Brefeldin A Effects in Plants

Are Different Golgi Responses Caused by Different Sites of Action?

During the past decade, BFA, a lipophilic fungal toxin, has become a major tool of cell biologists interested in studying vesicle-mediated trafficking in animal and plant cells. Its main attractions include its ability to block secretion and to induce profound morphological changes in the organelles of the secretory and endocytic pathways in a totally reversible manner. In the plant literature, however, discrepancies have arisen about the effects of BFA on the Golgi apparatus, with some laboratories reporting a breakdown of Golgi stacks and others finding no such effect (Satiat-Jeunemaitre and Hawes, 1994). The purpose of this communication is to propose a hypothesis that can explain the two types of Golgi responses.

EFFECTS OF BFA ON ANIMAL CELLS

In BFA-sensitive mammalian cells (cells that respond to 1-10 μg mL⁻¹ BFA), the block in secretion has been traced to the ER-to-Golgi transport step and has been shown to be caused by the disassembly of the Golgi complex and the subsequent redistribution of resident Golgi enzymes into the ER (for review, see Klausner et al. [1992]). This redistribution, in turn, has been shown to involve membrane tubules that grow out of Golgi cisternae and then fuse specifically with the ER. Similar BFA-induced fusigenic membrane tubules have been reported to develop from early endosomes and the TGN, and to lead to the formation of hybrid early endosome-TGN membrane networks.

Biochemical studies have demonstrated that BFA interferes with the assembly of cytoplasmic coats on budding vesicles of ER, Golgi, and other membrane systems. In the case of Golgi membranes, BFA appears to interfere with the exchange of GTP for GDP on ADP-ribosylation factor, which is required for its binding to Golgi cisternae and coat assembly (for example, see Stamnes and Rothman [1993]). However, this effect might be indirect, and several lines of evidence suggest that BFA may have multiple targets in cells and may affect different cell lines in an organelle-specific manner (Pelham, 1991). Thus, whereas BFA inhibits protein secretion at the ER-to-Golgi step in most cell lines, it has no such effect in PtK1 cells or MDCK cells. Similarly, BFA seems to block transport to lysosomes in chick embryo fibroblasts but not in rat hepatocytes. A recent investigation of BFA-resistant mutant Chinese hamster ovary cell lines has provided additional support for the hypothesis that BFA can inhibit coated vesicle formation in an organelle-specific manner (Yan et al., 1994). Starting with a parent Chinese hamster ovary line in which endosomes and Golgi complex were equally sensitive to BFA, Yan et al. (1994) were able to select for mutants in which the relative sensitivities of these two organelles were dramatically altered. One explanation for the specificity of the BFA-induced fusion processes is that, although BFA has the ability to disrupt coat assembly on budding vesicles, it may not prevent the specific vesicle targeting types of molecules such as the v-SNARE complexes from becoming incorporated into the tips of the tubes that grow out of the vesicle budding sites (Staehelin and Moore, 1995).

EFFECT OF BFA ON PLANT CELLS

In plants BFA also seems to exert its primary effects through perturbations of vesicular transport in the secretory pathway. As first shown by Satiat-Jeunemaitre and Hawes (1992a, 1992b), treatment of maize and onion roots as well as maize and carrot suspension-cultured cells with 50 to 200 μg mL⁻¹ BFA causes a reversible vesiculation and disassembly of Golgi stacks. In contrast to animal cells, however, no evidence for fusion of the Golgi-derived vesicles with ER membranes was obtained when the Golgi-specific antibody JIM84 was used to follow the redistribution of Golgi proteins.

In another study Driouich et al. (1993) reported that when sycamore maple suspension-cultured cells were treated with 2.5 to 10 μg mL⁻¹ BFA, the treatment both blocked secretion and altered glycosylation patterns of glycoproteins and complex polysaccharides, but did not cause any breakdown of Golgi stacks. Instead, a loss of TGN cisternae, an increase in the number of trans-like Golgi cisternae, and the accumulation of large numbers of trans-Golgi-derived vesicles in the adjacent cytoplasm were seen. At 50 μg mL⁻¹ BFA the Golgi stacks of sycamore

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Abbreviations: BFA, brefeldin A; TGN, trans-Golgi network.
maple cells disintegrate (A. Driouich, unpublished results). These two types of BFA responses have since been confirmed in other plant systems and, as discussed by Satiat-Jeunemaitre and Hawes (1994), should be viewed as part of a continuum of BFA effects, and not as evidence for flawed experimental protocols. What has yet to be determined is why BFA sometimes leads to Golgi stack vesiculation and in other instances has little effect on Golgi stack morphology, while still inhibiting secretion. The fact that as little as 1 μg mL⁻¹ BFA can induce Golgi vesiculation in tobacco pollen tubes indicates that the BFA-caused breakdown of Golgi stacks (Rutten and Knuiman, 1993) is not simply due to nonspecific effects of high BFA concentrations on Golgi membranes.

