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G.A.T.T. (A General Agreement on Traffic and Transport) and Brefeldin A in Plant Cells

In the September 1993 issue of THE PLANT CELL, Gomez and Chrispeels (1993) reported an important advance in our understanding of membrane protein targeting in plant cells. Over the past few years, Chrispeels' group has made an invaluable contribution to the search for signals responsible for the targeting of soluble vacuolar proteins, and this latest paper questions the ubiquity of the sorting mechanisms for both the protein components of vacuolar membranes and soluble vacuolar proteins. The observation that the transport of a tonoplast protein (α-TIP) and a soluble vacuolar protein (phytohemagglutinin) to the vacuole could be experimentally uncoupled suggests that soluble and vacuolar proteins may follow different pathways to the same destination and may be targeted by different mechanisms to the vacuole. The data were derived from a pool of biochemical experiments using the drugs monensin and brefeldin A (BFA), which are known to perturb the Golgi-based secretory machinery. However, we are concerned about some of the hypotheses developed in the discussion of this paper, which are based partially on a misquotation of recent literature on the structural effects of BFA on the plant Golgi apparatus. In this letter, we wish to correct this quotation and to clarify what is known from experimental evidence from what is mere hypothesis regarding the subcellular targets of BFA in plants.

Mystery Still Surrounds the Mechanics of the Plant Golgi

An understanding of the mechanics of the Golgi stack is essential to our comprehension of membrane flow, protein targeting, and wall morphogenesis in plants. This organelle can be considered as a production line for the synthesis of complex polysaccharides and the sequential modification (glycosylation) of glycoproteins that also acts as a dispatch department sending vesicle-packaged material to various parts of the cell (Satiat-Jeunemaitre and Hawes, 1993a). Over the past few years, breathtaking progress has been made in the molecular dissection of the Golgi machinery in animal cells by use of a combination of marker proteins (e.g., viral coat proteins), cell-free systems, and a variety of pharmacological agents. Unfortunately, such an array of tools has yet to be fully developed to facilitate the study of the plant Golgi apparatus.

Electron microscopy has shown that the plant Golgi stack is an easily recognizable entity that is often well separated from the rest of the endomembrane system and, thus, amenable to morphological analysis. The effects of various drugs on the structure of the plant Golgi and on secretion and protein targeting have, therefore, been relatively straightforward to study. A range of antagonists, including herbicides, ionophores, and toxins have been used over the years; unfortunately, such drugs are never Golgi specific, and their effects often vary between different cell types. A good example is the ionophore monensin, for which various contradictory effects have been reported over the years, resulting in a variety of hypotheses on its effect on Golgi trafficking and targeting (Bednarek and Raikhel, 1992; Satiat-Jeunemaitre et al., 1994).

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Brefeldin A, the Latest Weapon for Studying Membrane Trafficking

And so to the latest weapon in our armory for taking the Golgi apart—BFA, a compound that some considered so important in unraveling the membrane flow story in animal cell Golgi that it could be termed the drug of the early nineties (for reviews, see Pelham, 1991; Klausner et al., 1992). Following the initial report of BFA as a Golgi disrupting agent (Fujiwara et al., 1988; Lippincott-Schwartz et al., 1989), the use of this drug has facilitated an impressive dissection of the molecular events involved in vesicle trafficking within cells (Donaldson et al., 1992; Helms and Rothman, 1992; Orci et al., 1993a; Stamnes and Rothman, 1993).

BFA was initially shown to inhibit secretion in animal cells (Misumi et al., 1986; Fujiwara et al., 1988; Lippincott-Schwartz et al., 1989), an effect associated with dramatic alterations to the Golgi apparatus and its neighboring organelles. The drug induces a progressive redistribution of Golgi cisternae and their constituent enzymes into the endoplasmic reticulum (ER), starting at the *cis* face. This is achieved by the formation of Golgi-derived tubules that fuse with the ER and by the subsequent reabsorption of the entire Golgi stack into the ER. These results highlight the efficiency of the retrograde transport pathway in animal cells. The *trans*-Golgi network (TGN) does not take part in this ER reabsorption process but fuses with the endosomal system, indicating that there are fundamental differences in the mechanics of this Golgi-associated organelle and that of the Golgi itself (Lippincott-Schwartz et al., 1991; Wood et al., 1991).

