The small G protein Arf1 regulates Golgi traffic and is activated by two related types of guanine nucleotide exchange factor (GEF). GBF1 acts at the cis-Golgi, whereas BIG1 and its close paralog BIG2 act at the trans-Golgi. Peripheral membrane proteins such as these GEFs are often recruited to membranes by small G proteins, but the basis for specific recruitment of Arf GEFs, and hence Arfs, to Golgi membranes is not understood. In this paper, we report a liposome-based affinity purification method to identify effectors for small G proteins of the Arf family. We validate this with the Drosophila melanogaster Arf1 orthologue (Arf79F) and the related class II Arf (Arf102F), which showed a similar pattern of effector binding. Applying the method to the Arf-like G protein Arl1, we found that it binds directly to Sec71, the Drosophila ortholog of BIG1 and BIG2, via an N-terminal region. We show that in mammalian cells, Arl1 is necessary for Golgi recruitment of BIG1 and BIG2 but not GBF1. Thus, Arl1 acts to direct a trans-Golgi–specific Arf1 GEF, and hence active Arf1, to the trans side of the Golgi.

Introduction

The members of the ADP ribosylation factor (Arf) family of small G proteins are essential regulators of membrane traffic and cytoskeletal systems (D’Souza-Schorey and Chavrier, 2006; Gillingham and Munro, 2007; Donaldson and Jackson, 2011). Distinct from the other members of the Ras superfamily of small G proteins, they are attached to membranes by an amphipathic N-terminal helix, which is often N myristoylated (Antonny et al., 1997; Pasqualato et al., 2002). The founding member of the family, Arf1, was initially shown to be required for the recruitment of COPI vesicle coats to Golgi membranes (Serafini et al., 1991; Donaldson et al., 1992). Arf1 is one of four close paralogs in humans, which are divided into class I (Arf1 and Arf3) and class II (Arf4 and Arf5), with a single member of each class being present in invertebrates (Tsuchiya et al., 1991; Lee et al., 1994). Most work has been performed on Arf1, although the other Arfs are thought to have similar roles in Golgi function but be less abundant. GTP-bound Arf1 has been shown to bind directly to vesicle coat proteins on both the cis-Golgi (COPI) and on the trans-Golgi (AP-1, AP-3, and GGAs; Stammes and Rothman, 1993; Traub et al., 1993; Boman et al., 2000; Dell’Angelica et al., 2000; Drake et al., 2000). In addition, Arf1 has been shown to be involved in the Golgi recruitment of a coiled-coil protein as well as proteins involved in lipid transport and metabolism (Brown et al., 1993; Cockcroft et al., 1994; Gillingham et al., 2004).

The fact that Arfs1–5 function throughout the Golgi requires that they are activated in multiple parts of the Golgi stack. Two distinct Arf guanine nucleotide exchange factors (GEFs) have been found: the Gea1/GBF1 family and the Sec7/BIG family (Morinaga et al., 1997; Claude et al., 1999). These large proteins are related over much of their length and share a conserved Sec7 domain, which mediates nucleotide exchange (Chardin et al., 1996; Morinaga et al., 1999). However, the proteins are clearly distinct, with members of both families being found in all eukaryotic kingdoms, implying that they diverged before the last common eukaryotic ancestor and, hence, that the two types of GEF have fundamentally different roles (Cox et al., 2004; Mouratou et al., 2005; Bui et al., 2009). GBF1 acts on the early parts of the Golgi stack, whereas on the trans-Golgi are BIG1 and its close paralog BIG2 (whose human orthologs are encoded by the genes ARFGEF1/2; Zhao et al., 2002; Ishizaki et al., 2012). This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/).
et al., 2008; Manolea et al., 2008). This raises the question of how the two proteins are recruited to different ends of the Golgi stack.

