. Both Golgi- and plasma membrane–specific adaptor complexes exist in animals and yeast. Plant counterparts are considerably less well characterized, but current evidence supports a role for γ- and β-type adaptins in plasma membrane recycling (Drucker et al., 1995; Keon et al., 1995). Another feature of animal adaptins is the possession of autophosphorylating and casein kinase activity, which recently has been shown in adaptin-like polypeptides from zucchini (Drucker et al., 1998).

A variety of other molecules is associated with the endocytotic pathway in animals and yeast, and some of these molecules are beginning to be described in plant systems. The GTP-binding protein dynamin is required for rapid endocytosis coupled to exocytosis in adrenal chromaffin cells

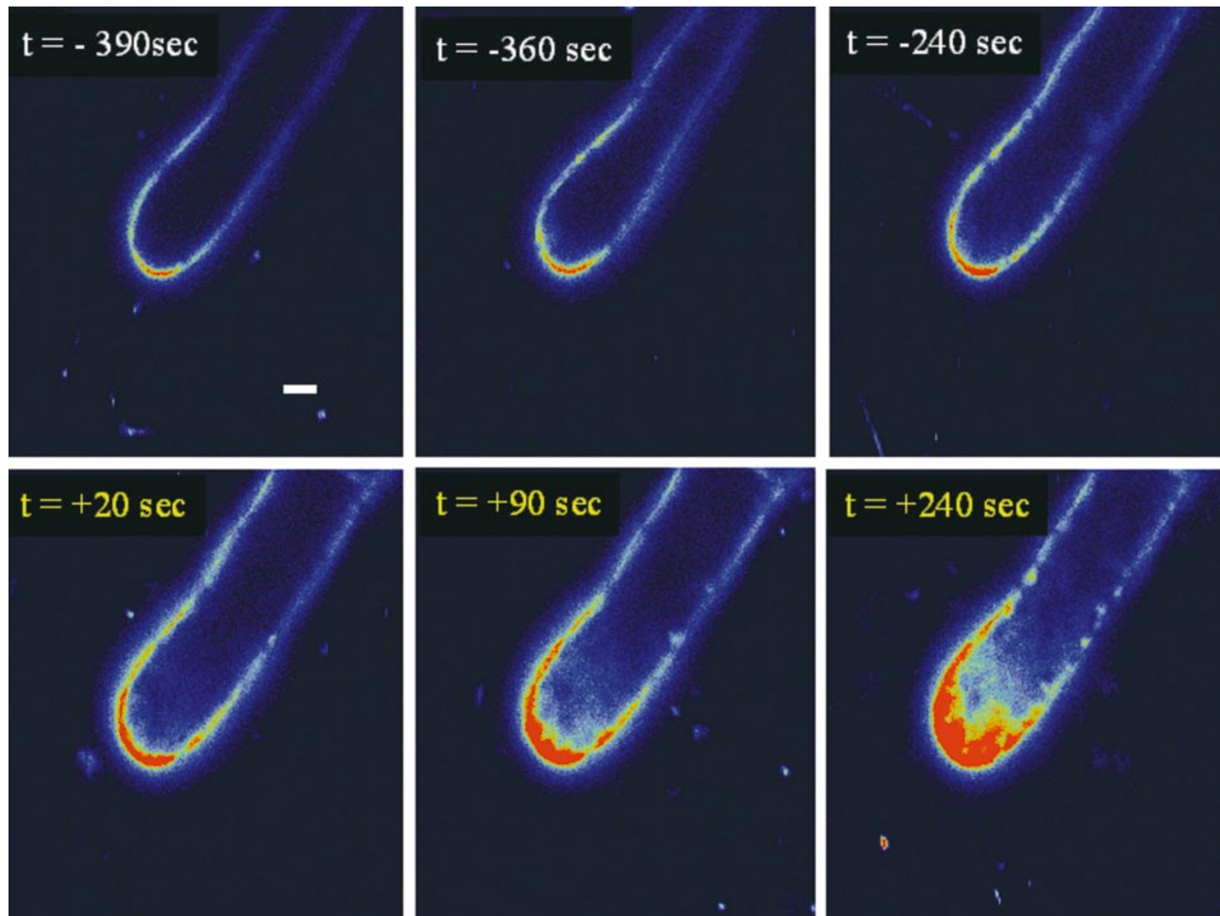

Figure 5. Confocal Images of the Pattern of Internalization of the Fluorescent Membrane Dye FM1-43 in a Rhizoid Cell of *Fucus serratus.*

Embryos were incubated in 1 μM FM1-43 for the duration of the experiment. Hypoosmotic treatment (from sea water to 50% sea water) was given at time (t) 0. Little or no dye internalization was observed for at least 390 sec before the hypoosmotic shock. However, dye internalization was apparent within 20 sec of the shock and continued for the duration of the recording (240 sec).

(Artalejo et al., 1995). Although this particular endocytotic system is independent of clathrin, dynamin is involved elsewhere in clathrin-mediated endocytosis, for instance, in the mammalian COS-7 cell line (Herskovits et al., 1993). One plant homolog of dynamin has been isolated in Arabidopsis with significant similarity to yeast Vps1p, rat dynamin, and murine Mx1 protein (Dombrowski and Raikhel, 1995). Another dynamin homolog, phragmoplastin, has been found at the cell plate in soybean (Gu and Verma, 1996). It appears that polymerized dynamin acts to "squeeze" vesicles from the plasma membrane during endocytosis (Takei et al., 1995), and so it is tempting to speculate that phragmoplastin acts in a similar way to allow the exocytotic release of vesicle contents during growth of the cell plate. Finally, an uncoating ATPase facilitates the removal of the clathrin coat from CCVs (Hannan et al., 1998), and preliminary evidence exists for such ATPases in plants (Beevers, 1996).

