Brefeldin A: Deciphering an Enigmatic Inhibitor of Secretion

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The fungal macrocyclic lactone brefeldin A (BFA) has proved to be of great value as an inhibitor of protein trafficking in the endomembrane system of mammalian cells (Sciaky et al., 1997). BFA has also often been used as an inhibitor of secretion and vacuolar protein transport in plant cells, but just exactly how BFA achieves these effects has been a matter of debate for some time (e.g. Satiat-Jeunemaitre et al., 1996; Staehelin and Driouich, 1997). The apparently broad spectrum of BFA responses, combined with a lack of understanding of the primary target of BFA, has made it difficult to develop a coherent explanation of BFA effects, which in turn has led to a series of misconceptions that riddle the plant literature. In the last few years, there has been a tremendous increase in our understanding of the molecular targets and primary effects of BFA in mammalian and yeast systems (see below). On the plant side, several papers have recently been published that, by applying new tools and technologies, have shed fresh light onto the BFA problem (Baluska et al., 2002; Brandizzi et al., 2002; Emans et al., 2002; Ritzenthaler et al., 2002; Saint-Jore et al., 2002). In this *Update*, we wish to evaluate these new findings and explore whether we are any closer to solving the dilemma of "what BFA really does."

WHAT IS THE PRIMARY TARGET OF BFA AND DOES THIS TARGET EXIST IN PLANT CELLS?

It is now well established that the target of BFA in mammalian cells is a subset of Sec7-type GTP-exchange factors (GEFs) that catalyze the activation of a small GTPase called Arf1p (Jackson and Casanova, 2000). Arf1, in turn, is responsible for the recruitment of coat proteins (coatomer, also called COPI; as well as clathrin via the adaptor complex AP-1) to membranes, resulting in the formation of transport vesicles (Scales et al., 2000). Arf1p and BFA-sensitive GEFs are localized to the Golgi apparatus of mammalian and yeast cells (Spang et al., 2001). Thus, one of the earliest and best characterized effects of BFA in nonplant organisms is the loss of COPI coats from the Golgi apparatus (Kreis et al., 1995).

In recent years, it has become apparent that the molecular targets for BFA also exist in plant cells. The Arabidopsis gene *GNOM* encodes an Arf GEF that falls into the subgroup of BFA-sensitive Sec7-like proteins (Steinmann et al., 1999). Although the intracellular localization of GNOM is not yet known, most data are consistent with its Golgi localization, in accordance with other members of the Gea/GNOM/GBF subfamily (Jackson and Casanova, 2000). Plants also possess Arf1p, coatomer, AP-1, and clathrin, all of which also localize to the Golgi apparatus (Pimpl et al., 2000), suggesting that the machinery that mediates vesicle transport in the endomembrane system is basically the same in all eukaryotes (Nebenführ and Staehelin, 2001). Moreover, the expression of a dominant-negative mutant form of Arf1p inhibits protein transport to and through the Golgi apparatus of Arabidopsis protoplasts in a BFA-like manner (Lee et al., 2002). Thus, it is reasonable to assume that BFA has similar primary effects in plants as in animals.

This prediction has been borne out by a recent study in which the effects of BFA on the intracellular localization of COPI coat proteins in tobacco (*Nicotiana tabacum*) bright yellow-2 (BY-2) suspension-cultured cells were examined (Ritzenthaler et al., 2002). In untreated cells, about one-half of the protein detected by an antibody raised against the γ subunit of Arabidopsis COPI (Movafeghi et al., 1999) was associated with Golgi stacks. This situation changed rapidly after BFA addition, and after only 5 min, essentially no anti-Atγ-COPI labeling was detected on the Golgi (Ritzenthaler et al., 2002). Thus, a rapid release of COPI coats from the Golgi into the cytosol in response to BFA is a universal feature of all eukaryotic cells, and suggests that the molecular target and mode of action of BFA is highly conserved. In other words, all BFA effects in plants most likely are consequences of an initial inhibition of Arf1p and its BFA-sensitive GEF.

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DOES BFA INDUCE/PROMOTE RETROGRADE GOLGI→ENDOPLASMIC RETICULUM (ER) TRANSPORT IN PLANTS?