Arfs1–5 are members of a larger Arf family that includes Sar1 and several Arf-like proteins (Arls; Pasqualato et al., 2002; Gillingham and Munro, 2007; Donaldson and Jackson, 2011). Some Arls have roles in membrane traffic, signaling, and cilia formation, although less is known about their regulation, and several lack known effectors. Two of the Arls, ARFRP1 and Arl1, are known to be localized on the trans-Golgi and to have been proposed to function in both exocytosis and in retrograde traffic from endosomes (Lowe et al., 1996; Behnia et al., 2004; Nishimoto-Morita et al., 2009; Cheryl Chia and Gleeson, 2011). Arl1 recruits several long coiled-coil golgins to the Golgi by binding to a conserved golgin-97, RanBP2ox, In1p1, and p230/golgin-245 (GRIP) domain at their C termini and also binds to the Bin–Amphiphysin–Rvs (BAR) domain protein ar-faptin (Lu and Hong, 2003; Panic et al., 2003a; Setty et al., 2003; Derby et al., 2004; Man et al., 2011). ARFRP1 is required for the localization of Arl1 to Golgi membranes but has no known effectors (Panic et al., 2003b; Setty et al., 2003; Shin et al., 2005; Zahn et al., 2006; Nishimoto-Morita et al., 2009). Effectors for Arfs and Arls have been typically found by affinity chromatography using GST fusion proteins or yeast two-hybrid screens. However, it has been observed that at least one Arf1 effector, the coiled-coil protein GMAP-210, only shows detectable binding in vitro when Arf1-GTP is present on liposomes rather than being attached to beads as a GST fusion (Gillingham et al., 2004; Drin et al., 2008). This is a result of an amphipathic helix next to the GRIP-related Arf-binding (GRAB) domain, which stabilizes the GRAB–Arf1–GTP interaction by binding to the adjacent lipid bilayer (Drin et al., 2008). Given that all members of the Arf family are likely to sit close to the membrane and that several Arf family effectors have been found to also bind or modify lipids, this requirement for additional lipid interactions for stable binding may be a feature of at least some of their effectors (Levine and Munro, 2002; Shin and Nakayama, 2004; Cohen et al., 2007; Hofmann et al., 2007; Ménétrey et al., 2007). Thus, we developed a method for using G protein–coated liposomes for affinity purification of Arf effectors. Applying this to Drosophila melanogaster cell extracts revealed a direct interaction between Arf1 and Sec71, the Drosophila ortholog of the trans-Golgi GEFs BIG1/2. In mammalian cells, knockdown of Arf1 affects the Golgi recruitment of BIG1 and BIG2 but not of GBF1. Thus, Arf1 acts upstream of the trans-Golgi population of Arf1 and may therefore coordinate the tethering of arriving vesicles with the recruitment of coats for vesicle departure.

**Results and discussion**

**Liposome-based isolation of Arf family effectors**

Arf family members bind to membranes only in the GTP-bound state because GTP binding drives the displacement of the amphipathic helix and N-terminal myristoyl group from a hydrophobic pocket (Goldberg, 1998; Pasqualato et al., 2002). This presents challenges for attaching native Arfs to liposomes, as they require an exchange factor to activate the protein before liposome binding, and mutant forms that are locked in the GTP-bound state are poorly soluble as a result of the exposed lipid-binding surface (Antonny et al., 1997; Béraud-Dufour et al., 1999). Thus, to uncouple nucleotide state from membrane attachment, we replaced the amphipathic N-terminal helices
of *Drosophila* Arf1 and ARFRP1 with an N-terminal His$_{10}$ tag. Such His-tagged forms of Sar1 and Arf1 have been previously shown to bind to liposomes containing low levels of a lipid with an Ni–nitrilotriacetic acid (NTA) head group and to then recruit effectors (Lee et al., 2005; Drin et al., 2008). For controls, we also expressed *Drosophila* orthologs of the class I and class II Arfs. The *Drosophila* genes for these four proteins are named CG7039 (ARFRP1), Arf72A (Arf1), Arf79F (class I Arf), and Arf102F (class II Arf), but to aid clarity, we will refer to the proteins by the names of their mammalian orthologs (ARFRP1, Arf1, Arf1, and Arf4).

All four G proteins were expressed with mutations previously shown to lock them in the GDP-bound (T to N) or GTP-bound (Q to L) states (Dascher and Balch, 1994; Lu et al., 2001) and then loaded with nucleotide and bound to Ni-NTA lipid-containing liposomes. The coated liposomes were suspended in extracts prepared from *Drosophila* S2 cells using a detergent-free protocol. The cell lysates were then adjusted to be of a higher density than the interior of the liposomes, and at the end of the binding reaction, the liposomes were floated out of the lysate by centrifugation (Fig. 1 A). A second flotation from washing buffer was used to reduce the background, and proteins were then extracted from the coated liposomes. Examination of the liposome-associated proteins showed relatively little background, and antibody staining revealed that the GRIP domain coiled-coil protein GCC88 was selectively bound to the Arf1-GTP–coated liposomes (Fig. 1 B). This demonstrates that Arf-coated liposomes can be used to enrich effectors from cell lysates.