Receptor-Mediated Endocytosis

Major functions of clathrin and associated proteins in animals and yeast are the internalization of membrane receptors and proteins and the correct targeting of CCVs to the endosomal lysosome, late-Golgi compartments, or vacuole. Such receptor-mediated endocytosis is an essential process in signal transduction whereby receptor–ligand complexes are internalized and processed (e.g., Egner and Kuchler, 1996). This process further involves the ubiquitination of target molecules before endocytosis and degradation (Egner and Kuchler, 1996; Hicke and Riezman, 1996), and it also

requires myosin and actin (Geli and Riezman, 1996). Evidence for receptor-mediated endocytosis in plants is currently very limited. Endocytotic internalization of *Rhizobium* involving lectin binding occurs in pea root hairs (Kijne et al., 1988), and receptor-mediated uptake of elicitor molecules (Low and Chandra, 1994) and auxin binding protein (Robinson, 1996) has been proposed.

Quantification of Endocytosis

Electrophysiological experiments allow direct measurements of protoplast membrane surface area and provide further evidence for membrane cycling, and although changes detected in cell capacitance reflect only the net result of exocytosis and endocytosis (Battey et al., 1996), such measurements with barley aleurone and maize root protoplasts reveal significant membrane internalization upon intracellular perfusion with solutions of very low [Ca^{2+}] (Zorec and Tester, 1992; Carroll et al., 1998). Rapid increases in barley aleurone protoplast surface area, induced by increasing internal pressure, are followed by a decline to resting values, suggesting the presence of a pressure-responsive exo-endocytosis cycle (Zorec and Tester, 1993). Furthermore, hypoosmotic treatment of stomatal guard cell protoplasts causes a reversible increase in plasma membrane area that is quantifiable as an increase in cell volume (Homann, 1998). This observation is of particular interest because the guard cell is generally regarded as nonsecretory and nongrowing; the vesicles involved in this response, therefore, may have a specific role in osmoregulation. In the marine alga *Pelvetia*, freeze-fracture studies have similarly identified a class of vesicles that is exocytosed in response to hypoosmotic treatment (Gilkey and Staehelin, 1989). Hypoosmotically induced cell expansion in *Fucus* zygotes is followed by volume regulation that is demonstrable by a dramatic increase in the internalization of FM1-43 at the growing rhizoid apex (Brownlee et al., 1998; Figure 5).

Taken together, the above evidence amply suggests that membrane recycling can occur rapidly (within seconds to minutes) during osmotically induced cell volume changes. This rate of recycling may be faster than during normal growth and secretion, suggesting that the usual membrane cycling pathway may be bypassed. Observation of vesicular material extruded from the plasma membrane after pollen tube plasmolysis (Kroh and Knuiman, 1985) and in osmotically regulating protoplasts (Steponkus, 1991) also suggests that atypical membrane structures may be an important mechanism to allow volume regulation (see also Thiel and Battey, 1998).

The simultaneous monitoring of exocytosis and endocytosis through measurements of cell capacitance and internalization of FM1-43, respectively, has been achieved with adrenal chromaffin cells (Smith and Betz, 1996). This study showed that after short exocytotic stimuli, endocytosis occurred after exocytosis had ceased, whereas prolonged exocytotic stimuli resulted in endocytosis that eventually balanced the rate of exocytosis. The potential of FM dyes for quantification of endocytosis also has been shown in hippocampal neurons, where unitary endocytotic events have been visualized and quantified (Ryan et al., 1997), and such high-resolution imaging could potentially provide unique quantitative information on both protoplasts and whole plant cells.

CONCLUSIONS AND FUTURE PROSPECTS

Our initial interest in exocytosis was triggered by the need to find mechanisms that underlie the pronounced effects of Ca^{2+} on plant cell development (Hepler and Wayne, 1985) and the circumstantial evidence that Ca^{2+} might control the exocytotic/endocytotic balance in plants (Steer, 1988; Battey and Blackbourn, 1993). Ca^{2+} has subsequently been shown to be needed for a sustained increase in the plasma membrane capacitance of plant protoplasts, reflecting the activation of exocytosis relative to endocytosis. This evidence supports the hypothesis that regulation of vesicle fusion at the plasma membrane is a critical control point for Ca^{2+}. It remains to be demonstrated that these effects of Ca^{2+} lead to developmental changes; indeed, effects on secretion (polysaccharide or protein) in addition to membrane growth have not been shown. Therefore, the electrophysiological measurement of membrane capacitance has enhanced our knowledge of the factors that influence exocytosis/endocytosis in plants, but the need to use protoplasts and the resultant lack of necessary relevance to plant physiology and development are serious limitations to progress. Nevertheless, the recent paper by Homann (1998), which relates changes in capacitance to guard cell osmotic regulation, is an important step in the right direction. Similarly, the ability of the dye FM1-43 to reveal membrane cycling in plants (Carroll et al., 1998) offers the opportunity, in conjuction with capacitance measurements, to monitor exocytosis and endocytosis independently. At a still finer level of detail, there is much potential in the ability of the attached-patch method to distinguish transient fusion from full fusion (Thiel et al., 1998). A major technical challenge that remains is to measure exocytosis/endocytosis in walled cells to relate these measurements to cell and tissue changes.

Those cells of most interest for understanding exocytosis and endocytosis are often highly differentiated and difficult subjects for biochemical analysis (e.g., the honeysuckle nectary or *Mimulus* glandular hairs; see Figure 1). The availability of a range of (labeled) protein probes whose trafficking pathways are known would provide the means to understand plasma membrane dynamics in such specialized cells, as is indicated by success in studies on bone-resorbing osteoclasts in animals, where the plasma membrane is divided into domains that are maintained by directed vesicle trafficking along the endocytotic pathway (Salo et al., 1996; Palokangas et al., 1997). In plants, probes that allow this

kind of analysis are unavailable at present, but the development of green fluorescent protein tagging offers potential for the future. An equivalent tool with which to probe polysaccharide distribution would be of great value in plant cells. It is already possible to visualize directly the transport of secondary metabolites in an exocytotic pathway that leads to the deposition of defense-related products in the cell wall (Stewart and Mansfield, 1985). This provides a useful means to understand this novel exocytotic pathway as well as to study an important aspect of the response of plants to fungal invasion.