As documented extensively for mammalian cells, a striking effect of BFA is the complete redistribution of Golgi enzymes into the ER (e.g. Sciaky et al., 1997). Recent work with different sets of Golgi markers has firmly established that this BFA response also occurs in plants (Boevink et al., 1998; Lee et al., 2002; Ritzenthaler et al., 2002; Saint-Jore et al., 2002). In these studies, green fluorescent protein (GFP)-tagged Golgi enzymes have been used to examine the fate of this organelle in living cells. This has allowed researchers to follow the fate of Golgi stacks in real time and show convincingly the redistribution of Golgi proteins into the ER network (for example, see the following video clips: http://www.brookes.ac.uk/schools/bms/research/molcell/hawes/BFAonGolgi.avi and http://web.utk.edu/~nebenfur/pubs/BFA-video.mov).

This phenomenon suggests, at face value, a stimulation of retrograde transport from the Golgi to the ER. However, it is well established that COPI-coated vesicles are involved in the normal recycling of escaped ER residents back from the Golgi (Letourneur et al., 1994). Therefore, BFA-inhibition of COPI recruitment should actually prevent Golgi→ER transport, rather than stimulate it. This apparent contradiction can be resolved by focusing on other proteins involved in Golgi→ER transport, the so-called tethering factors and SNARE complexes (Shorter et al., 2002). Whereas tethering involves a variety of peripheral membrane proteins and the activity of Rab-GTPases, fusion is dependent upon the interaction of a v-SNARE on the vesicle with its cognate t-SNARE on the target membrane (Rein et al., 2002). In vivo and in vitro data point strongly to SNAREs being concentrated in COPI-coated vesicles at the time of budding (Hay et al., 1998). However, when COPI vesicle formation is blocked by BFA, the population of v-SNAREs in the Golgi membranes presumably will rise temporarily, thereby increasing the chances of direct and uncontrolled fusion between neighboring Golgi and ER membranes (Elazar et al., 1994). Thus, we postulate that in plant as in mammalian cells, BFA leads to a breakdown of the physical separation of ER and Golgi compartments by allowing membrane fusion to occur in the absence of prior vesicle formation and not by a stimulation of the normal retrograde vesicular transport pathway.

It is important to realize that these are two different mechanisms, and part of the confusion stems from the use of the term "retrograde transport" for both processes. BFA-induced fusion is a pathological event that occurs only after prior dissociation of COPI from the Golgi (Scheel et al., 1997), and culminates in the physical continuity and mixing of the ER and the Golgi apparatus (Fig. 1).

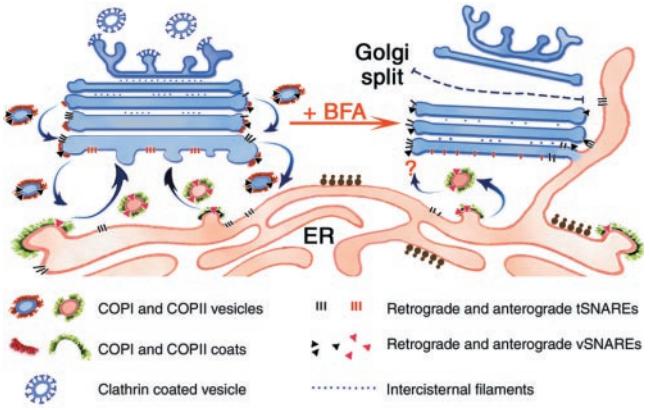

Figure 1. Effects of BFA on membrane trafficking between ER and Golgi. In untreated cells (left), export from the ER (pink) is mediated by COPII-coated vesicles (green). Fusion of these anterograde vesicles depends on anterograde v-SNAREs on the vesicles (red triangles) and t-SNAREs on the cis Golgi (red lines). Anterograde transport (at least to the PVC) continues on the trans side of the Golgi with clathrin-coated vesicles (crossed blue lines). Retrograde transport within the Golgi and from the Golgi to the ER depends on COPI coats (red) and retrograde v- and t-SNAREs (black triangles and lines). In BFA-treated cells (right), all vesiculation at the Golgi stops due to the inhibition of Arf1. As a result, retrograde v-SNAREs remain exposed on the Golgi and the cisternae fuse directly with the ER. Cisternal maturation continues in the presence of BFA so that early Golgi compartments assume a more trans-like morphology. At the same time, the later cisternae and the TGN are lost to the cytoplasm and, eventually, to the BFA compartment. COPII vesicle formation at the ER is initially not inhibited by BFA, but anterograde vesicles may no longer be able to fuse with the maturing cis cisterna, thus effectively blocking ER-to-Golgi transport.

