

Minireview

Brefeldin A and the endocytic pathway

Possible implications for membrane traffic and sorting

Walter Hunziker, J. Andrew Whitney and Ira Mellman

Department of Cell Biology, Yale University School of Medicine, New Haven, CT, USA

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A number of recent observations have suggested that the endocytic and biosynthetic pathways may share fundamentally similar transport mechanisms at the molecular level. Some of the more striking of these suggestions have come from a comparison of the effects of the macrocyclic lactone brefeldin A (BFA) on endosomes and the Golgi complex. BFA is thought to affect Golgi-specific coat proteins that may be involved in maintaining the structural integrity of the organelle and in regulating membrane transport in the secretory pathway. Many of the effects of BFA on the endocytic system, such as the guanine nucleotide and aluminum fluoride (AlF_4^-)-regulated induction of microtubule-dependent endosomal tubules, are strikingly reminiscent of the action of the drug on the Golgi complex. Therefore, the similar mechanisms of action of the drug on endosomes suggest that organelles of the endocytic pathway may be associated with similar cytoplasmic coats that could regulate endosome function and integrity.

Brefeldin A; Endocytosis; Membrane traffic

1. EFFECTS OF BFA ON THE GOLGI COMPLEX

Over the past few years, analysis of the action of the fungal antibiotic BFA on the Golgi complex has led to significant insights into the molecular mechanisms of membrane traffic in the secretory pathway. The first observed effects of BFA were the inhibition of protein secretion and the breakdown of the Golgi complex [1–4]. Concomitant with disassembly, components of the Golgi complex are delivered to the ER via retrograde transport involving tubular extensions and facilitated by intact cytoplasmic microtubules [5]. These dramatic effects of BFA appear to reflect the immediate dissociation of a non-clathrin-containing cytoplasmic coat from Golgi membranes [6–8]. The peripheral coat is assembled from cytoplasmic precursors and is believed to participate in the formation of Golgi-derived transport vesicles [9], and/or to play a role in regulating the formation of tubules that may be involved in transport [10,11].

BFA induces the redistribution of at least three components of this coat material, β -COP, ADP ribosylation factor (ARF) and γ -adaptin, from Golgi membranes to the cytosol, most likely by interfering with their ability to bind to the Golgi [7,12,13]. Both β -COP and γ -adaptin share significant homology to the β -subunit of

the clathrin adaptor complex of endocytic coated vesicles [6,8], already suggesting similarities in the transport mechanisms of the two pathways. Binding of β -COP, γ -adaptin and ARF (a low molecular weight GTP binding protein [14]) are directly or indirectly regulated by guanine nucleotides since pretreatment of cells or Golgi membranes with AlF_4^- or $\text{GTP}\gamma\text{S}$ inhibits the action of BFA [12]. Sequential addition experiments of β -COP and ARF suggest that ARF-binding is required for association of β -COP with Golgi membranes. Although $\text{GTP}\gamma\text{S}$ promotes membrane-association of both β -COP and ARF, only β -COP binding is enhanced by AlF_4^- . This differential effect of AlF_4^- has been taken to imply a role of trimeric G proteins in regulating membrane assembly of BFA sensitive coat proteins, in particular because AlF_4^- is thought to specifically target the trimeric G proteins without affecting the low molecular weight GTP binding proteins of which ARF is a member [14,15]. Such an involvement of trimeric G proteins has more directly been shown by the inhibitory effect of purified $\beta\gamma$ subunits of G proteins on AlF_4^- and $\text{GTP}\gamma\text{S}$ -induced binding of ARF and β -COP to Golgi membranes [12]. More recently, the trimeric G protein-activating peptide mastoparan has been shown to promote binding of β -COP to Golgi membranes and to antagonize the effect of BFA [16]. Since pre-treatment of the cells with pertussis toxin abolished the ability of mastoparan to inhibit the action of BFA, it is likely that activation of a pertussis toxin-sensitive $\text{G}\alpha$ regulates

Correspondence address: W. Hunziker, Department of Cell Biology, Yale University School of Medicine, New Haven, CT, USA.

binding of ARF and β -COP to the cytoplasmic face of the Golgi complex.

