

# Vesicle traffic in the endomembrane system: a tale of COPs, Rabs and SNAREs

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Recent years have seen remarkable progress in our understanding of the endomembrane system of plants. A large number of genes and proteins that are involved in membrane exchange between the different compartments of this system have been identified on the basis of their similarity to animal and yeast homologs. These proteins indicate that the endomembrane system in plants functions in essentially the same way as those in other eukaryotes. However, a growing number of examples demonstrate that the dynamic interplay between membrane-exchange proteins can be regulated differently in plant cells. Novel tools and a better understanding of the molecular effects of the inhibitor brefeldin A are helping to unravel these plant-specific adaptations.

## Addresses

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## Abbreviations

<b>BFA</b>	brefeldin A
<b>CCV</b>	clathrin-coated vesicles
<b>COPII</b>	coat protein II
<b>ER</b>	endoplasmic reticulum
<b>FRAP</b>	fluorescence recovery after photobleaching
<b>GEF</b>	guanine-nucleotide exchange factor
<b>GFP</b>	green fluorescent protein
<b>LV</b>	lytic vacuole
<b>PCR</b>	partially coated reticulum
<b>PIN1</b>	PIN-FORMED1
<b>PM</b>	plasma membrane
<b>PSV</b>	protein storage vacuole
<b>PVC</b>	pre-vacuolar compartment
<b>SNARE</b>	soluble <i>N</i> -ethylmaleimide sensitive factor adaptor protein receptor
<b>ST</b>	sialyltransferase
<b>TGN</b>	<i>trans</i> -Golgi network
<b>VTC</b>	vesiculo-tubular cluster

## Introduction

The endomembrane system of eukaryotic cells comprises the organelles of the secretory and endocytic pathways: the endoplasmic reticulum (ER), the Golgi apparatus, the *trans*-Golgi network (TGN), pre-vacuolar compartments (PVC), lytic compartments (vacuoles or lysosomes) and endosomes (Figure 1). Although this basic list of players are found in the endomembrane systems of all higher eukaryotes, a variety of specific adaptations are found only in certain lineages. For example, plants contain not only lytic vacuoles (LVs) but also protein storage vacuoles (PSVs) that perform radically different functions [1]. By

contrast, mammalian cells have an intermediate recycling compartment between the ER and the Golgi, the vesiculo-tubular cluster (VTC), that does not exist in plants.

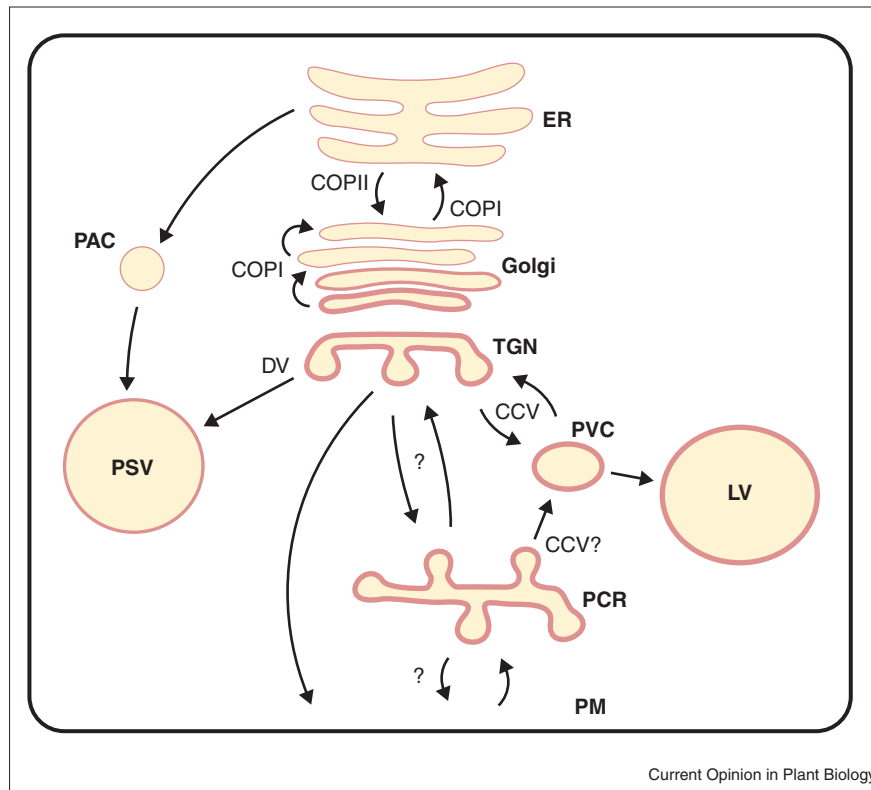
The transport of proteins and membranes between the different compartments of the endomembrane system is mediated by small carriers, the transport vesicles. The basic mechanisms involved in membrane-exchange reactions appear to be the same, irrespective of the organelles involved [2]. The initial step involves the formation of a proteinaceous membrane coat that plays a two-fold role. First, coat proteins deform the membrane so as to form a bud and eventually a vesicle, and second, they are involved in cargo selection. The assembly of the coat is controlled by a small GTPase of the Ras superfamily, which in turn is activated by a specific guanidine-nucleotide exchange factor (GEF). The fission of the transport vesicle from the membrane is probably catalyzed by a dynamin-like GTPase. Fusion of the vesicle with the target membrane depends on SNARE (soluble *N*-ethylmaleimide sensitive factor adaptor protein receptor) molecules [3•] in the vesicle (*v*-SNARE) and target (*t*-SNARE) membranes. The interaction of these proteins is regulated by a different class of small GTPases, belonging to the Rab/Ypt family, as well as by several other proteins such as Sec1.