It is interesting that the events lying between the BFA-induced loss of COPI coats from the Golgi on the one hand, and fusion of Golgi membranes with the ER on the other hand, are strikingly different between mammalian and plant cells. These distinct, secondary responses most likely reflect the different organization of the Golgi apparatus in the two organismal groups. In mammalian cells, the loss of COPI coats is followed by extensive formation of membrane tubules along microtubules (Sciaky et al., 1997; Hess et al., 2000), which eventually fuse with the ER. In contrast, BFA does not lead to extensive tubulation of the plant Golgi apparatus. Instead, at least in tobacco BY-2 cells, Golgi stacks initially maintain their morphology except for an apparent loss of cis cisternae (Ritzenthaler et al., 2002; see also below). The remaining Golgi cisternae eventually fuse with the ER, forming hybrid ER-Golgi stacks, or in some cases, they fuse laterally with other Golgi cisternae, resulting in oversized Golgi complexes that are continuous with the ER (Ritzenthaler et al., 2002). These unusual hybrid structures display ER and Golgi characteristics, are unique to plants, and presumably result from a heightened coherence of the plant Golgi cisternae that is not found in their mammalian counterparts.

IF THE GOLGI FUSES WITH THE ER, THEN WHAT IS THE BFA COMPARTMENT?

The recent description of a BFA-induced ER-Golgi hybrid compartment in some plant cells (see above) is apparently at odds with the older literature that described a disintegration of the Golgi apparatus into a conglomerate of tubules and vesicles termed the "BFA compartment" (Satiat-Jeunemaitre et al., 1996). It is possible that these two responses are tissue (or even species) specific, but it is more likely that the apparently different fates of the Golgi membranes in these cell types are simply a question of which markers were monitored. In particular, those markers demonstrating Golgi-ER fusion all reside in earlier parts of the Golgi: Gm-Man1-GFP is limited to the cis Golgi (Nebenführ et al., 1999), and AtERD2-GFP has been shown to be distributed over the entire Golgi stack (Boevink et al., 1998). In contrast, JIM84 antibodies that recognize the Lewis-a epitope, a complex oligosaccharide that is added to glycoproteins very late in the Golgi (Fitchette et al., 1999), do not label the ER in BFA-treated cells (Henderson et al., 1994).

It is interesting that two sialyltransferase-based markers seem to give contradictory results. The sialyltransferase (ST)-based GFP marker, which consists of a GFP fused to the transmembrane domain of rat sialyltransferase, has been used to demonstrate Golgi-ER fusion in tobacco leaf epidermal and BY-2 cells (Boevink et al., 1998; Saint-Jore et al., 2002). The ST-myc version, on the other hand, where the small myc epitope had been fused to the full-length enzyme, accumulates in BFA compartments in Arabidopsis callus (Wee et al., 1998). This apparent discrepancy might be explained by the slightly different distribution of these heterologous proteins in plant Golgi stacks. The ST-GFP protein is broadly distributed over the trans one-half of the stack (Boevink et al., 1998), whereas the ST-myc protein is found only in the trans-most cisterna and the trans-Golgi network (TGN) (Wee et al., 1998). In support of this, we point to the recent data of Jin et al. (2001) who have demonstrated that the Golgi-associated dynamin homolog ADL6, a protein probably involved in fission of clathrin-coated vesicles at the TGN, colocalizes exactly with the JIM 84 epitope, but only partially overlaps with ST-RFP (red fluorescent protein)-labeled Golgi stacks. In Arabidopsis root tips, ADL6 accumulates in BFA compartments, as would be expected from this distribution. We would also expect the GDP-Man transporter GONST1 from Arabidopsis to reside in the trans-most Golgi cisterna or the TGN because it is also present in BFA compartments of onion (*Allium cepa*) epidermal cells (Baldwin et al., 2001).

