Background: Arfaptin-2 is recruited onto Golgi membranes through interacting with Arl1 and induces membrane deformation.

Results: A crescent-shaped homodimer of the Arfaptin-2 BAR domain is flanked by two Arl1 molecules, leaving the concave face open for membrane association.

Conclusion: Arl1 determines the membrane binding specificity of Arfaptin-2 BAR.

Significance: Similar mechanisms underlie targeting of various BAR domain proteins to specific membranes.

Membrane-sculpting BAR (Bin/Amphiphysin/Rvs) domains form a crescent-shaped homodimer that can sense and induce membrane curvature through its positively charged concave face. We have recently shown that Arfaptin-2, which was originally identified as a binding partner for the Arf and Rac1 GTPases, binds to Arl1 through its BAR domain and is recruited onto Golgi membranes. There, Arfaptin-2 induces membrane tubules. Here, we report the crystal structure of the Arfaptin-2 BAR homodimer in complex with two Arl1 molecules bound symmetrically to each side, leaving the concave face open for membrane association. The overall structure of the Arl1-Arfaptin-2 BAR complex closely resembles that of the PX-BAR domain of sorting nexin 9, suggesting similar mechanisms underlying BAR domain targeting to specific organelar membranes. The Arl1-Arfaptin-2 BAR structure suggests that one of the two Arl1 molecules competes with Rac1, which binds to the concave face of the Arfaptin-2 BAR homodimer and may hinder its membrane association.
and Rac1 GTPases mediated by Arfaptin-2 remains to be elucidated.

Arfaptins are known to localize to the Golgi region when exogenously expressed in cells (2, 13). We have recently shown that both Arfaptin-1 and Arfaptin-2 interact not only with Arf GTPases but also with Arf-like 1 (Arl1), yet are recruited to trans-Golgi membranes through interaction with Arl1, rather than with the Arf GTPases (14). Arfaptin-2 colocalizes with Arl1 at dynamic vesicular and tubular structures emanating from the Golgi, suggesting that Arl1 may regulate Arfaptin-mediated membrane deformation at the trans-Golgi (14). Here, we report the crystal structure of the BAR domain of Arfaptin-2 in complex with Arl1; in the present study, we selected Arfaptin-2, because in our previous study we characterized more extensively the interaction of Arfaptin-2 with Arl1 than that of Arfaptin-1 and because only the structure of Arfaptin-2 in complex with Rac1 was reported. The structure shows that two molecules of Arl1 bind symmetrically on each side of the crescent-shaped homodimer of Arfaptin-2 BAR, leaving the concave face open for membrane association. Thus, unlike Rac1, which interferes with membrane binding of Arfaptin-2 BAR, Arl1 can recruit Arfaptin-2 onto membranes. Our study provides the structural basis for recruitment of Arfaptins to Golgi membranes by Arl1 and initiation of membrane remodeling.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Site-directed Mutagenesis—A bacterial expression vector for full-length human Arfaptin-2 fused to glutathione S-transferase (GST) was constructed as described previously (14). An expression vector for the GRIP domain of human golgin-245 (residues 2170–2230) (GE Healthcare), Construction of mammalian expression vectors for C-terminal HA-tagged human Arl1, C-terminal EGFP- or mCherry-tagged human Arl1, and N-terminal EGFP-tagged human Arfaptin-2 was described previously (14–16). Point mutations were introduced into Arl1 and Arfaptin-2 cDNA using the QuikChange Lightning Site-directed Mutagenesis kit (Agilent Technologies). For crystallization, a bacterial expression vector for N-terminal truncated human Arl1(Q71L) (residues 14–181) (referred to as Arl1 in the context of protein purification and x-ray crystallography) was constructed by cloning a cDNA fragment into pGEX-6P-1. The GST tag was cleaved off on the column with PreScission protease (GE Healthcare) or thrombin (GE Healthcare). The eluted proteins were purified by gel filtration chromatography using Superdex-200 HR 10/30 (GE Healthcare) with gel filtration buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM MgCl2, and 0.1% β-mercaptoethanol). Prior to gel filtration, Arl1 was incubated in gel filtration buffer containing 5 mM disodium EDTA and 1 mM Gpp(NH)p (Sigma) overnight at 4 °C.

For the SPR experiments, cDNA fragments for human Arl1(Q71L) (residues 14–181) and full-length human Rac1(1–192) harboring a G12V mutation (17), a kind gift from Manabu Negishi, Kyoto University), were cloned into the expression vector pET28a (Novagen). The proteins were overexpressed in the E. coli BL21(DE3) strain by induction with 0.3 mM isopropyl β-D-thiogalactopyranoside at 26 °C. The harvested cells were resuspended in PBS containing 0.1% β-mercaptoethanol, lysed by sonication, and clarified by centrifugation. The supernatant was then purified with a nickel-nitritoltriacid-agarose column (Qiagen), and eluted with buffer containing 10 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM MgCl2, 0.1% β-mercaptoethanol and a gradient of imidazole.

Crystallization—Purified Arl1-Gpp(NH)p and Arfaptin-2 BAR were concentrated using Amicon Ultra 15 10,000 MWCO (Millipore) in 10 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM MgCl2, and mixed to yield final concentrations of 0.7 and 1.9 mg/ml, respectively (1:2 molar ratio). The crystallization condition was first screened using a protein crystallization system (18) and subsequently refined. The crystal of Arl1-Gpp(NH)p-Bar1 was obtained with sitting drop vapor diffusion with precipitant solution containing 100 mM HEPES, pH 7.5, 200 mM ammonium sulfate, and 25% (v/v) PEG3350 at 25 °C.

Structure Determination and Refinement—Crystals were initially screened at beamlines BL-5A and AR-NW12A at Photon Factory. The diffraction dataset was collected at the beamline BL41XU at Spring-8 (Hyogo, Japan). The dataset was processed with HKL2000 (19) and the CCP4 program suite (20). The crystal belongs to space group P212121. The initial phases were obtained with Phaser (21) using Arfaptin-2 (PDB code 1I49) and Arl1 from the Arl1-GRIP domain complex (PDB code 1UPT) as search models. The refinement was carried out using Refmac (22) and Coot (23). All molecular graphics were prepared with PyMOL (24). The final structure of Arl1-Arfaptin-2 BAR contained residues 15–179 of Arl1 (both chains) and residues 119–311 of Arfaptin-2 (PDB code 5219) and Arl1 from the Arl1-GRIP domain complex (PDB code 1UPT) as search models. The refinement was carried out using Refmac (22) and Coot (23). All molecular graphics were prepared with PyMOL (24). The final structure of Arl1-Arfaptin-2 BAR contained residues 15–179 of Arl1 (both chains) and residues 119–311 (chain C) and residues 120–315 (chain D) of Arfaptin-2 BAR. The model was refined to 3.0-Å resolution with crystallographic Rwork = 26.4% and Rfree = 33.3%. Data collection and refinement statistics are shown in Table 1. The coordinate and structure factor of Arl1-Gpp(NH)p-Arfaptin-2 BAR have been deposited to Protein Data Bank under accession code 4DCN.