This ‘generalized mechanism of vesicular transport’ is executed by different sets of specific proteins throughout the endomembrane system. Research in yeast and mammalian cells has identified both the protein families that are involved and, in many cases, the specific proteins that are responsible for individual reactions [4,5]. In recent years, plant cell biologists have made great strides in confirming the involvement of homologous proteins in the corresponding transport steps in their favorite organisms. This review highlights a few of the recent developments, with particular emphasis on plant-specific adaptations in pre-Golgi and post-Golgi membrane exchanges.

## Going round in circles: the ER-Golgi circuit

It is now well established that plants contain the same machinery for vesiculation at the ER and the Golgi as other eukaryotes. The proteins that mediate export from the ER are the COPII coats with their associated GTPase Sar1p and its GEF Sec12p, all of which are present in plants [6–8]. Interference with Sar1p activity has been shown to block anterograde ER-Golgi transport in plants [9–11]. The formation of retrograde transport vesicles at the Golgi depends on the activity of the COPI coat, which is recruited by the Arf1 GTPase after its activation by a Sec7-type GEF. COPI coats, as well as Arf1p, have been directly localized to the rim of Golgi cisternae by

Figure 1



The major organelles of the endomembrane system and the membrane exchange routes that connect them. Proteins are synthesized in the ER and travel through the Golgi to the TGN. There, they are sorted for transport to the PM, PVC or the PSV. The PVC is the antechamber of the LV and serves as a recycling compartment for sorting receptors. The PCR presumably corresponds to the endosomes of mammalian cells. The membrane coats that are involved in vesicle formation are indicated next to the arrows where known. Dense vesicles (DV) are not formed by coats but by aggregation of cargo molecules. An alternative route into the PSV for certain storage proteins leads directly from the ER via precursor accumulating vesicles (PAC). The relationship between the PCR/endosome and the other post-Golgi organelles is largely unknown. Anterograde transport through the Golgi and into the TGN does not involve vesicles but is mediated by cisternal maturation.

immunocytochemistry with both light and electron microscopy [12,13••]. Interestingly, Arf1-like epitopes are also present at the plasma membrane of maize root cells [14]. Furthermore, the recruitment of COPI coats to Golgi membranes from cauliflower and tobacco could be reconstituted *in vitro* [12], an important prerequisite for detailed biochemical studies of vesicle formation and cargo selection.