The full range of SNAREs and other proteins that regulate vesicle docking and fusion at the plant plasma membrane needs to be identified. Confirmation that SNAREs are important in exocytosis may be obtainable by using the clostridial toxins, such as tetanus and botulinum toxins, in patch–clamp experiments. These toxins are specific proteases that cleave syntaxin and SNAP-25 and synaptobrevin in human nerve terminals (Huttner, 1993; Hayashi et al., 1994), thereby preventing neurotransmitter release. It is apparently not known whether these toxins have similar effects on exocytosis in plant cells, although one line of evidence implicating a syntaxin in guard cell responses to abscisic acid is a specific inhibition by botulinum C toxin (Leyman et al., 1999). A related question is whether plant cells with pronounced polarity of exocytosis, such as the hypersecretory cells of the maize root cap (Roy and Vian, 1991), seed coat cells of *Plantago* (Hyde, 1970), or pollen tubes (Taylor and Hepler, 1997), achieve this polarity by using SNARE mechanisms, Ca^{2+} gradients, cytoskeletal targeting, or a combination of all three.

Interestingly, it seems that secreted polysaccharide may play an important role in perpetuating polarized growth. Thus, the inhibitor of Golgi secretion brefeldin A has been shown to disrupt polarized secretion of a rhizoid-specific polysaccharide in polarizing *Fucus* zygotes (Shaw and Quatrano, 1996). This treatment prevents fixation of the polar axis but is without effect on actin localization or cell wall synthesis associated with cell division, and it implies that localized exocytosis of cell wall components is an early step in polar axis fixation such that the cell wall is a source of position-dependent information. In Arabidopsis, mutations of the *GNOM* gene, which has homology with the yeast *SEC7* gene, similarly show disrupted apical–basal polarity and the absence of specific organs (Mayer et al., 1993; Shevell et al., 1994), further suggesting that vesicle-mediated trafficking of positional information to the cell wall compartment is a fundamental step determining embryonic polarity.

ACKNOWLEDGMENTS

We thank Ian Moore (Oxford University, UK) for Rab guidance and for comments on the manuscript; Gerhard Thiel (Göttingen University, Germany) for comments on the manuscript; James Carew and Paul Le Mière (Department of Horticulture, University of Reading) for help with Figure 1 and for drawing the diagrams in Figures 3 and 4; Jean Whiterow (Department of Horticulture, University of Reading) for her invaluable help in preparing the manuscript; and the United Kingdom Biotechnology and Biological Sciences Research Council (BBSRC), the Leverhulme Trust, the University of Reading Research Endowment Trust Fund, the Marine Biological Association, UK, and ZENECA Agrochemicals for financial support of the authors' labs. N.C.J. acknowledges the support of an Industrial CASE studentship from the BBSRC.

REFERENCES

Acton, S.L., and Brodsky, F.M. (1990). Predominance of clathrin light chain LC_b correlates with the presence of a regulated secretory pathway. J. Cell Biol. **111,** 1419–1426.

Alvarez de Toledo, G., Fernandez-Chacùn, R., and Fernandez, J.M. (1993). Release of secretory products during transient vesicle fusion. Nature **363,** 554–558.

Artalejo, C.R., Henley, J.R., McNiven, M.A., and Palfrey, H.C. (1995). Rapid endocytosis coupled to exocytosis in adrenal chromaffin cells involves Ca^{2+}, GTP and dynamin but not clathrin. Proc. Natl. Acad. Sci. USA **92,** 8328–8332.

Atkinson, A.H., Heath, R.L., Simpson, R.J., Clarke, A.E., and Anderson, M.A. (1993). Proteinase inhibitors in *Nicotiana alata* stigmas are derived from a precursor protein which is processed into five homologous inhibitors. Plant Cell **5,** 203–213.

Barr, P.J. (1991). Mammalian subtilisins: The long-sought dibasic processing endoproteases. Cell **66,** 1–3.

Battey, N.H., and Blackbourn, H.D. (1993). The control of exocytosis in plant cells. New Phytol. **125,** 307–338.

Battey, N.H., Carroll, A., van Kesteren, P., Taylor, A., and Brownlee, C. (1996). The measurement of exocytosis in plant cells. J. Exp. Bot. **47,** 717–728.

Beckers, C.J.M., and Balch, W.E. (1989). Calcium and GTP: Essential components in vesicular trafficking between the endoplasmic reticulum and the Golgi apparatus. J. Cell Biol. **108,** 1245–1256.

Beevers, L. (1996). Clathrin-coated vesicles in plants. Int. Rev. Cytol. **167,** 1–35.

Belanger, K.D., and Quatrano, R.S. (1998). Characterisation of membrane populations in *Fucus distichus* using the vital dye FM4-64. Keystone Symp. Plant Cell Biol. (abstr.).

Bevan, M., et al. (1998). Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. Nature **391,** 485–488.

Bilderback, D.E., and Sloane, J.H. (1987). The ultrastructure of the secreting ligule of *Selaginella kraussiana*. Bot. Gaz. **148,** 413–419.

Blackbourn, H.D., and Jackson, A.P. (1996). Plant clathrin heavy chain: Sequence analysis and restricted localisation in growing pollen tubes. J. Cell Sci. **109,** 777–787.

Blumenthal, R., Pak, C.C., Raviv, Y., Krumbiegel, M., Bergelson, L.D., Morris, S.J., and Lowry, R.J. (1995). Transient domains induced by influenza haemagglutinin during membrane fusion. Mol. Membr. Biol. **12,** 135–142.

Bohlmann, H., and Apel, K. (1991). Thionins. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42,** 227–240.

Broekaert, W.F., Cammue, B.P.A., De Bolle, M.F.C., Thevissen, K., De Samblanx, G.W., and Osborn, R.W. (1997). Antimicrobial peptides from plants. Crit. Rev. Plant Sci. **16,** 297–323.