Pulldown Assay—A pulldown assay of Arl1 with Arfaptin-2 or the golgin-245 GRIP domain fused to GST was performed as described previously (14). Monoclonal rat anti-HA antibody (clone 3F10) (Roche Applied Science) and horseradish peroxidase-conjugated secondary antibody (Jackson Immuno-Research Laboratories) were used for immunoblotting.

DNA Transfection, RNA Interference, Immunofluorescence Analysis, and Time-lapse Recording—Culture of HeLa cells and transfection of expression plasmids were carried out as described previously (14, 15). siRNA-mediated Arl1 knockdown and rescue experiments were performed as described previously (14, 16). Immunofluorescence analysis was per-
formed as described previously (14–16). Antibodies used were: polyclonal rabbit anti-Arfaptin-2 (Zymed Laboratories Inc.), monoclonal mouse anti-golgin-245 (BD Biosciences), polyclonal rabbit anti-TGN46 (25) (a kind gift from Minoru Fukuda, Sanford-Burnham Institute), and Alexa Fluor-conjugated secondary antibodies (Molecular Probes). Time-lapse analysis of cells expressing Arl1-mCherry and EGFP-Arfaptin-2 was performed using an A1R-MP confocal microscope (Nikon) as described previously (14).

Surface Plasmon Resonance Experiments—Surface plasmon resonance (SPR) experiments were performed using a Biacore2000 biosensor (GE Healthcare) at 25 °C. GST-fused Arfaptin-2 BAR was immobilized with anti-GST coupled to a CM5 sensor chip (GE Healthcare). Analyte proteins (Arl1 and/or Rac1) were injected for 80 s to achieve near equilibrium in buffer containing 20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 3 mM MgCl₂, and 0.005% surfactant P20 at a flow rate of 20 μl/min. A combination of N-terminal His-tagged Rac1(G12V) at 0, 0.5, 2, 6, or 10 μM and N-terminal His-tagged Arl1(Q71L:14–181) at 0, 3, 6, 12, or 18 μM was injected. The concentrations of analytes were adjusted by dilution with the buffer described above. Regeneration buffer containing 10 mM glycine, pH 2.2, was injected prior to each analyte injection. The measurement of each series was subtracted with a zero-concentration curve prior to further analysis. Dissociation constants were calculated from resonance units at equilibrium using the following equation: $R_{eq} = R_{max} \times \text{Concentration} / (\text{Concentration} + K_d)$. 

### RESULTS

**Crystal Structure of the Arl1-Arfaptin-2 BAR Complex**—To elucidate the molecular basis for Arl1-dependent recruitment of Arfaptins onto Golgi membranes and subsequent Arfaptin-dependent membrane tubulation (14), we determined the crystal structure of the complex formed between an N-terminal truncated form of Arl1(Q71L), a GTP-locked mutant (hereafter referred to as Arl1 in the context of the crystal structure) and the BAR domain of Arfaptin-2 in the presence of a GTP analog, Gpp(NH)p, at 3.0-Å resolution. The asymmetric unit of the crystal contained an Arfaptin-2 BAR homodimer flanked by two Arl1 molecules (Fig. 1, A and B). The dimeric structure of Arfaptin-2 BAR in the complex closely resembles the previously reported structure of the free form (12) with a root mean square deviation of 2.0 Å for all Cα atoms. Each Arfaptin-2 BAR monomer unit comprises an antiparallel three-helix bundle. The monomers associate to form a crescent-shaped dimer mainly through interactions between the α1 helices and between the N-terminal region of α2 from one monomer and the C-terminal region of α3 from the other. The two Arl1 molecules bind symmetrically to α2 and α3 of each Arfaptin-2 BAR monomer, around the kinks of the two helices. Unlike the previously reported Rac1-binding site (12), the Arl1-binding sites of the Arfaptin-2 BAR dimer are located outside of the concave membrane-binding face. The N-terminal amphipathic helical region of Arl1 (residues 1–13), which was removed in our construct for crystallization, is expected to adopt the same orientation as the concave face of the Arfaptin-2 BAR dimer. Because the amphipathic helix, along with the N-terminal attached myristoyl moiety, participates in membrane anchoring of Arl1, the crystal structure indicates that the two Arl1 molecules facilitate the direct association of the concave face of the Arfaptin-2 dimer with membranes (see “Discussion”).

The Arl1-Arfaptin-2 BAR interface is largely hydrophobic, with 725 Å² of buried surface area upon complex formation (26). The α3 helix of the Arfaptin-2 BAR domain interacts mainly with the switch 2 and interswitch regions of Arl1, and makes some peripheral interactions with the switch 1 region (Fig. 1, C and E). Phe-285 of α3 is surrounded by a hydrophobic pocket formed by Gly-50 of switch 1, Phe-51 of the interswitch region, and Ile-281 from switch 2 from the other. The two Arl1 molecules bind symmetrically to α2 and α3 of each Arfaptin-2 BAR monomer, around the kinks of the two helices. Unlike the previously reported Rac1-binding site (12), the Arl1-binding sites of the Arfaptin-2 BAR dimer are located outside of the concave membrane-binding face. The N-terminal amphipathic helical region of Arl1 (residues 1–13), which was removed in our construct for crystallization, is expected to adopt the same orientation as the concave face of the Arfaptin-2 BAR dimer. Because the amphipathic helix, along with the N-terminal attached myristoyl moiety, participates in membrane anchoring of Arl1, the crystal structure indicates that the two Arl1 molecules facilitate the direct association of the concave face of the Arfaptin-2 dimer with membranes (see “Discussion”).

The hydrophobic triad patch is conserved in the Arf/Arl and Rab families of GTPases and is generally involved in effector binding (27). The triad patch of Arl1, formed by Tyr-81 of Arl1 switch 2, whereas Ile-281 of α3 makes contact with Tyr-77 and Cys-80 of Arl1 switch 2 (Fig. 1, C and E). Apart from the hydrophobic pocket, there are also hydrogen bonds between the side chain Nδ atom of Asn-289 in BAR α3 and the main chain O atom of Phe-51 in the interswitch region, and between Nζ of Lys-292 in α3 (PDB chain D) and Oδ of Asn-52 in the interswitch region (PDB chain B) (Fig. 1, C and E).