The components that are involved in vesicle fusion at the ER and Golgi in mammals and yeast also appear to operate in similar fashion in plants. Although the SNARE proteins in this part of the endomembrane system have not been characterized in detail, the involvement of AtRab1b and NtRab2 proteins in ER-Golgi transport has been shown using dominant negative mutants [15,16]. It is currently not clear which transport step(s) are regulated by these Rab proteins. NtRab1b-GFP (for green fluorescent protein) fusion proteins are localized to the ER and the Golgi in leaf epidermal cells, whereas Rab2-GFP fusions are found on the Golgi in pollen tubes [16]. NtRab2 expression is limited to rapidly growing tissues, suggesting that it plays an accessory role in cells that have very active secretory systems [16].

From these studies, it is clear that the basic machinery for the production of vesicles at the ER-Golgi interface is the same in plants as in other eukaryotes. This is also illustrated by the early responses to the fungal toxin brefeldin A (BFA). BFA inhibits the activation of Arf1 by a subset of

Sec7-type GEFs [17], and thereby blocks the formation of COPI coats and retrograde traffic within the Golgi and back to the ER. Recently, Ritzenthaler *et al.* [13••] showed that this is also the case in tobacco Bright Yellow-2 (BY-2) cells, thus reinforcing the basic similarity between all eukaryotes. Interestingly, this identical primary BFA effect is followed in plants by secondary and tertiary consequences that only partially mimic the responses in mammalian cells. The mammalian Golgi quickly loses its structural integrity in response to BFA [18], forming elongated tubules that stretch along microtubules into the cell periphery [19]. By contrast, plant Golgi stacks initially maintain their structural identity after treatment with BFA, except for an apparent loss of *cis* cisternae [13••]. Eventually, Golgi membranes fuse with the ER in both mammalian and plant cells, resulting in the formation of an ER-Golgi hybrid compartment, but again, the morphological manifestation in plants is unique. In plant cells, the merger is more gradual than that in the cells of other eukaryotes, it does not depend on cytoskeletal elements [20] and, at least in cells with prominent intercisternal elements, it proceeds via unusual ER-Golgi hybrid stacks (Figure 2; [13••]). These stacks form by the fusion of individual Golgi cisternae with the ER, the intercisternal elements seem to prevent the new hybrid cisterna from drifting away. Thus, the ER-Golgi hybrid stacks are probably a reflection of the high structural stability of plant Golgi stacks, which is necessitated by their rapid stop-and-go movements [21]. It will be

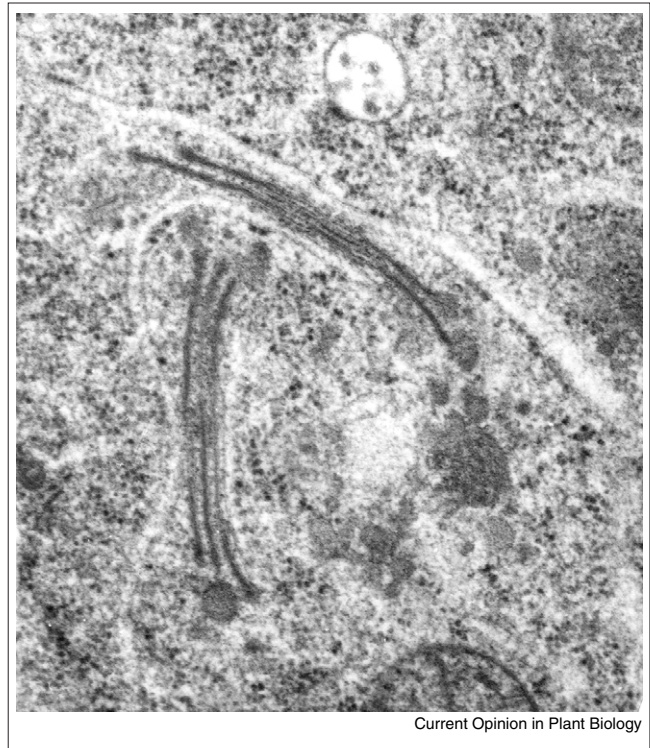
interesting to see whether this enhanced structural stability in plants is the result of novel proteins or whether it is simply brought about by the greater expression of common Golgi-matrix proteins.