**Isolation of effectors for class I Arf**

As Arf1 is likely to be the most abundant of the four small G proteins, we initially investigated whether the material bound to the Arf1-coated liposomes was in sufficient yield to allow mass spectrometric identification of individual effectors. Protein gels of the liposome-bound material were cut into three sections, and tryptic peptides from in-gel digestion were analyzed by liquid chromatography tandem mass spectrometry (MS/MS). This revealed that there was sufficient material for peptide sequences to be obtained from many different proteins. Comparison of the number of spectra from a particular protein that were obtained from the Arf1-GTP and the Arf1-GDP samples provides an approximate measure of the relative amount in each sample (Neilson et al., 2011). Several known Arf effectors were readily detectable on the Arf1-GTP liposomes, whereas they were represented by few, or no, peptides in the material from the Arf1-GDP liposomes (Fig. 1 C and Table S1). Several proteins were present at approximately similar levels on both liposomes, but many of these were also readily detectable on uncoated liposomes, indicating that they are nonspecific background (Table S1). Interestingly, the *Drosophila* ortholog of GBF1, Gartenzwerg, was the only protein showing a clear enrichment on the Arf1-GDP liposomes, consistent with Arf1-GDP being its substrate (Fig. 1 C).

Liposomes coated with the class II Arf, Arf4, showed similar results, and comparison of peptide spectral counts for known Arf1 effectors did not reveal any that were exclusive to Arf1 or to Arf4, although there may be some slight preferences in binding (Fig. 1 D and Table S1). This is consistent with a study in mammalian cells, which have indicated considerable redundancy between class I and class II Arfs (Volpicelli-Daley et al., 2005). Collectively, these results show that Arf1–coated liposomes can be used to enrich effectors with sufficient yield and purity to allow the effectors to be identified by mass spectrometry.

**Affinity chromatography with ARFRP1 and Arf1**

Next, we applied the same large-scale purification approach to liposomes coated with *Drosophila* ARFRP1 and Arf1. As with Arf1, mass spectrometry of bound material identified many proteins (Table S1). In the case of ARFRP1, the population of proteins bound to the GTP form appeared similar to that bound to the GDP form, and, thus, no candidate effectors could be identified (Fig. 2 A). However, several proteins showed preferential binding to Arf1-GTP over Arf1-GDP (Fig. 2 B). These included two GRIP domain proteins, as
the trans-Golgi protein while being clearly distinct from the cis-Golgi (Fig. 3 B). Thus, Sec71 binds to Arl1-coated liposomes in vitro and colocalizes with Arl1 on the trans-Golgi in vivo.

Sec71 binds directly to Arl1 via the region N terminal to the Sec7 domain

Sec71, like its mammalian counterparts, has large conserved regions flanking the central Sec7 domain (Fig. 4 A). It has
Mammalian Arl1 is required for the Golgi recruitment of BIG1 and BIG2

To test the relevance of these results for mammalian cells, we determined whether BIG1 and BIG2 require Arl1 for their Golgi localization. Arl1 could be efficiently knocked down in HeLa cells using two independent siRNAs, and in such cells, the GRIP domain golgins were displaced from the Golgi as expected (Fig. S1, A and B). Strikingly, knockdown of Arl1 also resulted in both BIG1 and BIG2 being displaced from the Golgi, whereas the levels of the proteins were not affected (Figs. 5 A and S1 A). Similar results were obtained with the two independent siRNAs and three independent antibodies to BIG1. Quantitation of the level of BIG1 staining relative to another Golgi marker demonstrated that the effect was seen throughout the cell population and was highly statistically significant (two-tailed unpaired t test). Error bars show the SEM. Bars, 50 µm. (C) As in A, except that the cells were stained for the indicated endogenous proteins to show that the cis-Golgi Arf GEF, GBF1, retains its Golgi localization when Arl1 is knocked down. Bars, 15 µm.
that the yeast ortholog Sec7 appears to remain at least partially Arf1-GDP binding to the Sec7 domain or by Arf1-GTP recruitment in vivo (Fig. S1 D). Alternatively, Arf1 displaces BIG1, suggesting that the latter may not rely on PtdIns(4)P for recruitment in vivo (Levine and Munro, 2002; Santiago-Tirado and Munro, 2010; Nordmann et al., 2010). Thus, Arf1 would be well placed to ensure that they are only active in the appropriate destination vesicles (Levine and Munro, 1998; Yamaji et al., 2000). GFP–GRIP [golgin-245 GRIP domain] and GFP–oxytosteryl-binding protein (OSBP) PH was as previously described (Levine and Munro, 1998; Munro and Nichols, 1999).