Brownlee, C., Manison, N.F.H., and Anning, R. (1998). Calcium, polarity and osmoregulation in *Fucus* embryos: One messenger, multiple messages. Exp. Biol. OnLine **3,** 11.

Bruns, D., Engers, S., Yang, C., Ossig, R., Jeromin, A., and Jahn, R. (1997). Inhibition of transmitter release correlates with the proteolytic activity of tetanus toxin and botulinum toxin A in individual cultured synapses of *Hirudo medicinalis*. J. Neurosci. **17,** 1898–1910.

Cai, G., Moscatelli, A., and Cresti, M. (1997). Cytoskeletal organization and pollen tube growth. Trends Plant Sci. **2,** 86–91.

Carroll, A.D., Moyen, C., van Kesteren, W.J.P., Tooke, F., Battey, N.H., and Brownlee, C. (1998). Ca^{2+}, annexins, and GTP modulate exocytosis from maize root cap protoplasts. Plant Cell **10,** 1267–1276.

Chaffey, N.J. (1985). Structure and function in the grass ligule: Optical and electron microscopy of the membranous ligule of *Lolium temulentum* L. (Poaceae). Ann. Bot. **55,** 65–75.

Chaffey, N.J. (1995). Structure and function in the grass ligule: The endomembrane system of the adaxial epidermis of the membranous ligule of *Lolium temulentum* L. (Poaceae). Ann. Bot. **76,** 103–112.

Chant, J. (1996). Generation of cell polarity in yeast. Curr. Opin. Cell Biol. **8,** 557–565.

Chavez, R.A., Miller, S.G., and Moore, H.-P.H. (1996). A biosynthetic regulated secretory pathway in constitutive secretory cells. J. Cell Biol. **133,** 1177–1191.

Cohn, J., Day, R.B., and Stacey, G. (1998). Legume nodule organogenesis. Trends Plant Sci. **3,** 105–110.

Cole, N.B., and Lippincott-Schwartz, J. (1995). Organization of organelles and membrane traffic by microtubules. Curr. Opin. Cell Biol. **7,** 55–64.

Coorssen, J.R., Schmitt, H., and Almers, W. (1996). Ca^{2+}-triggered massive exocytosis in Chinese hamster ovary cell. EMBO J. **15,** 3787–3791.

Craig, S., and Staehelin, L.A. (1988). High pressure freezing of intact plant tissues: Evaluation and characterization of novel features of the endoplasmic reticulum and associated membrane systems. Eur. J. Cell Biol. **46,** 80–93.

Craighead, M.W., Bowden, S., Watson, R., and Armstrong, J. (1993). Function of the ypt2 gene in the exocytic pathway of *Schizosaccharomyces pombe*. Mol. Biol. Cell **4,** 1069–1076.

Cram, W.J. (1980). Pinocytosis in plants. New Phytol. **84,** 1–17.

D'Esposito, M., Ciccodicola, A., Gianfrancesco, F., Esposito, T., Flagiello, L., Mazzarella, R., Schlessinger, D., and D'Urso, M. (1996). A synaptobrevin-like gene in the Xq28 pseudoautosomal region undergoes X inactivation. Nature Genet. **13,** 227–229.

Dombrowski, J.E., and Raikhel, N.V. (1995). Isolation of a cDNA encoding a novel GTP-binding protein of *Arabidopsis thaliana*. Plant Mol. Biol. **28,** 1121–1126.

Domozych, D.S. (1991). The Golgi apparatus and membrane trafficking in green algae. Int. Rev. Cytol. **131,** 213–253.

Driouich, A., Faye, L., and Staehelin, A. (1993). The plant Golgi apparatus: A factory for complex polysaccharides and glycoproteins. Trends Biochem. Sci. **18,** 210–214.

Drucker, M., Herkt, B., and Robinson, D.G. (1995). Demonstration of a β-type adaptin at the plant plasma membrane. Cell Biol. Int. **19,** 191–201.

Drucker, M., Happel, N., and Robinson, D.G. (1998). Localisation and properties of kinase activities in clathrin coated vesicles from zucchini hypocotyls. Eur. J. Biochem. **240,** 570–575.

Egner, R., and Kuchler, K. (1996). The yeast multidrug transporter PDR5 of the plasma membrane is ubiquitinated prior to endocytosis and degradation in the vacuole. FEBS Lett. **378,** 177–181.

Fahn, A. (1988). Secretory tissues in vascular plants. New Phytol. **108,** 229–257.

Fahn, A., and Rachmilevitz, T. (1970). Ultrastructure and nectar secretion in *Lonicera japonica*. In New Research in Plant Anatomy, N.K.B. Robson, D.F. Cutler, and M. Gregory, eds (London: Academic Press), pp. 51–56.

Fowke, L.C., Tanchak, M.A., and Galway, M.E. (1991). Ultrastructural cytology of the endocytotic pathway in plants. In Endocytosis, Exocytosis and Vesicle Traffic in Plants, C.R. Hawes, J.O.D. Coleman, and D.E. Evans, eds (Cambridge, UK: Cambridge University Press), pp. 15–40.

Franklin-Tong, V.E. (1999). Signaling and the modulation of pollen tube growth. Plant Cell **11,** 727–738.

Geli, M.I., and Riezman, H. (1996). Role of type-I myosins in receptor-mediated endocytosis in yeast. Science **272,** 533–535.

Gilkey, J.C., and Staehelin, L.A. (1989). A new organelle related to osmoregulation in ultrarapidly frozen *Pelvetia* embryos. Planta **178,** 425–435.

Goda, Y., and Südhof, T.C. (1997). Calcium regulation of neurotransmitter release: Reliably unreliable? Curr. Opin. Cell Biol. **9,** 513–518.

Goud, B., Salminen, A., Walworth, N.C., and Novick, P.J. (1988). A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. Cell **53,** 753–768.

Grabski, S., de Feijter, A.W., and Schindler, M. (1993). Endoplasmic reticulum forms a dynamic continuum for lipid diffusion between contiguous soybean root cells. Plant Cell **5,** 25–38.