2. EFFECTS OF BFA ON ENDOCYTIC ORGANELLES AND MEMBRANE TRAFFIC

Whilst not previously thought to affect any intracellular organelle other than the Golgi, it has recently become clear that BFA can also target several endocytic organelles in a fashion reminiscent of its action on the Golgi complex. BFA has been found to significantly increase the tubular appearance of the early endosomal compartment involved in recycling of internalized transferrin receptors. As visualized by EM, BFA-induced endosomal tubules display a diameter of ~100 nm and appear to emanate from the centriole region. As is the case for Golgi-tubules, the BFA-induced endosomal tubular network is stabilized by cytoplasmic microtubules and is not observed in the presence of AIF₄ [17-19].

The dramatic morphological changes induced by BFA do not, however, appreciably alter internalization to and recycling from endosomes [17,18]. At least in some cells, however, BFA appears to induce the fusion of the trans-Golgi network (TGN) with the plasma membrane and/or the early endosomal compartment [18,19]. As a result, TGN proteins were found to be delivered to the cell surface from where they were internalized and recycled through a compartment involved in internalization and recycling of transferrin.

Although BFA has been found to exhibit profound morphological effects on endocytic organelles, the drug does not markedly affect internalization, recycling, or delivery of internalized ligands from early endosomes to degradative endocytic compartments [17,18]. However, since degradation in the presence of the drug could also occur in late endosomes which are known to contain degradative enzymes, it remains unclear whether the drug affects transport from endosomes to lysosomes. Although the morphology of late endosomes was not altered by the presence of BFA, lysosomes acquired a more tubular appearance [18].

The morphological effects of BFA on endocytic organelles were even observed in two cell lines, MDCK and PtK1, where high BFA concentrations failed to have an effect on the Golgi complex as evidenced in the persistence of characteristic β -COP-coated Golgi stacks [17,18,20]. Although MDCK and PtK1 cells are derived from kidney of dog and rat kangaroo, respectively, the resistance of these cells to BFA does not reflect an insensitivity of kidney cells, or epithelial cells in general, since the Golgi of a porcine kidney cell line, LLC-PK₁ is sensitive to the drug (unpublished results). Whether resistance of the Golgi to BFA is a species-specific feature, or a more widespread phenomenon, remains to be determined. This restricted effect of the drug on endosomes but not on the Golgi suggests, however, that

the mechanism of the effect of the drug on the two pathways may be similar but certainly not identical.

Thus, while BFA elicits dramatic and widespread morphological alterations to endosomes and lysosomes in diverse cells, it is apparent that these organelles remain largely functional with endocytosis, recycling and delivery to degradative compartments continuing unabated. We have found, however, that the drug selectively and completely blocks one type of endocytic transport event, namely basolateral to apical transcytosis of dIgA mediated by the polymeric immunoglobulin receptor (pIgR) in polarized MDCK cells [17].

As found for transferrin, BFA did not affect internalization or recycling via the pIgR in transfected MDCK cells. Transcytosis of receptor-bound dimeric IgA, however, was almost completely blocked (see Fig. 1). The drug appears to target an early step in the pathway since maximum inhibition is only obtained within 5-10 min after internalization. Furthermore, it is likely that the drug affects transcytosis at a step prior to the microtubule-dependent translocation of transcytotic vesicles from the basolateral to the apical cytoplasm since incubation of dIgA bound to pIgR in the presence of the microtubule-disrupting drug nocodazole allows transport beyond the BFA-sensitive step of the pathway. Indeed, preliminary results suggest that dIgA and transferrin bound to their respective receptors are prevented from segregating in the presence of BFA (W. Hunziker et al., unpublished results). These observations therefore strongly suggest that either sorting of pIgR-dIgA into transcytotic vesicles, and/or the formation of transcytotic vesicles might be affected by BFA.

Intriguingly, despite the inhibitory effect on dIgA transcytosis, BFA has also been reported to increase the transcellular delivery of several normally non-transcytosed proteins [21]. These apparently contradictory findings suggest that transcytosis of membrane might still occur in the presence of BFA, but that the efficient sorting of transcytotic proteins such as the pIgR from early endosomes into transcytotic carriers might specifically be affected. Alternatively, more than one transcytotic pathway might exist and in the presence of BFA transport from basolateral early endosomes across the cell to the apical surface could possibly take place via late endosomes.