Despite the high level of structural integrity of plant Golgi stacks, it is clear that there is a constant anterograde flux of secretory products through their cisternae that has to be counterbalanced by a similar retrograde flux of Golgi-resident enzymes [22,23]. This recycling of Golgi proteins can reach as far back as the ER, and has been demonstrated in elegant experiments using fluorescence recovery after photobleaching (FRAP) [24<sup>•</sup>]. To observe FRAP, a small region of a cell is selectively bleached with high laser power. The loss of fluorescence of the reporter molecules in the affected area is irreversible, so any recovery of fluorescence has to be the result of new molecules entering the region. In the case of the Golgi, the most likely source of such molecules is the ER. Using this approach, Brandizzi *et al.* [24<sup>•</sup>] discovered that the Golgi-localized fluorescence of *Arabidopsis thaliana* ER-RETENTION DEFECTIVE2 (AtERD2)-GFP and rat sialyltransferase (ST)-GFP fusions can recover pre-bleach values within just five minutes. On the basis of data from mammalian cells, we can assume that this influx of new Golgi proteins is balanced by a recycling of old, bleached proteins back to the ER. These FRAP experiments therefore demonstrate a basic similarity between the Golgi of plants and mammals, namely that Golgi proteins cycle between their predominant localization in the Golgi and a temporary presence in the ER.

At the same time, however, these studies demonstrate a difference between the endomembrane systems of the two groups. In plants, unlike in mammals, the FRAP effect was observed in both the presence and the absence of microtubules [24<sup>•</sup>]. In fact, no cytoskeletal elements are needed for the replacement of Golgi proteins in plants as all of the experiments were conducted in the presence of latrunculin B to prevent rapid Golgi movements [24<sup>•</sup>]. It is possible that the rate of fluorescence recovery would be different in the presence of actin filaments, but active Golgi movement is not strictly required for ER-Golgi transport. This conclusion plays directly into the current debate on how efficient ER-Golgi transport is possible in a system with such a high degree of mobility [21]. Interestingly, the new data suggest yet another interpretation, namely that Golgi stacks and ER export sites travel together through the cytoplasm as functional units, and therefore are closely apposed when actin-based movements are disrupted [24<sup>•</sup>]. Clearly, the answer to the question of how vesicles that are exported from the ER reach the Golgi hasn't yet been found. It probably has to await a better characterization of the sites of vesicle formation at the ER.

The FRAP experiments have also resulted in two curious, unexpected observations. The first was that two markers with different, albeit overlapping, distribution in Golgi

**Figure 2**



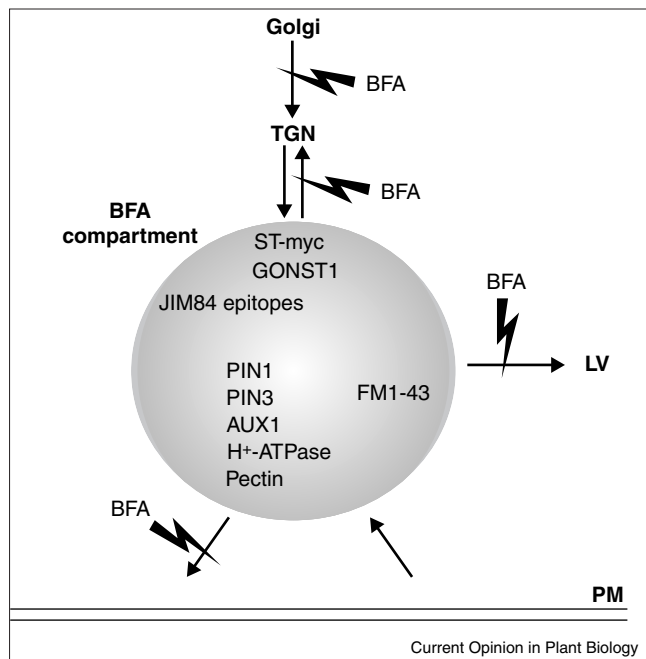
ER-Golgi stacks in a BFA-treated Bright Yellow-2 (BY-2) cell.