These morphological events are now known to result from the rapid and reversible dissociation of β-COP, one of the major proteins in the coat complex surrounding Golgi intercisternal vesicles, from budding vesicles (Pelham, 1991; Waters et al., 1991). Further molecular dissection suggests that the prime target of BFA is in fact an enzyme catalyzing guanine nucleotide exchange on ADP ribosylation factor (ARF), a small GTP binding protein. The GTP-bound ARF binds to a receptor in the cisternal membrane and this complex then binds β-COP to the cisternal periphery, permitting bud coating and vesicle scission (Donaldson et al., 1992; Helms and Rothman, 1992; Helms et al., 1993). By causing β-COP release, BFA inhibits the transport of non-clathrin-coated vesicles between the ER and *cis*-Golgi and down the Golgi stack.

BFA's effects on animal cells have also been found to extend to the clathrin-coated vesicles of the TGN. BFA inhibits the formation of these vesicles by interfering with the Golgi-specific β′ and τ adaptins, which mediate the attachment of clathrin to the TGN membranes (Robinson and Kreis, 1992; Wong and Brodsky, 1992). It has recently been shown that this adaptin binding is also mediated by ARF in a BFA-sensitive manner. Conversely, BFA has no effect on α and β adaptins, which are targeted to plasma membrane clathrin-coated pits (Robinson and Kreis, 1992; Wong and Brodsky, 1992). This most likely explains the earlier observations that BFA has no effect on endocytosis (Misumi et al., 1986; Miller et al., 1992).

Effects of Brefeldin A on Plant Cells: Experimental Facts

BFA is now being used by various groups to probe the functioning of the plant Golgi apparatus. Perhaps it would be more appropriate to say that BFA has re-emerged in the plant field because some of its effects have, in fact, previously been reported from biochemical and microscope observations of plant cells treated with this antibiotic, but under other names (see Betina, 1992; Robinson, 1993). We have, however, still to identify equivalents of the animal cell target molecules for BFA action. Preliminary reports indicating that BFA both inhibits the transport of vacuolar proteins (Holwerda et al., 1992) and perturbs the Golgi apparatus (Satiat-Jeunemaitre and Hawes, 1992a) suggest that BFA is a promising tool for the study of vesicle trafficking in plants.

The effects of BFA on plant cells are now becoming clearer. Both immunocytochemical (Satiat-Jeunemaitre and Hawes, 1993a; Driouich et al., 1993) and biochemical (Driouich et al., 1993; Jones and Herman, 1993) studies have shown that, as in most animal cells, the drug inhibits the transport pathway from the Golgi to the cell surface. It also causes a major reduction in wall deposition in red algae (Garbary and Phillips, 1993). Furthermore, transport of soluble proteins from the *trans*-Golgi to the vacuole is inhibited by BFA (Holwerda et al., 1992; Gomez and Chrispeels, 1993), but the drug does not affect movement of the tonoplast integral protein (α-TIP) (Gomez and Chrispeels, 1993). Our preliminary experiments also suggest that the drug does not inhibit the endocytosis of cationized ferritin in protoplasts (B. Satiat-Jeunemaitre and C. Hawes, unpublished data).

Using a marker antibody (JIM 84) for the plant Golgi, we have shown that the initial morphological effect of BFA on plant cells is a clustering of the Golgi stacks, a phenomenon not seen in animal cells (Satiat-Jeunemaitre and Hawes, 1992b, 1993a). A similar movement of cisternal stacks was also reported in sycamore cells (Driouich et al., 1993) and may be a common initial response to the drug. The clusters of Golgi stacks appear to surround highly vesiculated areas in the cytoplasm that show both an accumulation of the JIM 84 epitope, as shown in Figure 1, and secretory products (polysaccharides and glycoproteins). For want of a better terminology, we have called these "BFA compartments" (Satiat-Jeunemaitre and Hawes, 1992b, 1993a), but these should not be confused with the recently described "BFA bodies" of Orci et al. (1993b), which are subcompartments of BFA-modified ER in certain mammalian cells.