3. IMPLICATIONS ON ENDOCYTIC TRANSPORT AND SORTING MECHANISMS

BFA has been found to display striking similarities in its action on the exocytic and endocytic pathways, strongly suggesting fundamental similarities in the biochemical basis of membrane traffic in the two pathways. Most notably, the morphological integrity of organelles in both pathways is severely affected by the drug. This morphological effect is dependent on intact cytoplasmic microtubules and appears to be regulated by GTP γ S

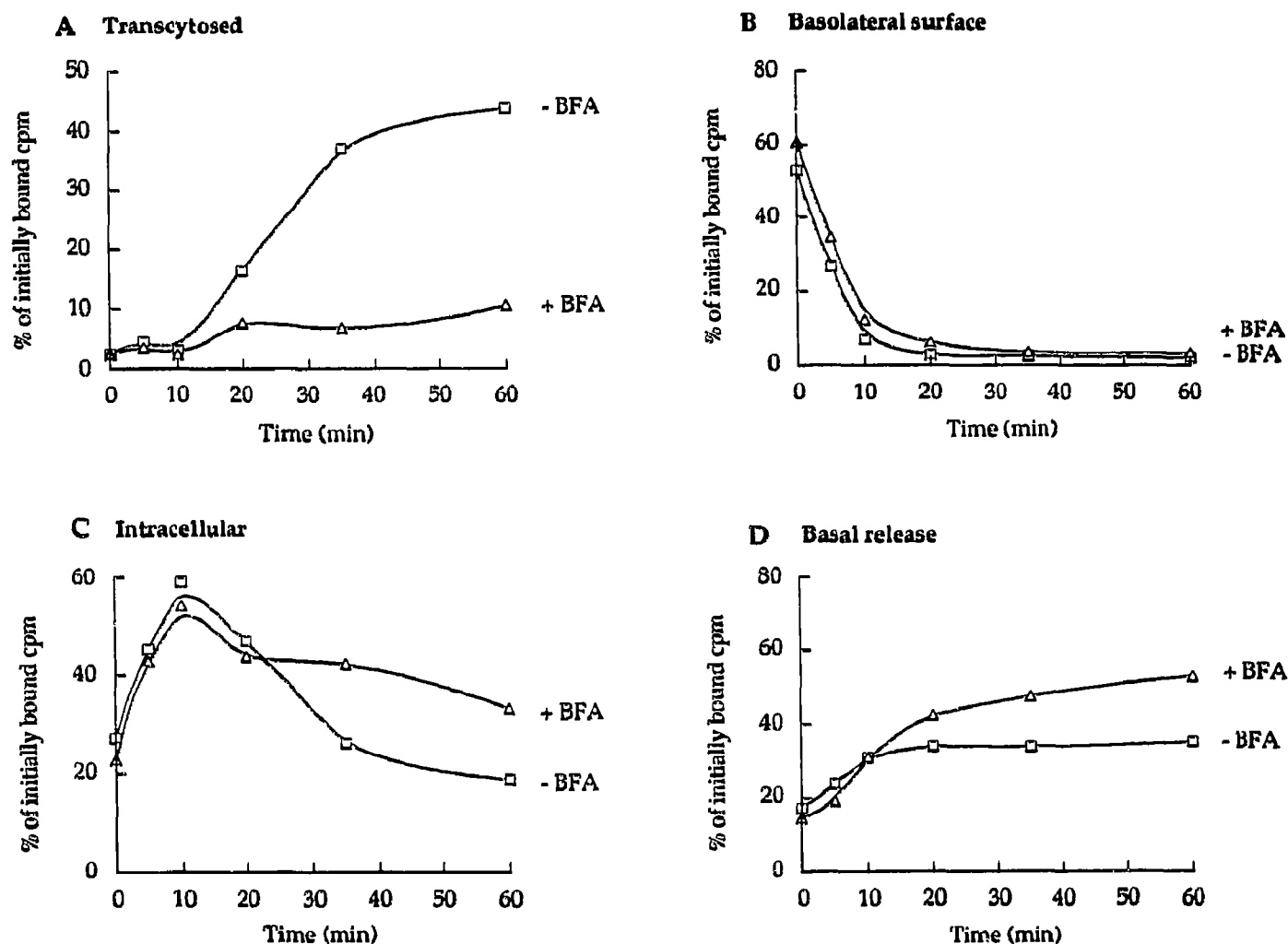


Fig. 1. BFA blocks transcytosis but not internalization or recycling of pIgR. 125 I-dIgA was prebound on ice from the basolateral surface of transfected MDCK cells. Cells were then transferred to 37°C in the presence or absence of BFA. dIgA transcytosed (released into the apical medium and present on the apical surface; panel A), present on the basolateral surface (acid sensitive; panel B), intracellular (acid resistant; panel C) or released into the basolateral medium (panel D) was determined at the indicated periods of time and plotted as % of initially bound dIgA. Points were determined in duplicate and % values differed by less than 15%.

