Chapter 6

BIG1 AND BIG2: BREFELDIN A-INHIBITED EXCHANGE FACTORS FOR ARFS
Actions of brefeldin A

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Abstract: Brefeldin A-inhibited guanine nucleotide exchange factor 1 (BIG1) and BIG2 were purified as components of a ~670 kDa protein complex. BIG1 and BIG2 partially colocalize with the Golgi 58 kDa protein and γ-adaptin, a component of the AP-1 complex. The distribution of BIG1 in the TGN is distinct from that of GBF-1, a brefeldin A (BFA)-resistant GEF that was confined to the cis-Golgi. Recently reported studies implicate BIG2 in the transport of lysosomal enzymes between the TGN and late endosomes. Recognition that BFA inhibition of BIG1 and Gea1 is non-competitive was the key to understanding its mechanism of action. [3H]BFA binding was used to define structural requirements for the formation of a BFA-Arf-Sec7 domain complex. A recently published synthesis of structure-function information includes a valuable model of the Arf-Sec7 domain interaction and the mechanism of BFA inhibition.

1. INTRODUCTION

Brefeldin A (BFA) is a fungal, fatty acid metabolite described over 30 years ago as an anti-viral agent (Tamura et al., 1968). Later, investigation of its effect on protein synthesis in cultured rat hepatocytes revealed that protein secretion was completely inhibited by BFA at concentrations that were without effect on protein synthesis (Misumi et al., 1986). This and subsequent work demonstrated that BFA reversibly blocked transport of protein from the ER to Golgi and caused apparent disintegration of Golgi structure (Fujiwara et al., 1988). Only three years later, studies of BFA action by several groups had resulted in significant clarification of the processes of ER-Golgi transport, anterograde and retrograde (Orci et al., 1991).
Since Palade (1975) described a vesicular transport process for protein secretion, the use of cell-free systems to characterize biochemically and microscopically individual steps in membrane traffic pathways has accelerated the recognition that analogous molecular mechanisms and machinery are employed at multiple sites in all eukaryotic cells. Present understanding of these processes is based on work of many investigators including major contributions, experimental and conceptual, from the Rothman laboratory. In the Rothman model (Rothman, 1994), inactive, cytosolic Arf\textbullet\text{GDP} interacts with a guanine nucleotide-exchange protein (GEF), which catalyzes the replacement of bound GDP with GTP generating Arf\textbullet\text{GTP} that moves to a Golgi membrane, where it recruits coatamer (a cytosolic complex of seven coat proteins) to initiate vesicle formation. Sequential accumulation of Arf\textbullet\text{GTP} and coatamer results in deformation of the membrane to form a bud. By fusion of the donor membrane at the base of the bud (fission), a transport vesicle is released. Demonstration of bud and vesicle formation from lipid model membranes (liposomes) later confirmed that the only additions required were Arf\textbullet\text{GTP} and coatamer (Spang et al., 1998).

After the arrival of a coated vesicle at its target membrane and before membrane fusion can complete the transport process, release of coatamer must occur. This was believed to be triggered by the hydrolysis of Arf-bound GTP, followed sequentially by dissociation of Arf\textbullet\text{GDP} and coatamer. Earlier this year, a publication by Lippincott-Schwartz and co-workers provided valuable new insight into the dynamics of Arf and coatamer interactions in vesicle formation (Presley et al., 2002). Based on detailed quantification and comparison of movements of fluorescently labeled Arf1 and ß-COP in living cells, they established clearly that inactivation of Arf and release of Arf\textbullet\text{GDP} is required for release of coatamer. In the cell, however, dissociation of Arf from Golgi membranes was much more rapid than that of coatamer, i.e., release of Arf did not “trigger” release of coatamer. As was pointed out, the continuing Arf-dependent recruitment and independently regulated release of coatamer during vesicle formation is consistent with the critical functions of coatamer in the modification of membrane morphology (composition) and the assembly/accumulation of appropriate cargo through direct and indirect interactions with phospholipids and other proteins (Presley et al., 2002). BFA was, in fact, employed to advantage in these studies to inhibit vesicle formation from Golgi membranes.

In 1992, two groups had reported simultaneously that Golgi membranes accelerated \textit{in vitro} binding of GTP to Arf, and this GEF activity was inhibited by BFA (Donaldson et al., 1992; Helms and Rothman, 1992). Purification of a BFA-inhibited GEF was finally reported in 1996 (Morinaga