Gradmann, D., and Robinson, D.G. (1989). Does turgor prevent endocytosis in plant cells? Plant Cell Environ. **12,** 151–154.

Gruenberg, J., and Maxfield, F.R. (1995). Membrane transport in the endocytic pathway. Curr. Opin. Cell Biol. **7,** 552–563.

Gu, X., and Verma, D.P.S. (1996). Phragmoplastin, a dynamin-like protein associated with cell plate formation in plants. EMBO J. **15,** 695–704.

Hannan, L.A., Newmyer, S.L., and Schmidt, L.A. (1998). ATP- and cytosol-dependent release of adaptor proteins from clathrin-coated vesicles. Mol. Biol. Cell **9,** 2217–2229.

Hay, J.C., and Scheller, R.H. (1997). SNAREs and NSF in targeted membrane fusion. Curr. Opin. Cell Biol. **9,** 505–512.

Hayashi, T., McMahon, H., Yamasaki, S., Binz, T., Hata, Y., Südhof, T.C., and Niemann, H. (1994). Synaptic vesicle membrane fusion complex: Action of clostridial neurotoxins on assembly. EMBO J. **13,** 5051–5061.

Heath, R.L., Barton, P.A., Simpson, R.J., Reid, G.E., Lim, G., and Anderson, M.A. (1995). Characterization of the protease processing sites in a multidomain proteinase inhibitor precursor from *Nicotiana alata*. Eur. J. Biochem. **230,** 250–257.

Heath, R.L., McDonald, G., Christeller, J.T., Lee, M., Bateman, K., West, J., van Heeswijck, R., and Anderson, M.A. (1997). Proteinase inhibitors from *Nicotiana alata* enhance plant resistance to insect pests. J. Insect Physiol. **43,** 833–842.

Hepler, P.K., and Wayne, R.O. (1985). Calcium and plant development. Annu. Rev. Plant Physiol. **36,** 397–439.

Herskovits, J.S., Burgess, C.C., Obar, R.A., and Vallee, R.B. (1993). Effects of mutant rat dynamin on endocytosis. J. Cell Biol. **122,** 565–578.

Hicke, L., and Riezman, H. (1996). Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis. Cell **84,** 277–287.

Hillmer, S., Depta, H., and Robinson, D.G. (1986). Confirmation of endocytosis in higher plant protoplasts using lectin–gold conjugates. Eur. J. Cell Biol. **41,** 142–149.

Hirai, Y. (1993). Molecular cloning of human epimorphin: Identification of isoforms and their unique properties. Biochem. Biophys. Res. Commun. **191,** 1332–1337.

Hohl, I., Robinson, D.G., Chrispeels, M.J., and Hinz, G. (1996). Transport of storage proteins to the vacuole is mediated by vesicles without a clathrin coat. J. Cell Sci. **109,** 2539–2550.

Homann, U. (1998). Osmotically-induced excursions in the surface area of guard cell protoplasts. Planta **206,** 329–333.

Homann, U., and Tester, M. (1997). Ca^{2+}-independent and Ca^{2+}/GTP-binding protein–controlled exocytosis in a plant cell. Proc. Natl. Acad. Sci. USA **94,** 6565–6570.

Horn, M.A., Heinstein, P.F., and Low, P.S. (1990). Biotin-mediated delivery of exogenous molecules into soybean cells. Plant Physiol. **93,** 1492–1496.

Huber, L.A., Pimplikar, S., Parton, R.G., Virta, H., Zerial, M., and Simons, K. (1993). Rab8, a small GTPase involved in vesicular traffic between the TGN and the basolateral plasma membrane. J. Cell Biol. **123,** 35–45.

Hubner, R., Depta, H., and Robinson, D.G. (1985). Endocytosis in maize root cap cells: Evidence obtained using heavy metal salt solutions. Protoplasma **129,** 214–222.

Hugueney, P., Bouvier, F., Badillo, A., D'Harlingue, A., Kuntz, M., and Camara, B. (1995). Identification of a plastid protein involved in vesicle fusion and/or membrane protein translocation. Proc. Natl. Acad. Sci. USA **92,** 5630–5634.

Huttner, W.B. (1993). Snappy exocytoxins. Nature **365,** 104–105.

Hyde, B.B. (1970). Mucilage-producing cells in the seed coat of *Plantago ovata*: Developmental fine structure. Am. J. Bot. **57,** 1197–1206.

Jahn, R., and Hansen, P.I. (1998). SNAREs line up in new environment. Nature **393,** 14–15.

Keller, P., and Simons, K. (1997). Post-Golgi biosynthetic trafficking. J. Cell Sci. **110,** 3001–3009.

Keon, J.P.R., Jewitt, S., and Hargreaves, J.A. (1995). A gene encoding γ-adaptin is required for apical extension growth in *Ustilago maydis.* Gene **162,** 141–145.

Kijne, J.W., Smit, G., Diaz, C.L., and Lugtenberg, B.J.J. (1988). Lectin enhanced accumulation of manganese-limited *Rhizobium leguminosarum* cells on pea root hair tips. J. Bacteriol. **170,** 2994–3000.

Kinal, H., Park, C.-M., Berry, J.O., Koltin, Y., and Bruenn, J.A. (1995). Processing and secretion of a virally encoded antifungal toxin in transgenic tobacco plants: Evidence for a Kex2p pathway in plants. Plant Cell **7,** 677–688.

Kiyosue, T., and Ryan, C.A. (1997). A novel gene of tomato preferentially expressed in tomato fruit encodes a protein with a Ca^{2+}-dependent lipid-binding domain. Plant Mol. Biol. **35,** 969–972.

Kroh, M., and Knuiman, B. (1985). Exocytosis in non-plasmolysed and plasmolysed tobacco pollen tubes. Planta **166,** 287–299.

Lancelle, S.A., Cresti, M., and Hepler, P.K. (1997). Growth inhibition and recovery in freeze-substituted *Lilium longiflorum* pollen tubes: Structural effects of caffeine. Protoplasma **196,** 21–33.