Materials and methods

Antibodies and plasmids
Human or Drosophila versions of Arf family proteins without the residues comprising the N-terminal amphipathic helix (typically 14 amino acids) were inserted into pET-based vectors for expression with an N-terminal His$_{10}$ tag. Full-length and truncated forms of Drosophila Sec71 (CG7578) were amplified and cloned into the pDONR221 vector and transferred to appropriate destination vectors (Invitrogen). The N-terminal 453 residues of Drosophila Sec71 were cloned into pASK-IBA3C (IBA Gmbh) for expression with a C-terminal S-tag.

GFP–human BIG1 and HA–human BIG1 constructs were gifts from M. Vaughan [National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD] and D. Stephens (University of Bristol School of Medical Sciences, Bristol, England, UK; Yamaji et al., 2000; Boal and Stephens, 2010), and GFP–arfaptin was a gift from Y. Vallis [Medical Research Council Laboratory of Molecular Biology, Cambridge, England, UK]. GFP–GRIP [golgin-245 GRIP domain] and GFP–oxytosteryl-binding protein (OSBP) PH was as previously described [Levine and Munro, 1998; Munro and Nichols, 1999].

Mouse antibodies were against GFP, human AP-1 [600/3; Sigma-Aldrich], human GM130 (55B712; BD), human golgin-245 (612130; BD), and human GBF1 (612116; BD). Rabbit anti-Arl1 was provided by W. Hong [Institute of Molecular and Cell Biology, Singapore, Singapore], and other rabbit antibodies were against Drosophila GM130 [ab30637; Abcam], Drosophila GRIP domain–containing proteins [Sinka et al., 2008], BIG1 [HPA023399 [Sigma-Aldrich] and H-200 [Santa Cruz Biotechnology, Inc.]], and BIG2 [HPA026078; Sigma-Aldrich]. Goat anti-giantin [N-18; Santa Cruz Biotechnology, Inc.], goat anti-BIG1 (K-19; Santa Cruz Biotechnology, Inc.), and sheep anti-TGN46 (APH500; AbD Serotec) were used.

Protein expression and purification
Expression constructs were transformed into E. coli BL21-GOLD (DE3; Agilent Technologies). Cells grown to OD$_{600}$ = 0.8–1.0 at 37°C were induced with 0.1 mM IPTG at 17°C overnight. Cell pellets were frozen in liquid nitrogen and stored at −80°C until use. To purify the His-tagged proteins, cell pellets from 1 L of culture were resuspended in 40 mL of lysis buffer (50 mM Heps, 120 mM K$_2$Ac, 1 mM MgCl$_2$, and 1 mM DTT) further containing 10 mM imidazole and protease inhibitors and lysed by Dounce homogenization and sonication. The lysate was precleared by centrifugation at 15,000 rpm [J25.5 rotor; Beckman Coulter] for 15 min and added to 1 mL of equilibrated Ni-NTA agarose beads (Qiagen). After 1 h of incubation at 4°C, the beads were washed three times in batch (two times with 25 mL of lysis buffer containing 20 mM imidazole and once in 25 mL of lysis buffer containing 40 mM imidazole), poured into columns, and washed once more with 10 mL of the same buffer. Protein was eluted using lysis buffer containing 250 mM imidazole. Fractions containing His-tagged protein (typically 8× 0.5-mL fractions) were collected, with protein eluted in fraction 2, 3, and 4) were combined and dialyzed overnight against lysis buffer without imidazole, containing either GDP or nonhydrolyzable guanosine 5'-(β,γ-imidodipropionate (Sigma-Aldrich).