stacks had identical FRAP rates [24<sup>•</sup>]. The AtERD2-GFP marker would have been predicted to recycle more quickly than ST-GFP because of its localization in the *cis* Golgi and its role in returning escaped ER residents. It remains to be seen whether this apparently indiscriminate recycling rate also holds true for endogenous proteins, or whether it results from the use of heterologous markers. The second unexpected observation was that BFA prevented ER-Golgi transport in about half of the Golgi stacks tested [24<sup>•</sup>]. The known BFA target resides on the Golgi and is involved in retrograde transport (see above), so any effect on anterograde transport is likely an indirect one. It has been proposed that this indirect effect acts by blocking COPII-mediated vesicle formation at the ER [24<sup>•</sup>]. Alternatively, it is conceivable that the inhibition occurs at the level of the Golgi, where BFA treatment may prevent the integration of transport vesicles into the maturing cisternae [25]. The FRAP experiments, by nature of their design, cannot distinguish between these possibilities and we will have to find other ways of addressing this problem, as well as the question of why only half of the stacks were affected by BFA.

### Grand central: the TGN-PVC-PCR sorting station

The situation on the far end of the Golgi is more complex than that on its *cis* side as it involves a branching of the anterograde pathway to the plasma membrane (PM) and the vacuoles, as well as the integration of the endocytic pathway. This complexity is illustrated by the plethora of syntaxin-like SNARE proteins that are found on the TGN

Figure 3



Hypothetical sites of BFA action in post-Golgi compartments. Many molecules that originate in either the (TGN) or the PM seem to accumulate in the same perinuclear aggregate, the BFA compartment (gray area). This accumulation suggests that export from the BFA compartment towards the PM, the TGN and the LV is sensitive to BFA. Input into the BFA compartment from the ER. AUX1, AUXIN INSENSITIVE 1; GONST1, GOLGI NUCLEOTIDE SUGAR TRANSPORTER1 [37]; ST-myc, rat sialyltransferase with myc epitope tag [38].

and the PVC. Four different syntaxin proteins (SYP41, SYP42, SYP51, and SYP61) have been localized to the TGN of *Arabidopsis* root cells by immuno-electronmicroscopy, and four (SYP21, SYP22, SYP51, and SYP61) have been detected on the PVC [26,27\*,28\*]. It is not likely that the high density of syntaxins on these organelles reflects redundancy as knockouts of either of the SYP4x or SYP2x genes are gametophytic lethals [29]. In addition, these syntaxins interact only with certain other members of the SNARE family, as demonstrated by pull-down assays [27\*,28\*]. The related syntaxins SYP41 and SYP42 are sorted into different sub-domains of the TGN [27\*]. All of these findings demonstrate a high degree of differentiation of the post-Golgi compartments, and the large number of specific membrane-recognition events that are possible.

Another indication that the post-Golgi and endocytic compartments are interconnected by a complex set of membrane-exchange reactions is provided by the effects of BFA on secreted and endocytosed proteins. Early work with this drug has established that glycoproteins that are secreted from the Golgi, as detected by the JIM84 antibody, accumulate in large perinuclear clusters in BFA-treated plant cells [30]. These highly vesiculated membrane aggregations have been termed BFA compartments, and

are thought to be remnants of the disintegrated Golgi apparatus [30]. Interestingly, recent work with plasma membrane proteins suggests that endocytic vesicles may accumulate in the same structures. Treatment of *Arabidopsis* seedlings with BFA leads to a mislocalization of the putative auxin-efflux carrier PIN-FORMED1 (PIN1) in root cells: the PIN1 signal at the PM is reduced and large perinuclear aggregates appear instead [31\*\*]. Similar results were obtained with the related protein PIN3 [32\*], the PM H<sup>+</sup>-ATPase [14,31\*\*], and the putative auxin-influx carrier AUXIN-INSENSITIVE1 (AUX1) [33\*]. Curiously, meristematic cells in the root tip of maize also accumulated a cell-wall-modified pectin in similar perinuclear aggregates [14]. The size, distribution, and dependence on drug treatment of these aggregates suggest that these are so-called BFA compartments. The new data on PM proteins thus suggest that the endocytic pathway also feeds into BFA compartments, although it has to be cautioned that double labeling with a *bona fide* BFA compartment marker, such as JIM84, has not been carried out so far.