Golgi stacks themselves can also be dramatically disrupted by the drug so that they are no longer recognizable (Satiat-Jeunemaitre and Hawes, 1992a, 1992b, 1993a; Rutten and Knuiman, 1993). The

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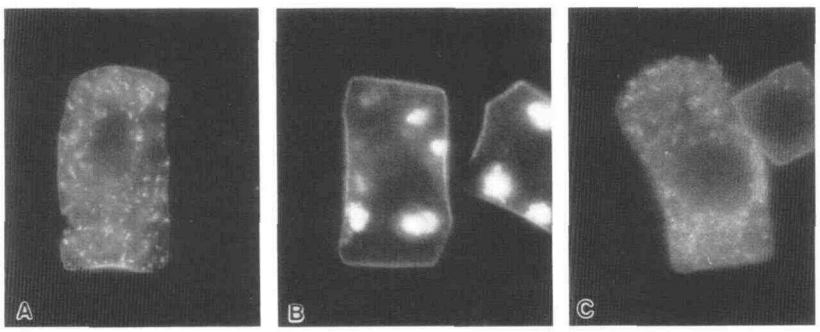

Figure 1. Reversible Effect of BFA on the Distribution of the Golgi Marker Epitope Recognized by JIM 84 in Maize Root Cells.

(A) Control root cell.
(B) Root cell after a 1-hr treatment with 50 μg/mL BFA. Staining is restricted to "BFA compartments."
(C) Root cell 2 hr after removal of BFA.

ultimate response to BFA seems to be the complete disintegration of the Golgi stacks into these vesiculated areas, a procedure that is reversible on removal of the drug (Figure 1). Concomitant with these BFA-induced effects on the Golgi compartment, the ER network has also been reported to be affected, becoming swollen (Driouich et al., 1993) or reorganized (Rutten and Knuiman, 1993; Satiat-Jeunemaitre et al., 1993). However, we have no evidence that any tubular structures arise from the Golgi toward the ER in response to the drug.

The G.A.T.T. Problem

Even at this early stage in the research on plant secretion and vesicle trafficking using BFA, discrepancies in the literature are becoming apparent. In essence, it appears that American laboratories do not report the BFA-induced Golgi disintegration (Chrispeels and Staehelin, 1992; Driouich et al., 1993) that has been described by European laboratories (Satiat-Jeunemaitre and Hawes, 1992a, 1992b, 1993a; Rutten and Knuiman, 1993; Schindler et al., 1994). These apparent discrepancies raise the question, do we have another G.A.T.T. (General Agreement on Traffic and Transport!!) problem? In our opinion, the answer is almost certainly no; because most of the results reported to date have come from work on a variety of plant material, using different techniques and different drug concentrations, a lack of consensus on the effects of BFA is not surprising.

Where a potential G.A.T.T. problem could arise is when published results are misquoted in subsequent literature. For instance, Gomez and Chrispeels (1993) stated that "this drug [BFA] has almost no effect on the structure of the plant Golgi (Satiat-Jeunemaitre and Hawes, 1992, 1993; Driouich et al., 1993)." Unfortunately, the pictures and text in our two papers (Satiat-Jeunemaitre and Hawes, 1992a, 1992b) demonstrate exactly the opposite. Indeed, BFA can significantly perturb the positioning and structure of the Golgi stacks in plant cells, as described above, and this effect is reversible (Figure 1).

It should also be pointed out that our results and those of Driouich et al. (1993) are not necessarily "contradictory" or "controversial," as several authors (Gomez and Chrispeels, 1993; Robinson, 1993) have described them. In our laboratory, we have found that treatment of maize and onion roots or maize and carrot suspension cells with 50 μg/mL or more BFA causes a vesiculation and dissociation of the Golgi stacks. This effect is reversible even with BFA concentrations as high as 200 μg/mL. However, at lower concentrations we are not able to detect Golgi dissociation. Similarly, in sycamore cells treated with 2.5–7.5 μg/mL BFA, no breakdown of the Golgi stack was reported, but there was an increase in the number of *trans*-like cisternae and in the number of *trans*-Golgi–derived vesicles (Chrispeels and Staehelin, 1992; Driouich et al., 1993). In addition, 10 μg/mL BFA did not result in the disintegration of the Golgi complex of rice cells (Kimura et al., 1993), and 20 μg/mL BFA reduced the Golgi stack of maize coleoptile segments to just two or three cisternae but did not cause it to disintegrate (Schindler et al., 1994). It is possible that the modification of cisternal structure caused by low BFA concentrations is simply a prelude to the further demolition of the stack observed at higher BFA concentrations.

