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Review

Multiple activities for Arf1 at the Golgi complex

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Abstract

The Arf family of GTPases regulates membrane traffic and organelle structure. At the Golgi complex, Arf proteins facilitate membrane recruitment of many cytoplasmic coat proteins to allow sorting of membrane proteins for transport, stimulate the activity of enzymes that modulate the lipid composition of the Golgi, and assemble a cytoskeletal scaffold on the Golgi. Arf1 is the Arf family member most closely studied for its function at the Golgi complex. A number of regulators that activate and inactivate Arf1 on the Golgi have been described that localize to different regions of the organelle. This spatial distribution of Arf regulators may facilitate the recruitment of the coat proteins and other Arf effectors to different regions of the Golgi complex.

Keywords: Arf1; Golgi complex; COPI; Adaptor protein; Membrane traffic; Phosphoinositide

1. Introduction

The Golgi complex, a series of flattened cisternal discs and complex tubular membranes, has intrigued cell biologists for over 100 years. This unique structure and organization can be attributed to the recruitment of a diverse group of scaffolding, tethering and structural coat proteins onto membranes that have a distinct membrane lipid composition [1,2]. The build-up of this complex organelle structure is dependent upon the activities of small Ras-related GTPases of the Rab and Arf families. GTP-binding proteins function through a cycle of GTP-binding and GTP-hydrolysis leading to the GTP-bound, active form and the GDP-bound inactive form of the protein, respectively. The Arf protein was originally identified and named as the "ADP-ribosylation factor" (Arf) due to its activity as a cofactor in the cholera toxin-catalyzed ADP-ribosylation of the α-subunit of the Gs heterotrimeric G protein [3]. Its role in Golgi membrane traffic, however, was first suggested in a landmark study that showed that Arf1 was essential for the growth and the secretory pathway in the yeast *Saccharomyces cerevisiae* and that Arf1 localized to the Golgi complex in mammalian cells [4]. Then, when Arf activation was shown to be required for the recruitment of many cytosolic coat protein complexes onto the Golgi membrane and the target of Brefeldin A-induced disassembly of the Golgi complex [5–7], its critical role in Golgi organization and function was well established. Since Arf1's role in the recruitment of various coat protein complexes to the Golgi will be covered in other reviews in this issue, here we will emphasize the spatial and temporal events that link Arf activation and inactivation with effectors at particular sites of function on the Golgi.

Arf proteins have been identified in every eukaryotic organism examined. All Arfs are myristoylated at the amino terminus, a lipid modification that is required for membrane binding and biological function. Arf proteins have been divided into three classes based on amino acid sequence analysis [8]. There are six mammalian Arf proteins. Arf1, 2 and 3 comprise Class I; Arf4 and 5 comprise Class II; and Arf6 is the sole member of Class III. All organisms have a Class I Arf; for *Giardia*, it is the sole Arf in that organism [9]. *S. cerevisiae* has two Class I (yArf1 and 2) and one

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Class III (yArf3) but no Class II Arf. Multicellular organisms appear to have at least one member of each class [9]. Class I Arfs are associated with and function at the Golgi complex and are the most thoroughly studied. Human Arf1 and Arf3 localize to the Golgi complex (humans, but not rodents, lack an Arf2) and as there are only 7 amino acid differences between these two, they might function interchangeably, although this needs to be examined. There is some evidence that Arf5, a Class II Arf, may function at the Golgi [10–12], however, the cellular localizations and functions of Arf4 and 5 have not been systematically studied. Arf6 and the yeast homologue, yArf3, on the other hand are found at the plasma membrane and do not localize to nor do they function at the Golgi complex [13–15]. There are also a number of Arf-like or Arl proteins that associate with the Golgi complex that will be discussed later.