### TABLE 1

| Data collection, phasing, and refinement statistics |
|-----------------------------------------------|
| **Data collection**                           |
| Space group                                   |
| P2₁,2,2,2                                    |
| Cell dimensions                               |
| a, b, c (Å)                                   |
| 62.7, 111.1, 119.8                           |
| α, β, γ (°)                                   |
| 90.0, 90.0, 90.0                             |
| Resolution (Å)                                |
| 43.3-3.0 (3.1-3.0)                           |
| Rmerge                                        |
| 9.4 (46.2)                                    |
| l/a1                                          |
| 11.4 (2.4)                                    |
| Completeness (%)                              |
| 96.5 (93.4)                                   |
| Redundancy                                    |
| 3.5 (3.4)                                     |
| **Refinement**                                |
| Resolution (Å)                                |
| 43.3-3.0                                      |
| No. reflections                               |
| 16567                                         |
| Rmerge/ Rwork                                |
| 26.4/33.3                                     |
| No. atoms                                     |
| Protein                                       |
| 5735                                         |
| Mg                                           |
| 2                                            |
| Gpp(NH)p                                     |
| 64                                           |
| Water                                        |
| 4                                            |
| B-factors                                    |
| Protein                                       |
| 51.6                                         |
| Mg                                           |
| 19.9                                         |
| Gpp(NH)p                                     |
| 47.3                                         |
| Water                                        |
| 25.6                                         |
| Root mean square deviations                  |
| Bond length (Å)                               |
| 0.007                                        |
| Bond angles (°)                               |
| 1.022                                        |

**Structure of the Arl1-Arfaptin-2 BAR Complex**
with the Nη Arg-19 side chain in Arl1 β1 (3.1 Å for chain A and 3.6 Å for chain B of Arl1) (Fig. 1, D and E). Lys-216 of BAR α2 makes van der Waals contacts with Gln-64 of the interswitch and may interact ionically with Glu-17 of the N-terminal loop of Arl1 (Fig. 1, D and E).

We previously showed that Arf1, Arf3, Arf5, and Arf6 also bind to Arfaptins (14). The residues of Arf1 involved in the interaction with Arfaptin-2 BAR are conserved in the Arf GTPases, except for Gln-64, Tyr-77, and Cys-80 (supplemental Fig. S1A), suggesting that Arfs bind to Arfaptin-2 BAR in essentially the same manner as Arl1. The residues of Arfaptin-2 that interact with Arl1 are well conserved in Arfaptin-1, also suggesting similar interactions with Arf/Arl GTPases (supplemental Fig. S1B).

Arl1-dependent Recruitment of Arfaptin-2 onto Golgi Membranes and Subsequent Arfaptin-dependent Membrane Tubulation Revealed by Structure-based Mutational Analysis—To verify the interface between Arl1 and Arfaptin-2 BAR in the crystal, we introduced mutations into Arl1 and Arfaptin-2. We focused on hydrophobic interactions between Phe-285 of Arfa-
ptin-2 and Gly-50 in the switch 1, Phe-51 in the interswitch, and Tyr-81 in the switch 2 of Arl1, because the latter two Arl1 residues, together with Trp-66, form the triad patch (27). We were also interested in a possible ionic interaction between Glu-17 within the Arl1 N-terminal loop and Lys-216 of Arfaptin-2, because the importance of the N-terminal residue of the Arf/Arl family GTPases in effector binding has not been reported.

As described previously (14), wild-type Arfaptin-2 fused to GST efficiently pulled down Arl1(Q71L) (Fig. 2A, lane 14), a GTP-locked active mutant, but not Arl1(T31N) (Fig. 2A, lane 15), a probable nucleotide-free mutant (28). Substitution of Glu-17 in the N-terminal loop (Fig. 2A, lane 11) or Phe-51 in the interswitch (Fig. 2A, lane 12) with alanine did not significantly affect the interaction of Arl1(Q71L) with GST-Arfaptin-2(WT). Substitution of Gly-50 in switch 1 with Thr did not significantly affect the interaction either (Fig. 2B, lane 13). In contrast, the Y81A mutation in switch 2 almost completely abolished the Arl1(Q71L) interaction with GST-Arfaptin-2(WT) (Fig. 2A, lane 13, and B, lane 12). The W66A mutation in the interswitch also extremely reduced the interaction (Fig. 2B, lane 10). When a K216A or F285A mutation was introduced into GST-Arfaptin-2, only the latter abolished the ability of GST-Arfaptin-2 to pull down Arl1(Q71L) (see Fig. 2A, lanes 19 and 24), suggesting that the possible ionic interaction between Glu-17 of Arl1 and Lys-216 of Arfaptin-2 is dispensable for complex formation. Together, these results indicate that the hydrophobic interaction between Tyr-81 in the switch 2 of Arl1 and Phe-285 of Arfaptin-2 and the hydrophobic interactions involving Trp-66 in the interswitch of Arl1, are critical for maintaining the overall Arl1-Arfaptin-2 complex, whereas Gly-50 in switch 1 and

FIGURE 2. Recruitment of Arfaptin-2 to Golgi membranes determined by interaction with Arl1. A, structure-based mutational analysis of residues required for the Arl1-Arfaptin-2 interaction. Lysates of HeLa cells expressing C-terminal HA-tagged Q71L/E17A, Q71L/F51A, Q71L/Y81A, Q71L, or T31N Arl1 mutant, as indicated, were pulled down with GST (lanes 1–5), GST-Arfaptin-2(WT) (lanes 11–15), GST-Arfaptin-2(K216A) (lanes 16–20), or GST-Arfaptin-2(F285A) (lanes 21–25), and subjected to SDS-PAGE and immunoblotting with anti-HA antibody. B, structure-based mutational analysis of residues required for the Arl1-Arfaptin-2 interaction. Lysates of HeLa cells expressing the C-terminal HA-tagged Q71L, Q71L/W66A, Q71L/G50T, or Q71L/Y81A Arl1 mutants, as indicated, were pulled down with GST (lanes 5–8) or GST-Arfaptin-2(WT) (lanes 9–12), then subjected to SDS-PAGE and immunoblotting with anti-HA antibody. C, F285A mutation abolishes association of Arfaptin-2 with the Golgi. HeLa cells were transfected with an expression vector for N-terminal EGFP-tagged Arfaptin-2(WT) (upper panel) or Arfaptin-2(F285A) (lower panel), and subjected to double immunostaining with antibodies against golgin-245 and TGN46. Cells expressing the EGFP-Arfaptin-2 construct are indicated by green asterisks. Note that exogenous expression of EGFP-Arfaptin-2(WT), but not EGFP-Arfaptin-2(F285A), resulted in a diminished signal for golgin-245 (but not TGN46) on the Golgi, probably due to sequestration by overexpressed Arfaptin-2 of endogenous Arl1, which is also required for membrane recruitment of golgin-245. Also note that the nuclear signal of EGFP-Arfaptin-2(F285A) may be due to an inherent property of EGFP.