At face value, this conclusion seems to indicate that recycled PM proteins and secretory cargo (JIM84) travel to the cell surface in the same carrier, or at least via similar mechanisms that both can be inhibited by BFA. However, our knowledge of how BFA achieves this effect is so far only conjecture (Figure 3). Export from the TGN in clathrin-coated vesicles (CCV), which is BFA-sensitive in animal cells, has only been shown for vacuolar proteins. It is not clear how BFA treatment could affect transport to the PM in plants as no membrane coat is known to be involved in this step. Furthermore, a recent study that demonstrated the importance of the dynamin-related protein AtADL6 in transport from the TGN to the PVC also illustrated that a block of the vacuolar branch does not interfere with traffic to the PM [34]. Similar uncertainty exists in the endocytotic pathway. Delivery of the FM1-43 tracer of endocytosis to the central vacuole is sensitive to BFA [35], but it is not clear at which stage of the transport the BFA block occurs. One candidate organelle for this block is the partially coated reticulum (PCR), which is thought to represent endosomes in plants [36]. Thus, the relationship between the endocytic, secretory and vacuolar pathways in plants is still unclear. If the large number of syntaxins at the TGN and the PVC are any indication, we can expect that many membrane exchange reactions await discovery.

## Conclusions

Although the bulk of the recent developments confirms the paradigm of membrane exchange that has been developed in mammalian and yeast cells, there is also a growing awareness of unique adaptations in plants. This reflects a trend away from 'inventory' studies, which catalogue proteins that are involved in membrane transport, towards functional studies that aim to elucidate the roles of individual proteins in the mechanisms of membrane exchange. This trend will likely accelerate in the future as more proteins are known and novel markers for the different compartments

are developed. The availability of the full genomic sequence of *Arabidopsis*, and soon rice, as well a range of molecular tools to interfere with individual genes and proteins will support this process significantly. We can expect to see rapid progress in our understanding of the endocytic pathway and its integration with secretion. The 'GFP revolution' will continue to provide important new insights to the dynamic interplay of the different transport steps as new experimental approaches are developed. It is hoped that the number of question marks in the next version of Figure 1 will be greatly decreased.