The above analysis of cisternal markers in plants reflects very much the situation in mammalian cells where TGN residents, e.g. ST and TGN38, have been shown not to redistribute to the ER upon BFA treatment (Chege and Pfeffer, 1990; Ladinsky and Howell, 1992). Instead, the remaining TGN membranes fuse/aggregate with elements of the endocytic pathway (Wood et al., 1991; Reaves and Banting, 1992). Taken together, these examples from the plant and animal literature strongly suggest that in response to BFA, the Golgi apparatus essentially splits horizontally, with most cisternae being absorbed into the ER, whereas the TGN, and possibly also the trans-most Golgi cisterna, contribute to the BFA compartment. This means that BFA compartments as well as ER-Golgi hybrids are always formed as a result of BFA treatment. Because Golgi-ER fusion is dependent upon SNARE-SNARE interactions, it also means that only those cisternae having the appropriate SNAREs will be fusion competent. As mentioned above, these SNAREs are normally concentrated into COPI vesicles, so one might speculate that the position of the "Golgi split" is indicated by cisternae no longer engaged in COPI vesicle budding (Fig. 1). In animal cells, "nonclathrin"-type vesicles have been shown to bud off every cisterna except for the last plus the TGN, where clathrin coats are found exclusively (Ladinsky et al., 1999). It is tempting to speculate that the difference between these two cisternae marks the "point of no return": Up to there, things can be recycled via COPI vesicles back to younger cisternae (and eventually the ER), and after this, it is only a matter of sorting into clathrin or secretory vesicles and delivering. As a consequence, this would also be the point where the Golgi is split during BFA treatment. It should be possible to test this hypothesis in plants by following the action of BFA on cells coexpressing Golgi-targeted GFP variants that reside at different levels in the stack.

The dual effect of BFA to create BFA compartments and ER-Golgi hybrids in the same plant cell has actually been described in a few instances. For example, the disruption of the ER structure, which in this case followed the formation of BFA compartments, has been revealed by double labeling maize (*Zea mays*) root cells for ER proteins and JIM84 epitopes (Henderson et al., 1994). In a similar manner, a subset of BY-2 cells formed clear BFA compartments in addition to the typical ER-Golgi hybrid (Ritzenthaler et al., 2002). Our new interpretation of BFA responses raises the question why the simultaneous formation of both novel compartments has not been described more often, and was discovered only in a relatively small fraction of the BY-2 cells examined (Ritzenthaler et al., 2002). As indicated earlier, this may have simply resulted from the use of single markers in most studies. In addition, it is possible that either of the effects could be small and therefore difficult to visualize without specific markers. Moreover, a modulating factor in determining the relative contribution of trans-Golgi elements to the BFA compartment is likely to be the rate of cisternal maturation within the Golgi stack. This process produces the TGN that would feed into the BFA compartment by sloughing of trans cisternae (Staehelin and Moore, 1995 and

refs. therein). Cisternal progression appears to continue during the early stages of BFA treatment (Ritzenthaler et al., 2002). In their minimal form, BFA compartments would consist only of the leftover TGN and maybe a trans cisterna or two that were sloughed off early on. These may be small and therefore may go unnoticed. It is also conceivable that very active Golgi stacks have a fast turnover of cisternae and therefore slough off more of them before the remaining ones fuse with the ER.

It is interesting that a number of apparently endocytosed cell surface markers also accumulate in perinuclear aggregates after BFA treatment. These include components of the auxin transport machinery (PIN1, Geldner et al., 2001; PIN3, Friml et al., 2002; and AUX1, Grebe et al., 2002), as well as a plasma membrane H^+-ATPase and pectins (Baluska et al., 2002). Although the intracellular membranes containing these molecules have thus far been characterized only at the light microscopic level, it is highly likely that they are BFA compartments. These new data strongly suggest that the endocytic pathway, in addition to the TGN, also contributes membranes to this unusual compartment (Baluska et al., 2002). Therefore, one might speculate that the size and prominence of a BFA compartment reflects the balance between secretory and endocytic activities of the cell in question. In this respect, it is interesting to note that in maize root tips, BFA compartments have not been detected in root cap cells or in elongating cells (Baluska et al., 2002), both of which can be regarded as being heavily engaged in secretion.

DOES BFA AFFECT EXPORT FROM THE ER?