and AlF_4^- in a similar way in both pathways. Since the morphological changes induced by BFA on the Golgi complex appear to directly reflect the association of a distinct non-clathrin coat, it is tempting to assume that the drug likewise targets a similar cytoplasmic coat associated with endocytic organelles. As is the case in the Golgi, such a BFA-sensitive coat could play a role in endosome function and in regulating organelle integrity by preventing tubule formation and homotypic fusion among related structures. In contrast to several BFA-sensitive coat proteins identified so far on Golgi membranes, however, none of the cytoplasmic proteins known to interact with the cytoplasmic face of endosomes are sensitive to BFA. Association of proteins of the rab family with early (rab 4 and rab 5) or late (rab 7) endosomes is not affected by the drug (P. van der

Sluijs, personal communication). Similarly, the α -subunit of the HA2 adaptor complex of the clathrin coat of early endocytic pits and vesicles is not targetted by the drug [13], even though the γ -subunit of the HA1 adaptor complex of the TGN clathrin coat is a target of BFA.

Of particular interest is the selective effect of BFA on pIgR-mediated basolateral to apical transcytosis, and several possible mechanisms can be postulated and experimentally tested (see Fig. 2). Conceivably, the recognition and sorting event that leads to sequestration of pIgR from basolateral early endosomes into transcytotic vesicles may involve an interaction with a β -adaptin/ β -COP-like endosomal coat protein. Alternatively, cytoplasmic coat proteins required for transcytotic carrier formation and/or function, such as the 108

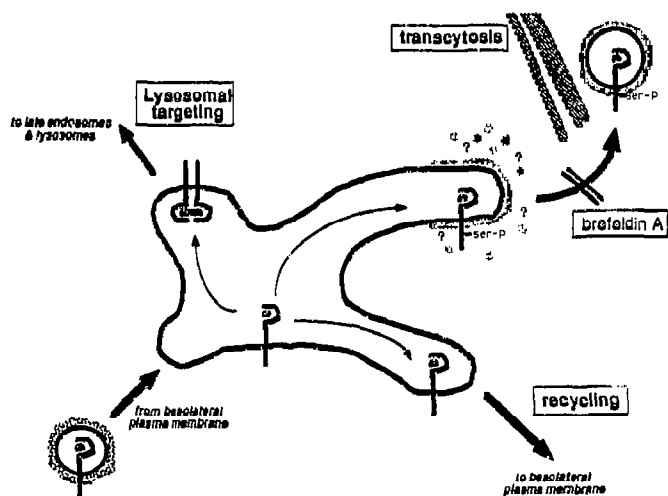


Fig. 2. BFA and sorting in basolateral early endosomes. Shown are the three possible fates of membrane proteins following endocytosis from the basolateral surface: recycling back to the surface of internalization, delivery to late endosomes and lysosomes, and transport across the cell to the opposite surface. BFA selectively affects transcytosis, suggesting that the drug might target a putative coat on endocytic organelles. Such a coat could be required for the segregation of a restricted set of proteins into transcytotic vesicles, or for the formation and/or function of transcytotic carriers.

kDa protein specific for transcytotic vesicles [22], may be affected by BFA.

Over the past few years a number of low molecular GTP-binding proteins of the rab family have been implicated in functions of the endocytic pathway such as early endosome fusion [23]. It has recently become clear that, perhaps not unexpected from their role in regulating coat binding and membrane transport in the exocytic pathway [24], heterotrimeric G proteins may also play a role during different steps in endocytosis. The trimeric G protein activating peptide mastoparan was found to inhibit early endosome fusion [25]. In addition, the stimulatory effect of GTP γ S on fusion was found to be antagonized by $\beta\gamma$ subunits of G proteins. Heterotrimeric G proteins are probably also involved in transcytosis, a pathway also reportedly affected by AIF $_4^-$ in a permeabilized cell system [26]. Whether G proteins also regulate binding of coat proteins to endocytic organelles, however, remains to be determined.

In conclusion, the similar effects of BFA and the involvement of trimeric G proteins in the function of the Golgi and endocytic organelles suggest that the molecular mechanisms of membrane transport in the exocytic and endocytic pathway may be fundamentally similar. Clearly, the identification and characterization of putative coat proteins on endocytic organelles will be critical

for a more detailed understanding of the biochemical basis of membrane transport and sorting during endocytosis.

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