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## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Vitale A, Raikhel NV: **What do proteins need to reach different vacuoles?** *Trends Plant Sci* 1999, 4:149-155.
  2. Robinson DG, Hinz G, Holstein SEH: **The molecular characterization of transport vesicles.** *Plant Mol Biol* 1998, 38:47-76.
  3. Sanderfoot AA, Assaad FF, Raikhel NV: **The *Arabidopsis* genome. • An abundance of soluble N-ethylmaleimide-sensitive factor adaptor protein receptors.** *Plant Physiol* 2000, 124:1558-1569.
- The authors present not only an exhaustive search of the *Arabidopsis* genome for SNARE proteins but also a good primer on membrane fusion events. In addition, they revise the nomenclature of plant syntaxins, which should help to reduce the confusion caused by borrowing names from yeast or mammals.
4. Béraud-Dufour S, Balch W: **Cell science at a glance: a journey through the exocytic pathway.** *J Cell Sci* 2002, 115:1779-1780.
  5. Sorkin A: **Cell science at a glance: the endocytosis machinery.** *J Cell Sci* 2000, 113:4375-4376.
  6. Bar-Peled M, Raikhel NV: **Characterization of AtSEC12 and AtSAR1. Proteins likely involved in endoplasmic reticulum and Golgi transport.** *Plant Physiol* 1997, 114:315-324.
  7. Andreeva AV, Kutuzov MA, Evans DE, Hawes CR: **Proteins involved in membrane transport between the ER and the Golgi apparatus: 21 putative plant homologues revealed by dbEST searching.** *Cell Biol Int* 1998, 22:145-160.
  8. Movafeghi A, Happel N, Pimpl P, Tai G-H, Robinson DG: ***Arabidopsis* Sec21p and Sec23p homologs. Probable coat proteins of plant COP-coated vesicles.** *Plant Physiol* 1999, 119:1437-1445.
  9. Andreeva AV, Zheng H, Saint-Jore CM, Kutuzov MA, Evans DE, Hawes CR: **Organization of transport from endoplasmic reticulum to Golgi in higher plants.** *Biochem Soc Trans* 2000, 28:505-512.
  10. Takeuchi M, Ueda T, Sato K, Abe H, Nagata T, Nakano A: **A dominant negative mutant of Sar1 GTPase inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus in tobacco and *Arabidopsis* cultured cells.** *Plant J* 2000, 23:517-525.
  11. Phillipson BA, Pimpl P, Pinto daSilva LL, Crofts AJ, Taylor JP, Movafeghi A, Robinson DG, Denecke J: **Secretory bulk flow of soluble proteins is efficient and COPII dependent.** *Plant Cell* 2001, 13:2005-2020.
  12. Pimpl P, Movafeghi A, Coughlan S, Denecke J, Hillmer S, Robinson DG: **In situ localization and in vitro induction of plant COPI-coated vesicles.** *Plant Cell* 2000, 12:2219-2236.
  13. Ritzenthaler C, Nebenführ A, Movafeghi A, Stussi-Garaud C, Behnia L, •• Pimpl P, Staehelin LA, Robinson DG: **Reevaluation of the effects of brefeldin A on plant cells using tobacco bright yellow 2 cells expressing Golgi-targeted green fluorescent protein and COPI antisera.** *Plant Cell* 2002, 14:237-261.
- A combination of approaches is used to characterize BFA responses in detail. These confirm that the primary response to BFA in plants is a loss of COPI from the Golgi. The subsequent fusion of the Golgi with the ER occurs via unusual ER-Golgi hybrid stacks that are unique to plants.
14. Baluska F, Hlavacka A, Samaj J, Palme K, Robinson DG, Match T, McCurdy DW, Menzel D, Volkmann D: **F-actin-dependent endocytosis of cell wall pectins in meristematic root cells: insights from brefeldin A-induced compartments.** *Plant Physiol* 2002, 130:422-431.
  15. Batoko H, Zheng H-Q, Hawes C, Moore I: **A Rab1 GTPase is required for transport between the endoplasmic reticulum and Golgi apparatus and for normal Golgi movement in plants.** *Plant Cell* 2000, 12:2201-2218.
  16. Cheung AY, Chen CY-h, Glaven RH, de Graaf BHJ, Vidali L, Heplaer PK, Wu H-m: **Rab2 GTPase regulates vesicle trafficking between the endoplasmic reticulum and the Golgi bodies and is important for pollen tube growth.** *Plant Cell* 2002, 14:945-962.
  17. Jackson CL, Casanova JE: **Turning on ARF: the Sec7 family of guanine-nucleotide exchange factors.** *Trends Cell Biol* 2000, 10:60-67.
  18. Hess MW, Müller M, Debbage PL, Vetterlein M, Pavelka M: **Cryopreparation provides new insight into the effect of brefeldin A on the structure of the HepG2 Golgi apparatus.** *J Struct Biol* 2000, 130:63-72.
  19. Sciaky N, Presley J, Smith C, Zaal KJM, Cole N, Moreira JE, Terasaki M, Siggia E, Lippincott-Schwartz J: **Golgi tubule traffic and the effects of brefeldin A visualized in living cells.** *J Cell Biol* 1997, 139:1137-1155.
  20. Saint-Jore CM, Evins J, Batoko H, Brandizzi F, Moore I, Hawes C: **Redistribution of membrane proteins between the Golgi apparatus and endoplasmic reticulum in plants is reversible and not dependent on cytoskeletal networks.** *Plant J* 2002, 29:661-678.
  21. Nebenführ A, Staehelin LA: **Mobile factories: Golgi dynamics in plant cells.** *Trends Plant Sci* 2001, 6:160-167.
  22. Pelham HRB: **Traffic through the Golgi apparatus.** *J Cell Biol* 2001, 155:1099-1101.
  23. Nebenführ A: **Intra-Golgi transport: escalator or bucket brigade?** *Annu Plant Rev* 2002, in press.
  24. Brandizzi F, Snapp E, Roberts A, Lippincott-Schwartz J, Hawes C: • **Membrane protein transport between the ER and Golgi in tobacco leaves is energy dependent but cytoskeleton independent: evidence from selective photobleaching.** *Plant Cell* 2002, 14:1293-1309.
- This is the first study to use FRAP to elucidate membrane dynamics in the plant endomembrane system. It demonstrates the continuous cycling of Golgi residents between the Golgi and the ER. The authors also discover that this recycling in plant cells, in contrast to that in animal cells, is not dependent on cytoskeletal elements.
25. Nebenführ A, Ritzenthaler C, Robinson DG: **Update on Brefeldin A: deciphering an enigmatic inhibitor of secretion.** *Plant Physiol* 2002, 130:in press.
  26. Sanderfoot AA, Kovaleva V, Zheng H, Raikhel NV: **The t-SNARE AtVAM3p resides on the prevacuolar compartment in *Arabidopsis* root cells.** *Plant Physiol* 1999, 121:929-938.
  27. Bassham DC, Sanderfoot AA, Kovaleva V, Zheng H, Raikhel NV: • **AtVPS45 complex formation at the trans-Golgi network.** *Mol Biol Cell* 2000, 11:2251-2265.
- This study combines thorough biochemical and cell-biological approaches to characterize SNAREs and related proteins at the TGN and PVC. Immunogold double-labeling studies and pull-down assays demonstrate the distribution of the proteins on the compartments and, at the same time, suggest possible ways in which they can interact to carry out their functions.
28. Sanderfoot AA, Kovaleva V, Bassham DC, Raikhel NV: **Interactions • between syntaxins identify at least five SNARE complexes within the Golgi/prevacuolar system of the *Arabidopsis* cell.** *Mol Biol Cell* 2001, 12:3733-3743.
- Similarly to [27], this study reveals a variety of interactions of SNARE molecules at the TGN and PVC, and highlights the large number of different interactions that are possible.
29. Sanderfoot AA, Pilgrim M, Adam L, Raikhel NV: **Disruption of individual members of *Arabidopsis* syntaxin gene families indicates each has essential function.** *Plant Cell* 2001, 13:659-666.
  30. Satiat-Jeunemaitre B, Cole L, Bouret T, Howard R, Hawes C: **Brefeldin A effects in plant and fungal cells: something new about vesicle trafficking?** *J Microsc* 1996, 181:162-177.