TGN, trans-Golgi network. Bar, 10 μm.
Phe-51 in the interswitch of Arl1 are less important for complex formation.

Using the Arfaptin-2 mutant, we next examined whether the interaction of Arfaptin-2 with Arl1 is critical for its association with Golgi membranes. When EGFP-tagged Arfaptin-2(WT) was expressed in HeLa cells, it exhibited a perinuclear Golgi-like distribution (Fig. 2C, upper panels). Notably, in cells with exogenous Arfaptin-2 expression (indicated by green asterisks), Golgi association of endogenous golgin-245 was significantly diminished, probably due to sequestration of endogenous Arl1 by overexpressed Arfaptin-2, as described previously (14). In contrast, EGFP-Arfaptin-2(F285A) was distributed throughout the cytoplasm and did not show distinct Golgi localization (Fig. 2C, lower panels). Furthermore, localization of golgin-245 was not affected by expression of the Arfaptin-2 mutant (cells indicated by green asterisks). Thus, association of Arfaptin-2 with the Golgi correlates well with its ability to bind Arl1. In the previous study (14), we showed that exogenously expressed Arfaptin-2 induces vesicular and tubular intermediates from Golgi membranes. To directly show that the membrane tubulation is coupled to the Arfaptin-2 recruitment onto Golgi membranes, we compared the dynamic distributions of wild-type and mutant Arfaptin-2 by time-lapse recording and semi-quantification of the frequency of the vesicle and tubule formation. When expressed alone (see Ref. 14) or in combination with Arl1-mCherry (Fig. 3, A and C, and supplemental Video S1), EGFP-Arfln-2(WT) was found on dynamic vesicular and tubular structures emanating from the Golgi region, as described previously (14). It is also noteworthy that incorporation of Arl1-mCherry into the vesicular and tubular intermediates was significantly increased in the EGFP-Arfln-2-expressing cells, compared with Arl1-mCherry coexpressed with EGFP as a negative control (Fig. 3C and supplemental Video S1). In stark contrast, EGFP-Arfln-2(F285A) was not observed on any membranous structures including the Golgi but distributed throughout the cytoplasm, and the frequency of Arl1-mCherry-positive vesicle and tubule formation when coexpressed with EGFP-Arfln-2(F285A) was not significantly different from that coexpressed with EGFP (Fig. 3B and C, and supplemental Video S2). Thus, association of Arfaptin-2 with Golgi membranes through interacting with Arl1 is essential for Golgi membrane tubulation.

Differential Effects of Arl1 Mutations on Interaction with Arfaptin-2 and Golgin-245 and Their Golgi Association—We then examined whether mutations of the Arl1 residues described above (except for E17A) differentially affect its interaction with Arfaptin-2 and golgin-245, because previous studies showed that these residues are important for the interaction between Arl1 and the GRIP domain of golgin-245 (29, 30). Introduction of the Y81A mutation, but not the F51A mutation, into Arl1(Q71L) greatly diminished its ability to bind Arfaptin-2 (Fig. 4A, lanes 9 and 10), as described above. In striking contrast, both mutations abolished the binding of Arl1(Q71L) to the golgin-245 GRIP domain (lanes 13 and 14) (see “Discussion”).

The crystal structure of the Arl1-GRIP complex demonstrated that the hydroxyl group of the tyrosine residue is critical for making a hydrogen bond with the hydroxyl group of Tyr-2177 of golgin-245 (29, 30), but does not participate in hydrogen bonding with any Arfaptin-2 residues (Fig. 1, C and E). Therefore, we constructed another Arl1 mutant, Y81F. As shown in Fig. 4A, lane 11, Arl1(Q71L/Y81F) retained the ability to bind Arfaptin-2, in contrast to Arl1(Q71L/Y81A) (lane 10). On the other hand, the Y81F mutation of Arl1(Q71L), like the Y81A mutation, abolished its interaction with golgin-245 GRIP (lanes 14 and 15).

We next explored whether the differential in vitro binding ability of the Arl1 mutants correlated with their capacity to recruit Arfaptin-2 and golgin-245 to Golgi membranes in cells. HeLa cells were first transfected with Arl1 siRNAs to deplete cellular Arl1, then transfected with an expression vector for wild-type or mutant Arl1-EGFP. After fixation, cells were dou-
bly immunostained with anti-Ardaptin-2 and anti-golgin-245 antibodies (Fig. 4, B and C, also see Fig. 4D for semi-quantification of the immunofluorescence data). Expression of wild-type or mutant Ard1-EGFP in control cells did not affect Golgi localization of Ardaptin-2 or golgin-245 (Fig. 4, cells with green asterisks). Treatment of cells with Arl1 siRNAs resulted in loss of distinct Golgi localization of both Ardaptin-2 and golgin-245 (Fig. 4, cells without green asterisks). Expression of wild-type Ard1-EGFP restored the Golgi localization of both proteins (Fig. 4, top row, cells with green asterisks) as described previously (14, 16). In contrast, exogenous expression of Ard1(F51A)-EGFP rescued the Golgi localization of Ardaptin-2 but not golgin-245 (middle row, cells with green asterisks). Furthermore, neither Ardaptin-2 nor golgin-245 localization was restored by expression of Ard1(Y81A)-EGFP, even though the mutant Ard1 protein was able to localize to the Golgi (bottom row, cells with green asterisks). Thus, the differential ability of Ard1 mutants to rescue Golgi localization of Ardaptin-2 and golgin-245 matched their distinct in vitro binding abilities.