A common supposition found in a number of publications is that BFA blocks export from the ER. This notion apparently finds its origin in the much-cited review of Klausner et al. (1992) where it is stated, ". . . in BFA-treated cells, protein secretion is inhibited at an early step in the secretory pathway . . . the block occurred in a pre-Golgi compartment." Recent experiments in plant cells seemingly support this concept (Brandizzi et al., 2002). These experiments have taken advantage of a technique called fluorescence recovery after photobleaching (FRAP), which measures the mobility of molecules in a living cell by selectively bleaching all fluorescent markers in a certain area with high light intensities and then determining the rate of recovery of fluorescence in that region. Because bleaching of fluorophores is an irreversible process, new fluorescence in the bleached area has to come from molecules that are newly synthesized or enter the area from another part of the cell. Using this approach, it has been possible to show that GFP-tagged Golgi enzymes are continuously cycling in and out of the Golgi, because fluorescence levels returned to normal within about 5 min of bleaching (Brandizzi et al., 2002). It is interesting that this replenishment of Golgi enzymes can be blocked in about one-half of the Golgi stacks in a given cell by the addition of BFA to the medium (Brandizzi et al., 2002). In a superficial way, this observation would therefore appear to indicate that BFA prevents export out of the ER. However, it must be pointed out that these FRAP experiments do not directly test for release from the ER, but instead measure arrival at the Golgi apparatus. In other words, the FRAP experiments can only establish that ER→Golgi transport is blocked in BFA-treated plant cells.

The central question that arises from this conclusion is at which step in ER to Golgi transport does BFA exert its effect? The first candidate is obviously at the level of ER export. However, vesiculation at the ER is driven by a different protein machinery than at the Golgi. In particular, exit from the ER, at least in yeast and mammalian cells, requires the two COPII coat dimers, Sec13/31p and Sec23/24p, as well as the GTPase, Sar1p (Antonny et al., 2001). Sar1p is activated by a different kind of GEF, Sec12p, which does not belong to the Sec7 family. Although COPII vesicles remain to be isolated and characterized from plants, Sec13p, Sec23p, Sar1p, and Sec12p homologs have been identified (e.g. Movafeghi et al., 1999). Moreover, indirect evidence for COPII-mediated transport in plants has now been published (Phillipson et al., 2001). Consistent with the absence of Arf1 and Sec7-type GEFs from the ER, there are no reports in the literature whatsoever of BFA directly interfering with the assembly of COPII coats. In fact, just the contrary has recently been demonstrated by the failure of BFA to prevent cargo recruitment to ER export sites in mammalian normal rat kidney (NRK) cells and the near-normal recovery rates of ER-bound Sec13-YFP after photobleaching in the presence of BFA (Ward et al., 2001). In conclusion, the effect of BFA on ER→Golgi transport most likely does not occur at the stage of export from the ER (see also Fig. 1).

Therefore, the target for BFA action has to reside on a compartment that lies after COPII vesicle formation. This organelle is well known for mammalian cells, where the vesicles formed at the ER quickly fuse with vesicular-tubular complexes (VTCs; Bannykh et al., 1996; sometimes also called ER-Golgi intermediate compartments). These structures are absent from plants and most other eukaryotes (see Fig. 2), and they have very high densities of ER-Golgi v- and t-SNARES (Hay et al., 1998) and function as recycling stations by sending ER residents back to the ER via COPI vesicles (e.g. Stephens et al., 2000). This recycling step appears to be a prerequisite for the subsequent transport of VTCs along microtubules to the perinuclear Golgi complex of mammalian cells (Scales et al., 1997). Consistent with this interpretation, it has been found that a BFA block of COPI formation (i.e. block of recycling from VTCs to the ER) leads to the accumulation of early Golgi markers in structures also labeled with VTC/ER-Golgi inter-