To purify the Strep-tagged proteins, the same procedure was following to lyze and preclar the lysate, with the exception that the lysate buffer did not contain imidazole. Protein was bound to equilibrated Strep-Tactin

(Fig. S1 D), consistent with Arl1 binding to BIG1 in a manner mutually exclusive with binding to the GRIP domain or arfaptin.

In contrast to these results with BIG1 and BIG2, other Golgi markers were relatively unaffected by Arl1 removal, including GBF1, the Arf GEF that is localized to the cis-Golgi (Fig. 5 C). Thus, it seems that, as in Drosophila, Arl1 is required for the recruitment of the Arf exchange factors of the trans-Golgi. This finding is consistent with several previous reported observations on mammalian Arl1 and BIG1/2. First, when cells are treated with the Arf GEF inhibitor Brefeldin A, BIG1 and BIG2, like Arl1, are displaced from the Golgi more slowly than Arf1 effectors, suggesting that all are upstream of Arf1 activity on the trans-Golgi (Lowe et al., 1996; Yamaji et al., 2000). Second, overexpression of either BIG1 or the GTP-locked form of Arl1 increases the Golgi recruitment of Arf effectors AP-1 and GGAs and blocks their Brefeldin A–induced redistribution, indicating that Arl1 and BIG1/2 both act upstream of Arf1-GTP (Lu et al., 2001; Shinotsuka et al., 2002). These previous observations fit well with the notion of a Golgi Arf cascade from ARFRP1 to Arl1 and then to the TGN Arfs, with at least the latter step being mediated by a direct interaction between a G protein and a downstream GEF. Such cascades of a small G protein recruiting the GEF for a second G protein have been found to occur with some members of the Rab family of G proteins (Ortiz et al., 2002; Kinchen and Ravichandran, 2010; Nordmann et al., 2010). In addition, the Arf GEFs of the ARF nucleotide–binding site opener/cytoesin family have been shown to be recruited to the plasma membrane by the Arf family members Arf4 and Arf6 (Cohen et al., 2007; Hofmann et al., 2007; Stalder et al., 2011). Thus, such G protein cascades may be a dominant theme in the organization of internal membranes.

Our data show that Arf GEFs require Arl1 for recruitment to liposomes and to the trans-Golgi. Our results also show that liposome-based binding can be used to identify novel effectors from total cell extracts, and so it may be of use for analyzing at least some of the other Arf family members. However, it does have the limitation that detergent washing is impossible, and so it may only be applicable to more abundant or higher affinity effectors, and it did not produce an effector for ARFRP1. Although Arl1 is sufficient for Sec71 recruitment when present at high levels on liposomes, it is likely that further interactions are required to stabilize binding to the trans-Golgi. This is perhaps not surprising, given the large size of these GEFs and the importance of ensuring that they are only active in the correct compartment. Several other trans-Golgi proteins bind PtdIns(4)P (Levine and Munro, 2002; Santiago-Tirado and Bretscher, 2011), but overexpression of a pleckstrin homology (PH) domain that binds the Golgi pool of PtdIns(4)P did not displace BIG1, suggesting that the latter may not rely on PI(4)P for recruitment in vivo (Fig. S1 D). Alternatively, Arf1 itself could contribute to Sec71/BIG1 recruitment either via Arf1-GDP binding to the Sec7 domain or by Arf1-GTP recognizing Sec71 as an effector and so enhancing recruitment in a positive feedback loop. This might also explain that fact that the yeast ortholog Sec7 appears to remain at least partially Golgi localized when yeast Arl1 is deleted (Panic et al., 2003b; Behnia et al., 2004). We did detect Drosophila Sec71 bound to Arf1-GTP liposomes (Table S1), but we did not detect binding of the overexpressed Sec71-GFP (Fig. 2 C), suggesting that the association may be indirect. Nonetheless, if there are any such additional interactions, they are clearly not sufficient for Golgi targeting of BIG1 and BIG2 in the absence of Arl1. The notion that Arl1 is the critical determinant of BIG1/2 localization is appealing, as this G protein also recruits golgins that are believed to tether carriers arriving from endosomes (Yoshino et al., 2005; Cheryl Chia and Gleeson, 2011; Munro, 2011). Thus, Arl1 would be well placed to ensure that maturing Golgi cisternae acquire the ability to receive traffic from endosomes, they are also able to generate the vesicles that will return recycling receptors to endosomes.
Sepharose beads [JBA GmbH] by gravity flow over a column. The column was washed five times with one column volume of lysis buffer, and protein was eluted with lysis buffer containing 2.5 mM dehistiobatin (Sigma-Alrich). Dehistiobatin was removed from the purified protein using protein binding columns [Zeba; Thermo Fisher Scientific].