**HYPOTHESIS: DIFFERENT RESPONSES OF PLANT GOLGI STACKS TO BFA ARE CAUSED BY MULTIPLE BFA-BINDING SITES**

Based on the evidence presented above, we postulate that BFA in plants can block vesicular transport in at least two sites in the secretory pathway, between the ER and the Golgi, and between the Golgi and the TGN (Fig. 1). BFA inhibition of vesicular transport "upstream" from the Golgi site leads to inhibition of secretion and to the disintegration of Golgi stacks, whereas inhibition of the "downstream" site causes inhibition of secretion without Golgi breakdown. The specific postulates are: (a) the downstream site is relatively more sensitive than the upstream site to BFA inhibition; (b) at lower concentrations of BFA the stronger inhibition of the downstream site results in a block in secretion without causing a breakdown of Golgi stacks; (c) at higher concentrations of BFA the additional strong blockage of the upstream site without a concomitant inhibition of Golgi vesiculation processes leads to Golgi breakdown; and (d) disintegration of the Golgi stacks is caused by a BFA-insensitive and coatamer-protein-independent vesiculation process that is driven by polysaccharide synthesis.

Support for our hypotheses comes from the following observations. When secretion is blocked by relatively low BFA concentrations (2.5–10 μg mL⁻¹ for sycamore maple suspension-cultured cells), the individual Golgi stacks retain both their normal architecture and their ability to produce polysaccharide-filled vesicles at their trans-cisternae (Driouich et al., 1993). However, these vesicles differ from normal secretory vesicles in several respects. They are larger, like those in mucilage-secreting cells, they form apparently without the aid of coatamer-protein coats, and they are filled with darkly staining polysaccharide contents. They also seem to be unable to exit the Golgi matrix zone (Driouich et al., 1993; Staehelin and Moore, 1995), consistent with the measured block in secretion. What can prevent these vesicles from traveling to the cell surface? Driouich et al. (1993) have also observed that, whereas the Golgi stacks show no signs of breakdown, the associated TGN units seem to disappear, which may prevent the maturation of the large, polysaccharide-filled, trans-Golgi cisterna-derived vesicles into normal secretory vesicles. Based on these data, we conclude that the more sensitive BFA site in plant cells is located between the Golgi stacks and the cell surface, i.e. downstream of the Golgi stacks. The simplest explanation for why the Golgi stacks do not disassemble under these conditions is that, because of its lower sensitivity to BFA, the ER-to-Golgi transport site is inhibited to a lesser extent than the post-Golgi site. This would then permit enough influx of new membrane from the ER to the Golgi to compensate for the loss of membrane from the Golgi stacks to the polysaccharide-filled vesicles that bud from the cisternae.

Our explanation for why Golgi stacks disintegrate and vesiculate at higher BFA concentrations (usually >20 μg mL⁻¹ BFA) is that at higher concentrations the second, less sensitive upstream site of BFA action also becomes blocked to a significant extent. When
this occurs, ER-to-Golgi transport is inhibited, but the polysaccharide synthesis-driven formation of Golgi vesicles continues, causing the cisternae to bud themselves into obliteration. The most convincing evidence for BFA’s ability to block ER-to-Golgi transport comes from a study of the O-glycosylation patterns of a sweet potato vacuolar protein, sporamine, expressed in transgenic tobacco plants (Matsuoka et al., 1995). In plants O-glycosylation has been shown to be initiated in cis-Golgi cisternae (Moore et al., 1991), and thus the presence of O-linked sugars on vacuolar/secretory proteins can be used as a tool for studying ER-to-Golgi transport. In the presence of BFA, O-glycosylation of sporamine was found to be largely abolished, consistent with BFA blocking ER-to-Golgi transport (Matsuoka et al., 1995). As mentioned above, without the input of new membrane to compensate for the loss of membrane to budding trans-Golgi vesicles, the Golgi stacks disappear by the conversion of cisternal membranes to vesicles. This type of Golgi breakdown response has been observed recently in sycamore maple suspension-cultured cells treated with the BFA analog 7-oxo-BFA (Driouich et al., 1997). Both BFA and 7-oxo-BFA are toxic agents produced by the plant pathogen Alternaria carthami, and both cause the same disease symptoms in safflower, Carthamus tinctorius (Tietjen et al., 1983). When applied at a concentration of 10 μg mL⁻¹, 7-oxo-BFA was found to be a more potent disruptor of Golgi stacks than BFA. Electron micrographs of cryofixed cells demonstrated that the cis and medial Golgi cisternae disappeared first, whereas the remaining trans-cisternae were seen budding large vesicles containing xyloglucan, a cell wall polysaccharide that is assembled in trans-Golgi cisternae (Staehelin and Moore, 1995).

Taken together, the presented theories can explain why BFA has been found to cause a breakdown of Golgi stacks in some experiments, while having only minor effects on Golgi morphology in others. Further studies are now needed to identify the target molecules at the specific sites of action of BFA and 7-oxo-BFA in plant cells.

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