On the other hand, it is clear that the varying results are not all attributable to differences in the BFA concentrations used. For instance, Driouich et al. (1993) point out that "maize suspension-cultured cells [treated with the same low concentration of BFA] . . . show much more Golgi fragmentation and vesiculation than is evident in the sycamore maple cells." In addition, a recent report on the effect of BFA on pollen tubes has shown Golgi dissociation at a BFA concentration as low as 5 μg/mL (Rutten and Knuiman, 1993). Thus, it is probable that in plant cells, as in animal cells, sensitivity to BFA depends on the cell type, although the threshold of morphological response to the drug appears to be generally higher in plants than in animals, as is well known for other drugs that affect secretion.

An interesting observation to come out of studies of BFA-treated plant cells is that low concentrations of BFA can perturb secretion and modify glycosylation of secretory proteins (Driouich et al., 1993; Gomez and Chrispeels, 1993) without the manifestation of any dramatic breakdown of the Golgi stack (Driouich et al., 1993).

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If the molecular target of BFA and the cisternal transport mechanisms in plants were similar to those in animal cells, BFA treatment should result in a cessation of ER-to-Golgi and intercisternal traffic, and morphological and biochemical effects should occur concomitantly. Because the reported biochemical effects appear temporally uncoupled from any breakdown of the Golgi apparatus, we must not dismiss the possibility that we are in fact dealing with two distinctly separate effects of the drug on secretion and Golgi function in plants.

Occurrence of a Retrograde Pathway between Golgi and ER in Plants—Experimental Facts and Hypotheses

As for the possibility that, in plant cells, BFA induces a redistribution of Golgi membrane and/or processing enzymes back into the ER by interfering with anterograde transport but not with a putative retrograde transport pathway, it is important to realize that here we start to deal with hypotheses rather than experimental facts. The presence of such a retrograde pathway from the Golgi to the ER has been postulated based on biochemical data showing a change in the quantity of the various glycosylation forms of phytohemagglutinin in transgenic tobacco plants (Gomez and Chrispeels, 1993). Rutten and Knuiman (1993) have even suggested a reabsorption of Golgi into the ER based on electron micrographs in BFA-treated pollen tubes. Although in animal cells this is the fate of the Golgi (but not of the TGN), there is as yet no hard experimental evidence that this occurs in plant cells.

The facts that no redistribution of the Golgi membrane marker JIM 84 into the ER occurs after BFA treatment (Satiat-Jeunemaitre and Hawes, 1992b) and that immunofluorescence studies have never shown tubular structures emerging from Golgi to ER during BFA treatment actually militate against the efficient operation of such a retrograde pathway. Furthermore, the reported morphological changes to the ER could simply represent another effect of the drug on plant cell structure (Driouich et al., 1993) and not be inextricably linked to a blockage of ER-to-Golgi transport and/or to putative backflow from the Golgi to the ER. It is also possible that Golgi transferases are redistributed to the "BFA compartment," as suggested by the presence of secretory products within this accumulation of vesicles (Satiat-Jeunemaitre and Hawes, 1993a), and we await the development of suitable antibody probes to further test this hypothesis. In short, we consider that it is premature to postulate further on the possibility of a retrograde transport pathway from plant Golgi to ER.

The differences between the ER/Golgi pathway in plant and animal cells have often been a matter of debate. For instance, structural evidence for ER/Golgi continuity is sparse in plants (but it may be more apparent in specific protein-secreting cells), although a KDEL/HDEL retention system operates in the ER (Napier et al., 1992). However, nothing is known regarding the putative recycling of the KDEL/HDEL receptor from the *cis*-Golgi or from an intermediate compartment, as has been described for animal cell systems. Moreover, the unique features of the plant Golgi (e.g., separate stacks, wall matrix synthesis, and lack of fragmentation during cell division) that distinguish it from the animal Golgi are well known and often emphasized in the literature (Driouich et al., 1993; Satiat-Jeunemaitre and Hawes, 1993b). Taking these factors into account, it is not surprising that the BFA-induced transformation of the endomembrane pathway is different from that described for animal cells.

Obviously, these are early days in our dissection of the plant Golgi apparatus using drugs such as BFA, and the results presented to date simply illustrate the end result of what is presumably a complex series of molecular events. It will naturally be tempting to draw parallels with the effect of BFA on animal cells and to search for a molecular target of BFA equivalent to ARF and for the coat components of the vesicles involved in secretory events. However, in our quest for unraveling the remaining mysteries of the Golgi stack, it will be important not to forget the unique nature of this organelle in plant cells.

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