Lauber, M.H., Waizenegger, I., Steinmann, T., Schwarz, H., Mayer, U., Hwang, I., Lukowitz, W., and Jürgens, G. (1997). The Arabidopsis KNOLLE protein is a cytokinesis-specific syntaxin. J. Cell Biol. **139,** 1485–1493.

Lazzaro, M.D., and Thomson, W.W. (1992). Endocytosis of lanthanum nitrate in the organic acid–secreting trichomes of chickpea (*Cicer arietinum*). Am. J. Bot. **79,** 1113–1118.

Leyman, B., Geelan, D., Quintero, F.J., and Blatt, M.R. (1999). A tobacco syntaxin with a role in hormonal control of guard cell ion channels. Science **283,** 537–540.

Lin, Y., and Yang, Z. (1997). Inhibition of pollen tube elongation by microinjected anti-Rop1Ps antibodies suggests a crucial role for Rho-type GTPases in the control of tip growth. Plant Cell **9,** 1647–1659.

Lin, Y., Wang, Y., Zhu, J.-K., and Yang, Z. (1996). Localization of a Rho GTPase implies a role in tip growth and movement of the generative cell in pollen tubes. Plant Cell **8,** 293–303.

Low, P.S., and Chandra, S. (1994). Endocytosis in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43,** 609–631.

Lukowitz, W., Mayer, U., and Jürgens, G. (1996). Cytokinesis in the Arabidopsis embryo involves the syntaxin-related *KNOLLE* gene product. Cell **84,** 61–71.

Lynch, M.A., and Staehelin, L.A. (1992). Domain-specific and cell-type specific localization of two types of cell wall matrix polysaccharides in the clover root tip. J. Cell Biol. **118,** 467–480.

Lynch, M.A., and Staehelin, L.A. (1995). Immunocytochemical localization of cell wall polysaccharides in the root tip of *Avena sativa.* Protoplasma **188,** 115–127.

Martin, T.F.J. (1997). Stages of regulated exocytosis. Trends Cell Biol. **7,** 271–276.

Mayer, U., Buttner, G., and Jürgens, G. (1993) Apical–basal pattern formation in the Arabidopsis embryo: Studies on the role of the *GNOM* gene. Development **117,** 149–162.

McFadden, G.I., and Melkonian, M. (1986). Golgi apparatus activity and membrane flow during scale biogenesis in the green flagellate *Scherffelia dubia* (Prasinophyceae). I. Flagellar regeneration. Protoplasma **130,** 186–198.

McFadden, G.I., Preisig, H.R., and Melkonian, M. (1986). Golgi apparatus activity and membrane flow during scale biogenesis in the green flagellate *Scherffelia dubia* (Prasinophyceae). II. Cell wall secretion and assembly. Protoplasma **131,** 174–184.

Melkonian, M., McFadden, G.I., Reize, I.B., and Becker, D. (1986). Secretion of organic scales in green algae: Secretory products are transported through the Golgi apparatus by cisternal progression. Ber. Deutsch. Bot. Ges. **99,** 263–280.

Monck, J.R., and Fernandez, J.M. (1996). The fusion pore and mechanisms of biological membrane fusion. Curr. Opin. Cell Biol. **8,** 524–533.

Moore, P.J., Swords, K.M.M., Lynch, M.A., and Staehelin, L.A. (1991). Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the Golgi apparatus of plants. J. Cell Biol. **112,** 589–602.

Morimoto, T., Popov, S., Buckley, K.M., and Poo, M.-M. (1995). Calcium-dependent transmitter secretion from fibroblasts: Modulation by synaptotagmin I. Neuron **15,** 689–696.

Murthy, V.N., and Stevens, C.F. (1998). Synaptic vesicles retain their identity through the endocytic cycle. Nature **392,** 497–501.

Nathke, I.S., Heuser, J., Lupas, A., Stock, J., Turck, C.W., and Brodsky, F.M. (1992). Folding and trimerization of clathrin subunits at the triskelion hub. Cell **68,** 899–910.

Neiman, A.M. (1998). Prospore membrane formation defines a developmentally regulated branch of the secretory pathway in yeast. J. Cell Biol. **140,** 29–37.

Nüsse, O., Serrander, L., Lew, D.P., and Krause, K.-H. (1998). Ca^{2+}-induced exocytosis in individual human neutrophils: High- and low-affinity granule populations and submaximal responses. EMBO J. **17,** 1279–1288.

Oberhauser, A.F., Monck, J.R., and Fernandez, J.M. (1992). Events leading to the opening and closing of the exocytotic fusion pore have markedly different temperature dependencies: Kinetic analysis of single fusion events in patch-clamped mouse mast cells. Biophys. J. **61,** 800–809.

O'Driscoll, D., Wolson, G., and Steer, M.W. (1991). Lucifer yellow and fluorescein isothiocyanate uptake by cells of *Morinda citrifolia* in suspension cultures is not confined to the endocytotic pathway. J. Cell Sci. **100,** 237–241.

Palokangas, H., Mulari, M., and Väänänen, H.K. (1997). Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts. J. Cell Sci. **110,** 1767–1780.

Pongs, O., Lindemeier, J., Zhu, X.R., Theil, T., Engelkamp, D., Krah-Jentgengs, I., Lambrecht, H.G., Koch, K.W., Schwemer, J., Rivosecchi, R., Mallart, A., Galceran, J., Canal, I., Barbas, J.A., and Ferras, A. (1993). Frequenin—A novel calcium-binding protein that modulates synaptic efficacy in the *Drosophila* nervous system. Neuron **11,** 15–28.

Raikhel, N.V., Lee, H.-I., and Broekaert, W.F. (1993). Structure and functions of chitin-binding proteins. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44,** 591–615.

Raven, J.A. (1987). The role of vacuoles. New Phytol. **196,** 357–422.

Record, R.D., and Griffing, L.R. (1988). Convergence of endocytosis and lysosomal pathways in soybean protoplasts. Planta **176,** 425–432.