Interaction Involving a Charged Residue in the Ard1 N-terminal Region Stabilizes the Ard1-BAR Complex—Although the above pulldown assay (Fig. 2A) indicated that Glu-17 of Ard1 is not critical for its interaction with Ardaptin-2, we were still interested in potential ionic interactions involving the N-terminal charged residues of Ard1, because these residues are conserved between Ard1 and Arf1-Arf6 (supplemental Fig. S1A). Therefore, we focused on the interaction between Arg-19 of Ard1 and Asp-220 of Ardaptin-2 suggested by the crystal structure (Fig. 1, D and E). As shown in Fig. 5A, introduction of an R19A mutation into Ard1(Q71L) greatly diminished its binding to GST-Ardaptin-2 (compare lanes 11 and 13). Furthermore, substitution of both Arg-19 and Glu-17 by alanine almost completely abrogated the interaction of Ard1(Q71L) with Ardaptin-2 (lane 14). We then turned to Asp-220 of Ardaptin-2, whose side chain potentially interacts with the Arg-19 side chain of Ard1 (Fig. 1, D and E). As expected, D220A mutation of Ardaptin-2 abolished its interaction with Ard1(Q71L) (Fig. 5B, compare lanes 3 and 4). Thus, the interaction between Arg-19

Structure of the Ard1·Ardaptin-2 BAR Complex

**Figure 4. Differential effects of Ard1 mutations on recruitment of Ardaptin-2 and golgin-245 to Golgi membranes.** A, mutations of residues of Ard1 differentially affect its interaction with Ardaptin-2 and the GRIP domain. Lysates of HeLa cells expressing C-terminal HA-tagged Q71L/F51A, Q71L/Y81A, Q71L/Y81F, or Q71L Ard1 mutants, as indicated, were pulled down with GST (lanes 1–4), GST-Ardaptin-2 (lanes 9–12), or GST-golgin-245 GRIP (lanes 13–16), and subjected to SDS-PAGE and immunoblotting with anti-HA antibody. B and C, interaction with Ard1 is required for Golgi localization of Ardaptin-2 and golgin-245. HeLa cells were transfected with a pool of siRNAs for LacZ as a control (B) or for the 3’-untranslated region of Ard1 mRNA (C) and then with an expression vector for C-terminal EGFP-tagged Ard1(WT), Ard1(F51A), or Ard1(Y81A). After 72 h, the cells were fixed and doubly immunostained with antibodies against Ardaptin-2 and/or golgin-245 signals on the Golgi. Percentages of cells with Ardaptin-2 and golgin-245 signals are expressed as bar graphs.
Structure of the Arl1·Arfaptin-2 BAR Complex

A, mutation of Arl1 Arg-19 abolishes the Arl1·Arfaptin-2 interaction. Lysates of HeLa cells expressing C-terminal HA-tagged Q71L, Q71L/E17A, Q71L/R19A, Q71L/E17A/R19A, or T31N Arl1 mutants as indicated, were pulled down with GST, GST-Arfaptin-2(WT), GST-Arfaptin-2(D220A), or GST-Arfaptin-2(K216A) as indicated, and subjected to SDS-PAGE and immunoblotting with anti-HA antibody. B, mutation of Arfaptin-2 Asp-220 abolishes the Arl1·Arfaptin-2 interaction. Lysates of HeLa cells expressing Arl1(Q71L)-HA (lanes 1–5) or Arfaptin-2(BAR) (lanes 6–10) were pulled down with GST, GST-Arfaptin-2(WT), GST-Arfaptin-2(D220A), or GST-Arfaptin-2(K216A) as indicated, and subjected to SDS-PAGE and immunoblotting with anti-HA antibody.

of Arl1 and Asp-220 of Arfaptin-2 makes a critical contribution to the Arl1·Arfaptin-2 complex formation, although there remains a possibility that the effect of the D220A mutation could also be due, at least in part, to loss of the hydrophobic interaction of the aliphatic portion of Asp-220 with the essential Trp-66 residue in the interswitch (Figs. 1E and 2B, lane 10). To our knowledge, this is the first demonstration that a residue in the N-terminal region, apart from residues in the switch and interswitch regions, of a small GTPase plays a critical role in effector binding.

Competitive Interaction of Arl1 and Rac1 with Arfaptin-2—Previous work has demonstrated that Arf1 is able to outcompete Rac1 in complex formation with Arfaptin-2 (12). However, competition between Arl1 and Rac1 for Arfaptin-2 binding has not been studied. We superimposed the structure of the Arl1·Arfaptin-2 BAR complex on the reported GDP-bound Rac1·Arfaptin-2 BAR complex structure (12) (PDB code 1I4L), using the main chain of Rac1-binding residues in Arfaptin-2 BAR (supplemental Fig. S1B) as a reference. We found that Asn-60 and Leu-61 in the β2-β3 loop of the proximal Arl1 molecule clashed with Pro-106 and Asn-107 in the α4-α5 loop of Rac1 (Fig. 6A), whereas the distal Arl1 molecule remained undisturbed. Rac1 is thus likely to interfere with only one of the two Arl1 molecules bound to the Arfaptin-2 BAR homodimer. Given the structural similarity between Arl1 and Arf1, it is reasonable to assume that Arf1 binds to Arfaptin-2 in essentially the same manner as Arl1. Thus, our structural data provide a mechanism for the competition between Arl1 and Rac1 for binding to Arfaptin-2.

To investigate whether Arl1 and Rac1 bind simultaneously or exclusively to Arfaptin-2 BAR, we performed SPR experiments using Arl1(Q71L) and Rac1(G12V), both of which are GTP-locked, constitutively active mutants, as analytes, and GST-fused Arfaptin-2 BAR immobilized on a sensor chip. The resonant signals were measured in the presence of varying concentrations of Arl1(Q71L) and Rac1(G12V) (supplemental Fig. S2). We first analyzed the data obtained with injection of Arl1(Q71L) alone to characterize the interaction between Arl1 and Arfaptin-2 BAR (Fig. 6E, models b and c). The dissociation constant was 3.3 ± 0.2 μM and the maximum binding value was 126 ± 2 resonance units (RU) (Fig. 6B and "Experimental Procedures"). For the Rac1·Arfaptin-2 BAR interaction (Fig. 6E, model a), the dissociation constant was 1.3 ± 0.1 μM and the maximum binding was 66 ± 2 RU (Fig. 6C). The maximum binding of Rac1 to Arfaptin-2 BAR is approximately one-half of that of Arl1, being in good agreement with the molecular stoichiometry of the complex crystal structures, 2:2 for Arl1·Arfaptin-2 BAR and 1:2 for Rac1·Arfaptin-2 BAR (12). The dissociation constant of Rac1·Arfaptin-2 BAR interaction by our SPR measurement is slightly lower than that previously reported (4.5 μM) by isothermal titration calorimetry (12); the difference could be due, at least in part, to the different assay systems used. If two molecules of Arl1 and one Rac1 molecule could bind simultaneously to Arfaptin-2 BAR without interference, as illustrated in Fig. 6E, model f, simultaneous injection of the two GTPases would yield an RU equal to the sum of the two RUs obtained from two separate injections; i.e., 192 (126 + 66) RU. However, the value observed was 125.9 RU, which is significantly lower than the sum of the values from two independent injections (Fig. 6D). Instead, it is close to 129 RU, the sum of the maximum binding of Rac1 and half of the maximum binding of Arl1, as illustrated in Fig. 6E, models d or e, or 126 RU, the maximum binding of two Arl1 molecules, as shown in Fig. 6E, model c.