Upon activation, Arf1 associates with Golgi membranes and, upon inactivation, Arf1 is released from Golgi membranes into the cytosol (Fig. 1). This cycle of association and dissociation is regulated by Golgi-associated, guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) (see below). The membrane/cytosol cycling of Arf1 has been observed in biochemical assays [5] and in living cells [16,17] raising the question as to how cytosolic Arf1 is recruited specifically back on to Golgi membranes. Do other Arfs (i.e. Arfs 3, 4, and 5) also associate with the Golgi and how are they recruited there? There is evidence in biochemical assays for a weak association of Arf1-GDP with membranes through the myristoyl moiety that precedes nucleotide exchange catalyzed by GEF on the membrane [18]. Although it is possible that the Golgi-associated GEFs recruit Arf1-GDP to the Golgi membrane, there are studies suggesting that other membrane proteins could serve as initial Arf1-GDP receptors (Fig. 1). The p23/24 proteins are abundant integral membrane proteins associated with the ER and early Golgi and are thought to act as cargo receptors for secretory proteins that exit the ER [19]. Evidence for an association of the cytoplasmic domain of p23/24 with Arf1-GDP has been found in cross-linking experiments in vitro [20] and in live cell FRET interactions [17]. Another study demonstrated that the yeast ER–Golgi SNARE proteins, including Sec22, bos1 and bet1, could bind to yArf1-GDP in vitro [21]. Recently, we reported that human Arf1-GDP is targeted to the early Golgi by binding to membrin, the human homolog of Bos1p [22]. This interaction detected in vitro and in vivo involves conserved amino acid residues in the α-3 helix in Arf1 [22]. This binding of Arf1 to membrin is likely to bring Arf1 to the early Golgi where it could also associate with p23/24 prior to activation at the membrane.

2. Arf GEFs associated with the Golgi complex

Although a guanine nucleotide exchange factor (GEF) activity towards Arf1 had been demonstrated in Golgi membrane preparations in the early 1990s [5], it was not until 1996 that the Arf GEFs were identified by Jackson and colleagues in both yeast and mammalian genomes [23,24]. All Arf GEFs contain a Sec7 domain that comprises the catalytic core responsible for nucleotide exchange. There are four families of Arf GEFs, the Gea/GBF1, Sec7/BIG1/BIG2, ARNO/cytohesin and EFA6 [25]. The Gea/GBF1 and Sec7/BIG members localize to the Golgi and generally are inhibited by BFA whereas the ARNO and EFA6 families are not. Although GBF1 was originally identified as a GEF resistant to BFA [10], recent studies indicate that GBF1 is in fact sensitive to BFA in cells [26,27].

In animal cells and in yeast, the Gea/GBF1 and Sec7/BIG GEFs associate with different regions of the Golgi complex (Fig. 2A). In mammalian cells, in particular, GBF1 is associated with tubular-vesicular structures associated with

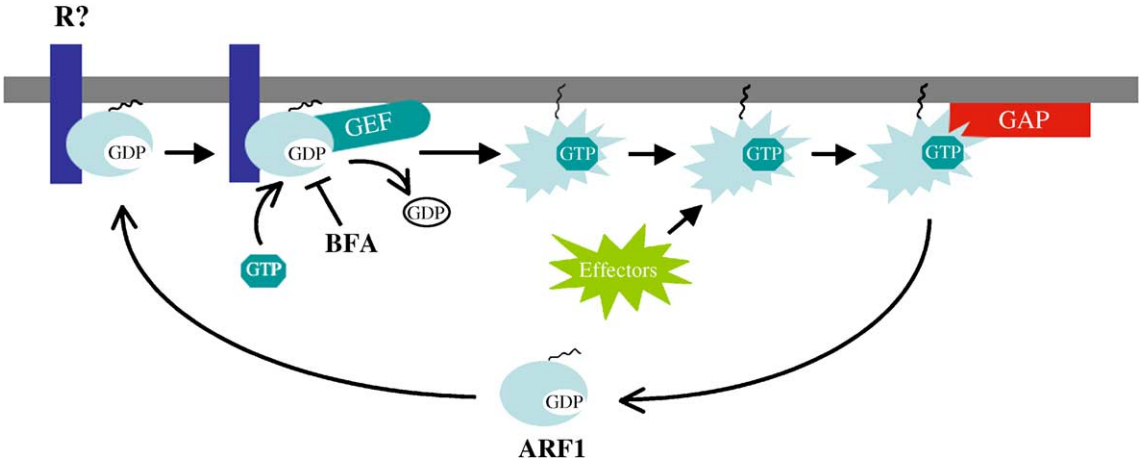

Fig. 1. Membrane–cytosol cycle of Arf1. Cytosolic myristoylated Arf1-GDP initially binds to the membrane surface weakly and possibly in combination with binding to a transmembrane receptor (R) (such as p23/24 or SNARE protein) prior to the nucleotide exchange of GTP for GDP catalyzed by membrane-associated, BFA-inhibited GEFs (GBF1 or BIG1,2). BFA inhibits nucleotide exchange by forming an uncompetitive complex of GEF–Arf–GDP–BFA [117]. Once GTP-bound, Arf1 binds tightly to Golgi membranes and recruits and interacts with effectors prior to termination by GTP hydrolysis catalyzed by Arf GAP proteins.