Ribeiro, A., Akkermans, A.D.L., van Kammen, A., Bisseling, T., and Pawlowski, K. (1995). A nodule-specific gene encoding a subtilisin-like protease is expressed in early stages of actinorhizal nodule development. Plant Cell **7,** 785–794.

Robinson, D.G. (1996). Clathrin-mediated trafficking. Trends Plant Sci. **1,** 323–361.

Rodriguez, A., Webster, P., Ortego, J., and Andrews, N.W. (1997). Lysosomes behave as Ca^{2+}-regulated exocytic vesicles in fibroblasts and epithelial cells. J. Cell Biol. **137,** 93–104.

Rothman, J.E. (1994). Mechanisms of intracellular protein transport. Nature **372,** 55–63.

Rougier, M. (1981). Secretory activity of the root cap. In Encyclopaedia of Plant Physiology, Vol. 13B, Plant Carbohydrates. II. Extracellular Carbohydrates, W. Tanner and F.A. Loewus, eds (Berlin: Springer-Verlag), pp. 542–574.

Roy, S., and Vian, B. (1991). Transmural exocytosis in maize root cap: Visualization by simultaneous use of a cellulose-probe and a fucose-probe. Protoplasma **161,** 181–191.

Ryan, T.A., Reuter, H., and Smith, S.J. (1997). Optical detection of a quantal presynaptic membrane turnover. Nature **388,** 478–482.

Rybin, V., Ullrich, O., Rubino, M., Alexandrov, K., Simon, I., Seabra, M.C., Goody, R., and Zerial, M. (1996). GTPase activity of Rab5 acts as a timer for endocytic membrane fusion. Nature **383,** 266–269.

Salo, J., Metsikkö, K., Palokangas, H., Lehenkari, P., and Väänänen, H.K. (1996). Bone-resorbing osteoclasts reveal a dynamic division of basal plasma membrane into two different domains. J. Cell Sci. **109,** 301–307.

Samuels, A.L., and Bisalputra, T. (1990). Endocytosis in elongating root cells. J. Cell Sci. **97,** 157–165.

Samuels, A.L., Giddings, T.H., Jr, and Staehelin, L.A. (1995). Cytokinesis in tobacco BY-2 and root tip cells: A new model of cell plate formation in higher plants. J. Cell Biol. **130,** 1345–1357.

Sanderfoot, A.A., and Raikhel, N.V. (1999). The specificity of vesicle trafficking: Coat proteins and SNAREs. Plant Cell **11,** 629–641.

Sato, Y., Matsuoka, K., and Nakamura, K. (1997). A tobacco cDNA encoding a homologue of *N*-ethylmaleimide–sensitive fusion protein (NSF) (Accession No. D86506) (PGR97-063). Plant Physiol. **113,** 1464.

Saxton, M.J., and Breidenbach, R.W. (1988). Receptor-mediated endocytosis in plants is energetically possible. Plant Physiol. **86,** 993–995.

Schaller, A., and Ryan, C.A. (1994). Identification of a 50-kDa systemin-binding protein in tomato plasma membranes having Kex2p-like properties. Proc. Natl. Acad. Sci. USA **91,** 11802–11806.

Schaller, A., and Ryan, C.A. (1995). Systemin—A polypeptide defense signal in plants. Bioessays **18,** 27–33.

Schnepf, E., and Busch, J. (1976). Morphology and kinetics of slime secretion in glands of *Mimulus tilingii.* Z. Pflanzenphysiol. **79,** 62–71.

Schopfer, C.R., and Hepler, P.K. (1991). Distribution of membranes and the cytoskeleton during cell plate formation in pollen mother cells of *Tradescantia.* J. Cell Sci. **100,** 717–728.

Schweizer, F.E., Dresbach, T., DeBello, W.M., O'Connor, V., Augustine, G.J., and Betz, H. (1998). Regulation of neurotransmitter release kinetics by NSF. Science **279,** 1203–1206.

Shaw, S.L., and Quatrano, R.S. (1996). The role of targetted secretion in the establishment of cell polarity and the orientation of the division plane in *Fucus* zygotes. Development **122,** 2623–2630.

Shevell, D.E., Leu, W.-M., Gillmor, C.S., Xia, G., Feldmann, K.A., and Chua, N.-H. (1994). *EMB30* is essential for normal cell division, cell expansion and cell adhesion in Arabidopsis and encodes a protein that has similarity to Sec7. Cell **77,** 1051–1062.

Siezen, R.J., and Leunissen, J.A.M. (1997). Subtilases: The superfamily of subtilisin-like serine proteases. Protein Sci. **6,** 501–523.

Smith, C.B., and Betz, W.J. (1996). Simultaneous independent measurement of endocytosis and exocytosis. Nature **380,** 531–534.

Söllner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., and Rothman, J.E. (1993). A protein assembly–disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell **75,** 409–418.

Spruce, A.E., Breckenridge, L.J., Lee, A.K., and Almers, W. (1990). Properties of the fusion pore that forms during exocytosis of a mast cell secretory vesicle. Neuron **4,** 643–654.

Steer, M.W. (1988). The role of calcium in exocytosis and endocytosis in plant cells. Physiol. Plant. **72,** 213–220.

Steponkus, P.L. (1991). Behaviour of the plasma membrane during osmotic excursions. In Endocytosis, Exocytosis and Vesicle Traffic in Plants, C.R. Hawes, J.O.D. Coleman, and D.E. Evans, eds (Cambridge, UK: Cambridge University Press), pp. 103–128.

Stewart, A., and Mansfield, J.W. (1985). The composition of wall alterations and appositions (reaction material) and their role in the resistance of onion bulb scale epidermis to colonization by *Botrytis allii.* Plant Pathol. **34,** 25–37.

Steyer, J.A., Horstmann, H., and Almers, W. (1997). Transport, docking and exocytosis of single secretory granules in live chromaffin cells. Nature **388,** 474–478.

Südhof, T.C., and Rizo, J. (1996). Synaptotagmins: C_2-domain proteins that regulate membrane traffic. Neuron **17,** 379–388.