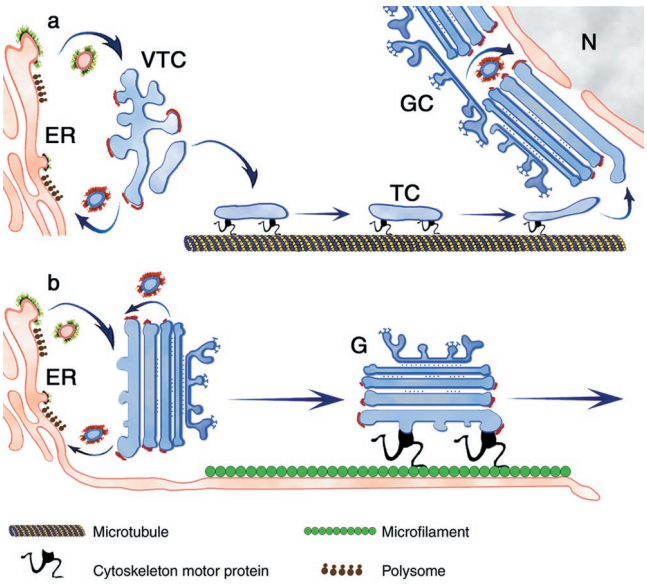

Figure 2. Comparison of ER-Golgi trafficking in animals and plants. In mammalian cells (a), COPII-coated vesicles fuse with VTC in close proximity to ER export sites. VTCs act as recycling stations from which retrograde COPI vesicles return escaped proteins to the ER. The remaining membranes then travel as transport containers (TC) along microtubules to the perinuclear Golgi complex (GC) where they form new cis cisternae. In plant cells (b), COPII vesicles directly fuse at the cis Golgi of individual stacks because the intermediate compartment of VTCs does not exist. Long-distance transport is achieved by active movement of intact Golgi stacks (G) along actin filaments. Retrograde transport from the Golgi to the ER may occur near ER export sites as depicted, or randomly during translational movements of Golgi stacks. The precise relationship between ER export sites and Golgi stacks in plant cells is currently not known. n, Nucleus.

mediate compartment markers (Füllekrug et al., 1997; Ward et al., 2001). It is interesting that these Golgi-VTC hybrids are not stable entities, but undergo a constant turnover as demonstrated by more FRAP experiments (Ward et al., 2001), suggesting that they are continuously exchanging material with the ER. Thus, in mammalian cells, BFA affects ER→Golgi transport at a post-ER compartment, and this compartment is not present in plants.

What do these results tell us about the plant secretory pathway? In principle, we should assume that ER export also still occurs in BFA-treated plant cells. In support of this, nonclathrin-type-coated buds have been identified on the ER-Golgi hybrid compartment of BFA-treated BY-2 cells (Ritzenthaler et al., 2002). The lack of a VTC should then result in the direct transport of these vesicles to the cis Golgi, which is the first post-ER compartment in plants (Fig. 2). This has been observed in the FRAP experiments of Brandizzi et al. (2002), albeit only for about one-half of the Golgi stacks tested. The big question is why the other one-half of the stacks in the experiments did not receive new proteins from the ER. What is different in these stacks that prevents accumulation of ER→Golgi transport vesicles at the first post-ER compartment, i.e. the cis Golgi? Although we do not have a conclusive answer to this question, we would like to offer a hypothesis that is consistent with our current understanding of the eukaryotic endomembrane system. In particular, the described difference between plants and animals with respect to BFA effects may simply be a result of the different nature of the target organelle for ER export.

In plants, this target organelle is the cis Golgi, whereas in animals, it is the VTC (see Fig. 2). Ultrastructural analysis of BFA effects in BY-2 cells has revealed that cis-Golgi cisternae are gradually lost during BFA treatment (Ritzenthaler et al., 2002). This loss has been interpreted as the continued maturation of Golgi cisternae combined with a lack of formation of new cisternae. This interpretation is primarily based on the observation that the cis-Golgi marker Gm-Man1-GFP (Nebenführ et al., 1999) is lost from the stacks at a later time point, namely when the "trans" Golgi cisternae disappear (Ritzenthaler et al., 2002). This shift of a cis Golgi protein to medial and trans cisternae in BFA-treated cells highlights the role of COPI vesicles in recycling Golgi enzymes to younger cisternae, as predicted by the cisternal progression/maturation model (e.g. Pelham, 2001). This model of intra-Golgi transport also predicts that new Golgi cisternae form by the fusion of anterograde ER→Golgi (COPII-) vesicles and retrograde intra-Golgi (COPI-) vesicles. A lack of these recycling vesicles (combined with a continued maturation of existing cis cisternae) may prevent the ER→Golgi transport vesicles from docking at the Golgi. This could explain the lack of FRAP in about one-half of the Golgi stacks tested (Brandizzi et al., 2002), whereas the other stacks presumably retained a normal ER→Golgi transport through the continued presence of cis cisternae. Therefore, our interpretation assumes that ER→Golgi transport in the presence of BFA cannot be observed for some plant Golgi stacks simply because the target organelle (cis Golgi) is no longer present. The situation in mammalian cells is different in that the first post-ER compartments are the VTCs, which do not mature in the presence of BFA and therefore continue to serve as a target for ER export.