Thus, our SPR analysis clearly shows that one molecule of Rac1 and two molecules of Arl1 cannot coexist on the Arfaptin-2 BAR dimer, as in Fig. 6E, model f. Because there is no structural interference between the distal Arl1 molecule and Rac1 on the Arfaptin-2 BAR homodimer, it is likely that one Arl1 molecule and one Rac1 molecule occupy the corresponding binding sites, as shown in Fig. 6E, model d.

DISCUSSION

Structural and Functional Similarities Between the Arl1-BAR Complex and the PX-BAR Domain—Because BAR domains generally do not show distinct specificities for different membrane lipids, their membrane binding specificities are often determined by additional modules to control spatial and temporal membrane deformation in cells (4). For example, the PX domain of SNX9 confers preferential binding of the adjacent BAR domain to membranes enriched in phosphatidylinositol 4,5-bisphosphate (7, 31). However, Arfaptins have no additional membrane-binding modules other than the BAR domain; rather, they are recruited specifically to trans-Golgi
membranes through interaction with Arl1 (14). Our study reveals an unexpected similarity between the overall structures of the Arl1/H18528 Arfaptin-2 BAR complex and the PX-BAR domain of SNX9 (7, 8) (Fig. 7, A and B), suggesting similar mechanisms underlying the BAR domain targeting to specific organellar membranes. The PX domain of SNX9 associates with the last two helices of the BAR domain of SNX9, and Arl1 similarly binds to the last two helices of Arfaptin-2 BAR. Furthermore, when the overall structure of the Arl1-Arfaptin-2 BAR complex is superimposed on that of SNX9 PX-BAR, the phosphoinositide-binding site of the PX domain (Fig. 7A, shown in red) coincides with the approximate position of the N terminus of the Arl1 construct (Gly-14; Fig. 7B, shown in red), from which the myristoylated amphipathic helix for membrane anchoring is predicted to extend. Thus, given the close structural similarity between the Arl1-Arfaptin-2 BAR complex and the SNX9 PX-
BAR domain, it is likely that Arl1 is positioned to determine membrane targeting specificity of the BAR domain.

The concave face of SNX9 PX-BAR engaged in membrane binding is predominantly positively charged (Fig. 7C). SNX9 PX-BAR is known to effectively tubulate phosphoinositide-containing liposomes (7, 31). Similarly, the concave surface of Arl1/H18528/Arfaptin-2 BAR is positively charged, although less extensively (Fig. 7D). Thus, like SNX9 PX-BAR, the Arl1/H18528/Arfaptin-2 BAR complex appears to be suitable for associating with negatively charged membranes.

As demonstrated by cryo-EM of the CIP4 F-BAR domain (32), BAR domains appear to oligomerize into lattices by lateral tip-to-tip interactions and longitudinal interactions along the cylindrical membrane tubules. The structural similarity between Arl1/Arfaptin-2 BAR and SNX9 PX-BAR suggests that, in addition to recruiting Arfaptin-2 to Golgi membranes, Arl1 may participate in membrane deformation. However, Arfaptin-2 alone has been shown to cause membrane tubulation in vitro (2). Furthermore, whereas overexpression of Arfaptin-2 alone in cells causes membrane tubulation, co-expression of Arl1 does not enhance Arfaptin-2-driven tubulation (14). Thus, it is likely that in the Arl1/Arfaptin-2 complex, Arl1 determines the membrane binding specificity of Arfaptin-2, whereas Arfaptin-2 itself plays the major role in membrane sculpting (33).

Like Arl1, the Rab5 small GTPase is also engaged in membrane recruitment of a BAR domain protein. The early endosomal protein APPL1 interacts with Rab5 through its BAR and PH domains, and these interactions are required for APPL1 targeting to endosomal membranes (34, 35). Thus, these data support the view that, in addition to phospholipid binding modules such as the PX and PH domains, small GTPases also determine the membrane binding specificity of BAR domain proteins.

Comparison of the Structures of the Arl1-BAR and Arl1-GRIP Complexes—Arl1 has been implicated in tethering golgin family proteins, such as golgin-245 and golgin-97, to Golgi membranes by binding to the GRIP domain in the C-terminal region.
Structure of the Arl1-Arfaptin-2 BAR Complex

FIGURE 8. Comparison of Arl1-BAR and Arl1-GRIP interactions. A and B, the structures of the Arl1 golgin-245 BAR complex (PDB code 1R4A) (A) and the Arl1-Arfaptin-2 BAR complex (B). The α1, α2, and α3 helices of GRIP are colored in smoky green, pale orange, and violet-brown, respectively, and the colors used for representing Arl1 and Arfaptin-2 BAR regions are the same as those described in the legend to Fig. 1. C, comparison of detailed interactions around the hydrophobic pocket of Arl1. The structure around Tyr-81 of the Arl1-Arfaptin-2 BAR complex is superimposed on that of the Arl1-golgin-245 GRIP complex. The dashed yellow line represents the hydrogen bond between Tyr-81 of Arl1 and Tyr-2177 of golgin-245.

of golgins (36). The crystal structure of Arl1 in complex with the GRIP domain of golgin-245 revealed that the GRIP domain, which consists of three antiparallel helices, forms a dimer to which two Arl1 molecules bind with dyad symmetry (29, 30). The α1 helix of the GRIP domain interacts mainly with Arl1 switch 1, whereas α2 contacts switch 2. These two helices are oriented parallel to the interswitch β strands of Arl1 (Fig. 8A). The connecting loop between the β2 and β3 strands of the Arl1 interswitch is close to the membrane-anchored N-terminal helix, leaving the other end of the interswitch β sheet pointing toward the cytosol. Most Arf/Arl effector proteins have Arf/Arl-binding domains with helices oriented more or less parallel to the interswitch β strands, such that the helical effectors rise above the membrane surface (27). In contrast, the α2 and α3 helices of Arfaptin-2 BAR are oriented at an oblique angle to the interswitch β strands (Fig. 8B), enabling simultaneous membrane association of Arl1 and Arfaptin-2 BAR.