Swairjo, M.A., Roberts, M.F., Campos, M.-B., Dedman, J.R., and Seaton, B.A. (1994). Annexin V binding to the outer leaflet of small unilamellar vesicles leads to altered inner-leaflet properties: ^{31}P and ^{1}H-NMR studies. Biochemistry **33,** 10944–10950.

Takei, K., McPherson, P.S., Schmid, S., and Camilli, P.D. (1995). Tubular membrane invaginations coated by dynamin rings are induced by GTP-γS in nerve terminals. Nature **374,** 186–190.

Tanchak, M.A., Griffing, L.R., Mersey, B.G., and Fowke, L.C. (1984). Endocytosis of cationised ferritin by coated vesicles of soybean protoplasts. Planta **162,** 481–486.

Taylor, A.R., Manison, N.F.H., Fernandez, C., Wood, J.W., and Brownlee, C. (1996). Spatial organization of calcium signaling involved in cell volume control in the *Fucus* rhizoid. Plant Cell **8,** 2015–2031.

Taylor, L.P., and Hepler, P.K. (1997). Pollen germination and tube growth. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48,** 461–491.

Terras, F.R.G., Schoofs, H.M.E., De Bolle, M.F.C., Van Leuven, F., Rees, S.B., Vanderleyden, J., Cammue, B.P.A., and Broekaert, W.F. (1992). Analysis of two novel classes of plant antifungal proteins from radish (*Raphanus sativa* L.) seeds. J. Biol. Chem. **267,** 15301–15309.

Thiel, G., and Battey, N.H. (1998). Exocytosis in plants. Plant Mol. Biol. **38,** 111–125.

Thiel, G., Rupnik, M., and Zorec, R. (1994). Raising the cytosolic Ca^{2+} concentration increases the membrane capacitance of maize coleoptile protoplasts: Evidence for Ca^{2+}-stimulated exocytosis. Planta **195,** 305–308.

Thiel, G., Kreft, M., and Zorec, R. (1998). Unitary exocytotic and endocytotic events in *Zea mays* coleoptile protoplasts. Plant J. **13,** 101–104.

Ungermann, C., Nichols, B.J., Pelham, H.R.B., and Wickner, W.T. (1998). A vacuolar v–t-SNARE complex, the predominant form in vivo and on isolated vacuoles, is disassembled and activated for docking and fusion. J. Cell Biol. **140,** 61–69.

Van der Woude, W.J., Morré, D.J., and Bracker, C.E. (1971). Isolation and characterization of secretory vesicles in germinated pollen of *Lilium longiflorum.* J. Cell Sci. **8,** 331–351.

Vian, B., and Roland, J.-C. (1974). Cytochemical and ultrastructural observations on polysaccharides during secretion and exocytosis. Portulaca. Acta Biol. **14,** 475–482.

Vida, T.A., and Emr, S.D. (1995). A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. J. Cell Biol. **128,** 779–792.

Villanueva, M.A., Taylor, J., Sui, X., and Griffing, L.R. (1993). Endocytosis in plant protoplasts: Visualisation and quantitation of fluid-phase endocytosis using silver-enhanced bovine serum albumin-gold. J. Exp. Bot. **44,** 275–281.

Volkman, D. (1981). Structural differentiation of membranes involved in the secretion of polysaccharide slime by root cap cells of cress (*Lepidium sativum* L.). Planta **151,** 180–188.

Wacker, I., Kaether, C., Kröner, A., Migala, A., Almers, W., and Gerdes, H.-H. (1997). Microtubule-dependent transport of secretory vesicles visualized in real time with a GFP-tagged secretory protein. J. Cell Sci. **110,** 1453–1463.

Walent, J.H., Porter, B.W., and Martin, T.F.J. (1992). A novel 145-kD brain cytosolic protein reconstitutes Ca^{2+}-regulated secretion in permeable neuroendocrine cells. Cell **70,** 765–775.

Weber, T., Zemelman, B.V., McNew, J.A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T.H., and Rothman, J.E. (1998). SNAREpins: Minimal machinery for membrane fusion. Cell **92,** 759–772.

Weidenhaupt, M., Bruckert, F., and Satre, M. (1998). Identification of the *Dictyostelium discoideum* homolog of the *N*-ethylmaleimide–sensitive fusion protein. Gene **207,** 53–60.

White, J.M. (1996). Membrane fusion: The influenza paradigm. Cold Spring Harbor Symp. Quant. Biol. **60,** 581–588.

Whiteheart, S.W., Griff, I.C., Brunner, M., Clary, D.O., Mayer, T., Buhrow, S.A., and Rothman, J.E. (1993). SNAP family of NSF attachment proteins includes a brain-specific isoform. Nature **362,** 353–355.

Wilson, D.W., Wilcox, C.A., Flynn, G.C., Chen, E., Kuang, W.J., Henzel, W.J., Block, M.R., Ullrich, A., and Rothman, J.E. (1989). A fusion protein required for vesicle-mediated transport in both mammalian cells and yeast. Nature **339,** 355–359.

Winsor, B., and Schiebel, E. (1997). Review: An overview of the *Saccharomyces cerevisiae* microtubule and microfilament cytoskeleton. Yeast **13,** 399–434.

Wolfe, J., and Steponkus, P.L. (1983). Mechanical properties of the plasma membrane of isolated plant protoplasts. Plant Physiol. **71,** 276–285.

Zamski, E., Shoham, O., Palevitch, D., and Levy, A. (1987). Ultrastructure of capsaicinoid-secreting cells in pungent and nonpungent red pepper (*Capsicum annuum* L.) cultivars. Bot. Gaz. **148,** 1–6.

Zorec, R., and Tester, M. (1992). Cytoplasmic Ca^{2+} stimulates exocytosis in a plant secretory cell. Biophys. J. **63,** 864–867.

Zorec, R., and Tester, M. (1993). Rapid pressure driven exocytosis–endocytosis cycle in a single plant cell. FEBS Lett. **333,** 283–286.