This hypothesis leads to several predictions that can be tested experimentally. First, a block of ER export by means of a dominant-negative mutant of Sar1p should block fluorescence recovery in all Golgi stacks because it affects the first step in the transport process (Takeuchi et al., 1998). Second, the same effect should occur when vesicle fusion at the Golgi is prevented by means of a dominant-negative mutant of Rab1b (Batoko et al., 2000). Third, the percentage of Golgi stacks that do not show FRAP in the presence of BFA should increase over time because the loss of cis cisternae has been found to be gradual and, in BY-2 cells, to extend over 10 to 15 min (Ritzentha-

ler et al., 2002). This last point in particular should be informative because the other possible transport blocks can be expected to work much more rapidly and on an all-or-nothing basis.

ARE THERE OTHER SITES FOR BFA ACTION IN THE PLANT CELL?

The capture of endocytosed membranes and extracellular components in BFA compartments suggests that there is an additional site of BFA action beyond the Golgi, one that affects endosomes. This should not come as a surprise because endocytic and secretory pathways in mammalian and yeast cells are equally affected by expression of Arf1 mutants (Gaynor et al., 1998). Arf1p is also required for the retrograde transport of internalized toxins (Morinaga et al., 2001). It has been shown in mammalian cells that the formation of a transport intermediate between early and late endosomes involves an Arf1-dependent recruitment of a subset of COPI vesicle coat polypeptides (Gu and Gruenberg, 2000). Consistent with these observations, it has been known for some time that BFA in mammalian cells causes a tubulation of endosomes similar to that induced in the Golgi apparatus (Lippincott-Schwartz et al., 1991).

The endocytic pathway in plants, despite over 15 years of research effort, remains largely "uncharted territory" to this day. The plant equivalents of early and late endosomes have yet to be unequivocally identified, and the nature of prevacuolar compartments is still unclear (Robinson et al., 2000). However, the recent demonstration that BFA prevents the delivery of the styryl dye FM1–43 to the central vacuole in BY-2 cells (Emans et al., 2002) might indicate that an Arf1-dependent step lies somewhere along the plant endocytic pathway.

It has to be cautioned that it is not known whether activation of Arf1p at any of these post-Golgi organelles depends on BFA-sensitive GEFs (Jackson and Casanova, 2000). Any effect of BFA on trafficking in the endocytic pathway could, in principle, also be a secondary effect resulting from disruption of the Golgi apparatus. The complex interplay between anterograde and retrograde transport at the ER-Golgi interface (see above) serves as an example for the indirect effects of disrupting one step in a network of membrane exchange reactions. In addition, coat protein recruitment is not the only effect of Arf1p on the endomembrane system. Other effectors of Arf1p include phosphatidylinositol 4-phosphate 5-kinase and phospholipase D (Donaldson and Jackson, 2000), indicating that BFA can also have effects on membrane composition, which in turn might be expected to affect a variety of membrane activities. Such an effect has recently been demonstrated to occur in maize roots as well as BY-2 cells (Mérigout et al., 2002).

CONCLUSIONS

Based on recent progress in our understanding of the secretory system in plants and other eukaryotes, we suggest the following scenario of BFA responses. The molecular target for BFA appears to be the same in all eukaryotic cells, namely, a Sec7-type GEF that is necessary for activation of Arf1p. Its best-studied immediate effects are the inability to recruit COPI coat proteins onto Golgi membranes. As a result, the majority of Golgi cisternae fuse directly with the ER, leading to the formation of an ER-Golgi hybrid compartment. However, trans-Golgi elements and the TGN separate from the Golgi stack and merge with components of the endocytic pathway to form "BFA compartments." The relative prominence of these two responses depends on the physiological state of the secretory and endocytic systems. Therefore, BFA effects are not limited to the Golgi apparatus and early post-Golgi compartments. The apparent block in ER→Golgi transport due to BFA treatment is more likely to result from the inability of ER-derived transport vesicles to fuse with altered Golgi cisternae than to an inhibition of ER export per se.

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