When the structure of the Arl1-BAR complex is superimposed on that of the Arl1-GRIP complex, the Arl1 effector helices are nearly perpendicular to one another (Fig. 8, A and B). Nevertheless, the key residues responsible for Arl1 binding of the Arfaptin-2 BAR and golgin-245 GRIP domains (Phe-285 and Tyr-2177, respectively) occupy nearly the same position in the hydrophobic pocket of Arl1 (Fig. 8C). Within the Arl1 hydrophobic pocket, there is a significant shift in the side chain of Tyr-81 between the Arl1-BAR and Arl1-GRIP complexes, whereas the other residues overlap well. In its complex with golgin-245 GRIP, Tyr-81 of Arl1 makes a hydrogen bond with the side chain of the key Tyr-2177 residue of golgin-245 (Fig. 8C). This hydrogen bond is critical for the Arl1-GRIP interaction, because both the Y81A and Y81F mutations of Arl1 abrogate Arl1 binding to golgin-245 (Fig. 4A). Thus, within the hydrophobic pocket, the hydrogen bond appears to determine the specificity of the Arl1-GRIP interaction. On the other hand, the hydroxyl group at this position is dispensable, but the bulky hydrophobic side chain is required to maintain the Arl1-BAR interaction, because the Y81A mutation but not the Y81F mutation abolished Arl1 binding to Arfaptin-2 (Fig. 4A).

The residues in the Arl1 interswitch region that interact with golgin-245 GRIP are limited to the region around the hydrophobic triad patch (Fig. 8A). This renders Phe-51 of Arl1 dispensable for binding to the GRIP domain, as demonstrated by the failure of Arl1 F51A mutant to bind (Fig. 4A). In contrast, the same Arl1 mutation has no obvious effect on Arfaptin-2 binding, due to the extensive hydrophobic interactions not only in the hydrophobic triad patch, but also throughout the entire Arl1 interswitch region (Fig. 8B).

Competition between Arl1 and Rac1 for Arfaptin-2 BAR Binding—The modes by which Rac1 and Arl1 bind to Arfaptin-2 BAR are completely different. Even in the presence of a GTP analog, the conformation of Rac1 switch 1 is forced to open up, as in the GDP-bound conformation (12). Moreover, only one Rac1 molecule binds to the concave face of the Arfaptin-2 BAR dimer (12), which is engaged in membrane association. Thus, it seems unlikely that Arfaptin-2 BAR can stably associate with membranes through Rac1, although Rac1 is anchored to membranes via the geranylgeranyl group covalently attached to its C-terminal Cys residue. Our SPR measurements using soluble forms of GTases support model d in Fig. 6E, in which one Arl1 molecule and one Rac1 molecule can bind simultaneously to the Arfaptin-2 BAR homodimer. However, when the Arfaptin-2 homodimer is associated with membranes by interacting with myristoylated full-length Arl1 molecules, Rac1 binding is not allowed. Further study will be required to reveal the physiological relevance of the Rac1 interaction with Arfaptin-2 and the potential competition between Arl1 and Rac1 for Arfaptin-2 binding.

Our SPR data also revealed that Arl1 binds to Arfaptin-2 BAR with a $K_d$ value of $\sim 3.3$ μM, which is comparable with the documented $K_d$ value of Arf1 for Arfaptin-2 ($\sim 2.5$ μM), although there was no experimental data for estimation of the value in that literature (12). On the other hand, our previous study using siRNA-mediated depletion of the Arl1 or Arf protein(s) and using brefeldin A, an inhibitor for Arf-guanine nucleotide exchange factors, unequivocally showed that Golgi association of Arfaptin-2 is dependent on Arl1, but not on Arfs (14). Although we do not know the exact reason for the apparent discrepancy, factor(s) other than Arl1, such as a specific phospholipid and an accessory protein, may have an auxiliary role in determining the association of Arfaptin-2 with subdomains of Golgi membranes. This issue must be addressed in a future study.

Acknowledgments—We thank the staff at beamlines BL5A and NW12A at Photon Factory and BL41XU at SPring-8 for help with x-ray diffraction experiments, Yohei Katoh for technical advice, and Manabu Negishi and Minoru Fukuda for providing materials. We also thank members of our laboratories for discussion, constructive comments, and critical reading of the manuscript.
REFERENCES