**Liposome flotation assay**

All lipids were obtained from Avanti Polar Lipids, Inc. Lipids and cholesterol were combined in chloroform in the following ratio: 50% egg phosphatidylcholine, 19% liver phosphatidylethanolamine, 5% brain phosphatidylserine, 10% liver phosphatidylinositol, and 16% cholesterol. To allow binding of His-tagged protein, 5% Ni-NTA-labeled lipid (18:1 DGS-NTA(Ni)/1,2-di-oleoyl-sn-glycero-3-[N-(5-sulfo-1-carboxypentyl)limino] propanediol acid succinyl) was added as well as 0.2% NBD-labeled phosphatidylethanolamine to allow distinction of the Ni-NTA liposomes from cellular vesicles and unlabeled liposomes. Solvent was evaporated using argon flow, and the mixture was further dried for 1 h under vacuum. The mixture was rehydrated using lysis buffer lacking imidazole added to a lipid concentration of 1 mg/ml for at least 1 h. Liposomes were prepared with the Mini-Extruder (Avanti Polar Lipids, Inc.) using Nuclease track-etched membranes with 400-nm pores [GE Healthcare].

Preclearred cell lysate was prepared by lysing cells using Dounce homogenization and sonication in an equal-volume lysis buffer containing protease inhibitors and subsequent centrifugation for 2 h at 100g. Either 1.5 ml of precleared cell lysate and 0.5 ml of heavy liposomes (liposomes without Ni-NTA lipids, prepared in lysis buffer containing 30% OptiPrep [Axis-Shield]) or purified protein was added, and the mixture was incubated for 1 h at room temperature. OptiPrep was added to a final concentration of 30%, and the mixture was overlaid with 0.55 ml of a 10% OptiPrep solution in lysis buffer and a 0.3-ml layer of lysis buffer. Liposomes were floated in an SW60 rotor at 45k rpm for 30 min. The Ni-NTA liposome layer that floated to the 10%-0% OptiPrep interface was collected and resuspended in lysis buffer. OptiPrep was added again to 30%, and the mixture was overlaid as described before. Liposomes were floated again and collected. Bound protein was precipitated using methanol and chloroform and dissolved in SDS-PAGE sample buffer.

**Mass spectrometry and data analysis**

Samples obtained from the liposome flotation assay were loaded onto 6% Tris-glycine SDS-PAGE gels and run for a few centimeters. Proteins were stained with Coomassie brilliant blue. The entire gel lane between the stacking gel and the His fusion protein was excised in three parts [40–60, 60–90, and >90 kD], and each was submitted to in-gel trypptic digestion, and peptides were eluted.

Data-dependent liquid chromatography MS/MS was performed by nanoflow reverse-phase liquid chromatography [Dionex U3000; Thermo Fisher Scientific] coupled online to a mass spectrometer (LTQ Orbitrap XL; Thermo Fisher Scientific). In brief, the liquid chromatography separation was performed using a Dionex C18 PepMap column (75 µm ID × 150 mm; Thermo Fisher Scientific), and the peptides were eluted using a linear gradient from 5 to 50% B over 2 h at a flow rate of 200 nL/min (solvent A: 98% H2O and 2% acetonitrile in 0.1% formic acid; solvent B: 90% acetonitrile, 10% acetic acid, and 0.1% formic acid). A cycle of one full Fourier transform scan was followed by 10 data-dependent MS/MS acquired in the linear ion trap and the mass range from 400 to 2000 m/z. The mass spectrometric peptides from proteins bound to liposomes were nontargeting siRNA1 [D001810-01-20] or human ARL1 [J019265-12 and J019265-09].

**Online supplemental material**

Fig. S1 shows experiments in mammalian cells to validate the Arf1 siRNA, demonstrates the rescue of BIG1 displacement by siRNA-resistant Arf1, and shows the displacement of BIG1 by other Arf1 effectors. Table S1 lists the mass spectrometric peptides from proteins bound to liposomes coated with GDP- and GTP-bound forms of Drosophila Arf1, Arf4, Arf11, and ARFRP1. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201107115/DC1.

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