31. Geldner N, Friml J, Stierhof Y-D, Jürgens G, Palme K: **Auxin transport inhibitors block PIN1 cycling and vesicle trafficking.** *Nature* 2001, **413**:425-428.

See annotation for [33\*].

32. Friml J, Wisniewska J, Benkova E, Mengden K, Palme K: **Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*.** *Nature* 2002, **415**:806-809.

See annotation for [33\*].

33. Grebe M, Friml J, Swarup R, Ljung K, Sandberg G, Terlou M, Palme K, Bennett MJ, Scheres B: **Cell polarity signaling in *Arabidopsis* involves a BFA-sensitive auxin influx pathway.** *Curr Biol* 2002, **12**:329-334.

The authors of these three papers [31\*\*,32\*,33\*] demonstrate that the polarized distribution of PM proteins in plants is probably maintained by continuous endocytosis and re-targeting to the cell surface. This dynamic view is a dramatic departure from the old conjecture that endocytosis is impossible in cells that have high turgor pressure.

34. Jin JB, Kim YA, Kim SJ, Lee SH, Kim DH, Cheong G-W, Hwang I: **A new dynamin-like protein, ADL6, is involved in trafficking from the trans-Golgi network to the central vacuole in *Arabidopsis*.** *Plant Cell* 2001, **13**:1511-1525.
35. Emans N, Zimmermann S, Fischer R: **Uptake of a fluorescent marker in plant cells is sensitive to brefeldin A and wortmannin.** *Plant Cell* 2002, **14**:71-86.
36. Staehelin LA, Moore I: **The plant Golgi apparatus: structure, functional organization and trafficking mechanisms.** *Annu Rev Plant Physiol Plant Mol Biol* 1995, **46**:261-288.
37. Baldwin TC, Handford MG, Yuseff M-I, Orellana A, Dupree P: **Identification and characterization of GONST1, a Golgi-localized GDP-mannose transporter in *Arabidopsis*.** *Plant Cell* 2001, **13**:2283-2295.
38. Wee EG-T, Sherrier DJ, Prime TA, Dupree P: **Targeting of active sialyltransferase to the plant Golgi apparatus.** *Plant Cell* 1998, **10**:1759-1768.