1. Masuda, M., and Mochizuki, N. (2010) Structural characteristics of BAR domain superfamily to sculpt the membrane. Semin. Cell Dev. Biol. 21, 391–398
2. Peter, B. L.,Kent, H. M., Mills, I. G., Vallis, Y., Butler, P. J., Evans, P. R., and McMahon, H. T. (2004) BAR domains as sensors of membrane curvature. The amphiphysin BAR structure. Science 303, 495–499
3. Suetsugu, S., Toyooka, K., and Senju, Y. (2010) Subcellular membrane curvature mediated by the BAR domain superfamily proteins. Semin. Cell Dev. Biol. 21, 340–349
4. Yao, Y., and Hauve, V. (2011) Membrane shaping by the Bin/amphiphysin/Rvs (BAR) domain protein superfamily. Cell. Mol. Life Sci. 68, 3982–3993
5. Traer, C. I.,Rutherford, A. C., Palmer, K. J., Wassmer, T., Oakley, J., Attar, N.,Carlton, J. G., Kremerskothen, J., Stephens, D. J., and Cullen, P. J. (2007) SNX4 coordinates endosomal sorting of TfR8 with dyneme-mediated transport into the endocytic recycling compartment. Nat. Cell Biol. 9, 1370–1380
6. Carlton, J., Bujny, M., Peter, B. J., Oorschot, V. M., Rutherford, A., Mellor, H., Klumperman, J., McMahon, H. T., and Cullen, P. J. (2004) Sorting nexin-1 mediates tubular endosome to TGN transport through coincidence sensing of high curvature membranes and 3-phosphoinositides. Curr. Biol. 14, 1791–1800
7. Pylypenko, O., Lundmark, R., Rasmuson, E., Carlsson, S. R., and Rak, A. (2007) The PX-BAR membrane-remodeling unit of sorting nexin-9. EMBO J. 26, 4788–4800
8. Wang, Q., Kaan, H. Y., Hooda, R. N., Goh, S. L., and Sondermann, H. (2008) Structure and plasticity of endophilin and sorting nexin-9. Structure 16, 1574–1587
9. van Weering, J. R., Verkade, P., and Cullen, P. J. (2010) SNX-BAR proteins in phosphoinositide-mediated, tubular-based endosomal sorting. Semin. Cell Dev. Biol. 21, 371–380
10. Van Aelst, L., Joneson, T., and Bar-Sagi, D. (1996) Identification of a novel Rac1-interacting protein involved in membrane ruffling. EMBO J. 15, 3778–3786
11. D’Souza-Schorey, C., Boshans, R. L., McDonough, M., Stahl, P. D., and Van Aelst, L. (1997) A role for POR1, a Rac-interacting protein, in ARF-mediated cytoskeletal rearrangements. EMBO J. 16, 5445–5454
12. Tarricone, C., Xiao, B., Justin, N., Walker, P. A., Rittinger, K., Gamblin, S. J., and Smerdon, S. J. (2001) The structural basis of Arfaptin-mediated cross-talk between Rac and Arf signaling pathways. Nature 411, 215–219
13. Kanoh, H., Williger, B. T., and Exton, J. H. (1997) Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes. J. Biol. Chem. 272, 5421–5429
14. Man, Z., Kondo, Y., Koga, H., Umino, H., Nakayama, K., and Shin, H. W. (2011) Arfaptins are localized to the trans-Golgi by interaction with Arl1, but not Arfs. J. Biol. Chem. 286, 11569–11578
15. Shin, H. W., Kobayashi, H., Kitamura, M., Wargau, S., Suganuma, T., Uchiyama, Y., and Nakayama, K. (2005) Roles of ARFPRP1 (ADP-ribosylation factor-related protein 1) in post-Golgi membrane trafficking. J. Cell Sci. 118, 4039–4048
16. Nishimoto-Morita, K., Shin, H. W., Mitsuhashi, H., Kitamura, M., Zhang, Q., Johannes, L., and Nakayama, K. (2009) Differential effects of depletion of ARL1 and ARFRP1 on membrane trafficking between the trans-Golgi network and endosomes. J. Biol. Chem. 284, 10583–10592
17. Katoh, H., Yasui, H., Yamaguchi, Y., Aoki, I., Fujita, H., Mori, K., and Negishi, M. (2000) Small GTPase RhoG is a key regulator for neurite outgrowth in PC12 cells. Mol. Cell. Biol. 20, 7378–7387
18. Hiraki, M., Kato, R., Nagai, M., Satoh, T., Hirano, S., Ihara, K., Kudo, N., Nagae, M., Kobayashi, M., Inoue, M., Uejima, T., Oda, S., Chavas, L. M., Akutsu, M., Yamada, Y., Kawaski, M., Matsuaki, N., Igarashi, N., Suzuki, M., and Wakatsuki, S. (2006) Development of an automated large-scale protein crystallization and monitoring system for high-throughput protein-structure analyses. Acta Crystallogr. D 62, 1058–1065
19. Otwinowski, Z., Minor, W., and Carter, C. W., Jr. (1997) Processing of x-ray diffraction data collected in oscillation mode. Meth. Enzymol. 276, 307–326
20. Collaborative Computational Project, Number 4 (1994) The CCP4 suite. Programs for protein crystallography. Acta Crystallogr. D 50, 760–763
21. McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C., and Read, R. J. (2007) Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674
22. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr. D 53, 240–255
23. Emsley, P., Lohkamp, B., Scott, W. G., and Cowtan, K. (2010) Features and development of Coot. Acta Crystallogr. D 66, 486–501
24. Delano, W. L. (2010) The PyMOL Molecular Graphics System, version 1.3, Schrödinger, LLC, New York
25. Kain, R., Angata, K., Kerjaschki, D., and Fukuda, M. (1998) Molecular cloning and expression of a novel human trans-Golgi network glycoprotein, TGN51, that contains multiple tyrosine-containing motifs. J. Biol. Chem. 273, 981–988
26. Krisinel, E., and Henrick, K. (2007) Inference of macromolecular assemblies from crystalline state. J. Mol. Biol. 372, 774–797
27. Kawasaki, M., Nakayama, K., and Wakatsuki, S. (2005) Membrane recruitment of effector proteins by Arf and Rab GT-Pases. Curr. Opin. Struct. Biol. 15, 681–689
28. Sahin, A., Espiau, B., Tetaud, E., Cuvillier, A., Cartonnier, T., Ambit, A., Robinson, D. R., and Merlin, G. (2008) The Leishmanial ARL-1 and Golgi traffic. PLoS One 3, e1620
29. Panic, B., Persic, O., Veprintsev, D. B., Williams, R. L., and Munro, S. (2003) Structural basis for Arl-dependent targeting of homodimeric GRIP domains to the Golgi apparatus. Mol. Cell 12, 863–874
30. Wu, M., Lu, L., Hong, W., and Song, H. (2004) Structural basis for recruitment of GRIP domain golgin-245 by small GT Pase Arl1. Nat. Struct. Mol. Biol. 11, 86–94
31. Yarar, D., Surka, M. C., Leonard, M. C., and Schmid, S. L. (2008) SNX9 activities are regulated by multiple phosphoinositides through both PX and BAR domains. Traffic 9, 133–146
32. Frost, A., Perera, R., Roux, A., Spasov, K., Destaing, O., Egelmans, E. H. D., Camilli, P., and Unger, V. M. (2008) Structural basis of membrane invagination by F-BAR domains. Cell 132, 807–817
33. Shin, H. W., Takatsu, H., and Nakayama, K. (2012) Mechanisms of membrane curvature generation in membrane traffic. Membranes 2, 118–133
34. Zhu, G., Chen, J., Liu, J., Brunzelle, J. S., Huang, B., Wakeham, N., Terzyan, S., Li, X., Rao, Z., Li, G., and Zhang, X. C. (2007) Structure of the APPL1-ARL-1 and Golgi network glycoprotein, TGN51, that contains multiple tyrosine-containing motifs. J. Biol. Chem. 282, 4039–4048
35. Miaczynska, M., Christoforidis, S., Giner, A., Shevchenko, A., Uttenweiler-Joseph, S., Habermann, B., Wilm, M., Parton, R. G., and Zerial, M. (2004) APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. Cell 116, 445–456
36. Goud, B., and Gleeson, P. A. (2010) TGN golgins, Rab and cytoskeleton. Regulating the Golgi trafficking pathways. Trends Cell Biol. 20, 329–336