The ARF-like GTPases Arl1p and Arl3p Act in a Pathway that Interacts with Vesicle-Tethering Factors at the Golgi Apparatus

Bojana Panic,[1] James R.C. Whyte,[1]
and Sean Munro*
MRC Laboratory of Molecular Biology
Hills Road
Cambridge CB2 2QH
United Kingdom

Summary

The ARLs are a diverse family of GTPases that are related to ADP-ribosylation factors (ARFs), but whose function is poorly understood. There are at least ten ARLs in humans, two of which have homologs in the yeast *Saccharomyces cerevisiae* (ARL1/Arl1p and ARFRP1/Arl3p). The function of ARFRP1 is unknown, but mammalian ARL1 has recently been found to interact with a number of effectors including the GRIP domain that is present in a family of Golgi-localized long coiled-coil proteins [1]. We find that in yeast, the intracellular targeting of Imh1p, the only yeast GRIP domain protein, is dependent on both Arl1p and Arl3p, but not on the ARF proteins. A recombinant form of the Imh1p GRIP domain binds to Arl1p in a GTP-dependent manner, but not to Arl3p. Yeast also contain a relative of SCOCO, a protein proposed to bind human ARL1, but this yeast protein, Slo1p, appears to bind Arl3p rather than Arl1p in vitro. However, Imh1p is not the sole effector of Arl1p since affinity chromatography of cytosol with immobilized Arl1p:GTP revealed an interaction with the GARP/VFT complex that is thought to act in the tethering of vesicles to the Golgi apparatus [2, 3]. Finally, we find that Arl3p is required in vivo for the targeting of Arl1p, explaining its requirement for the normal distribution of Imh1p.

Results and Discussion

The ARF and Rab families of small GTPases are known to play many key roles in intracellular membrane traffic. However, there is another class of GTPase found on intracellular organelles whose function is less well understood. These are the so-called ARF-like (ARL) proteins, which show considerable divergence from ARFs and from each other (40%–60% identity), but nonetheless are even more divergent from any other GTPase [4]. There are at least ten such proteins in humans, and most share with the ARFs a consensus sequence for N-terminal myristoylation [4, 5]. ARL1 is localized to the Golgi in mammalian cells and is essential for viability of *Drosophila* [6–8]. A recent yeast two-hybrid screen with human ARL1 identified several putative effectors, including golgin-245, a large coiled-coil protein of the Golgi apparatus, although this interaction was not confirmed biochemically [1]. Golgin-245 is a peripheral membrane

protein and is targeted to the Golgi via a C-terminal GRIP domain that is also found in several other Golgi coiled-coil proteins and is conserved from mammals to yeast and protozoa [9–12].

To investigate further the role of the ARL proteins, and in particular their interaction with the GRIP domain, we have made use of the yeast *S. cerevisiae*, which has five ARF and ARF-like proteins (see Supplementary Figure S1 available with this article online). Of these, the closely related Arf1p and Arf2p are class I/II ARFs involved in Golgi transport, based on both sequence and functional analysis [13, 14], while Arf3p appears to be related to the class III ARF, ARF6 (although also named Arl2p) [15]. Of the two remaining proteins, sequence comparisons indicate that Arl1p is a homolog of mammalian ARL1, while Arl3p is closest to the mammalian protein ARFRP1 [16, 17]. Mutants lacking Arl1p or Arl3p are still viable, but show partial defects in sorting of proteins to the vacuole, suggesting a role in post-Golgi membrane traffic [17–19]. The only protein in yeast with a GRIP domain is Imh1p (Sys3p), a nonessential coiled-coil protein identified as a suppressor of defects in a strain lacking Ypt6p, the yeast Rab6 GTPase [20, 21]. *IMH1* shows synthetic lethal genetic interactions with the genes encoding Ypt6p or its exchange factor subunit Ric1p, again implying a role in membrane traffic [20–22].

To examine the targeting of Imh1p, we constructed a version of the protein with GFP fused to the N terminus. When expressed from the *IMH1* promoter, the level of the fusion protein was insufficient for ready detection by fluorescent microscopy, and so it was instead expressed from the stronger *TPI1* promoter (Figure 1A). The resulting GFP-Imh1p was able to maintain the growth of strains lacking endogenous copies of Imh1p and either Ypt6p or Ric1p, indicating that the fusion protein is functional (Figure 1B). Fluorescence microscopy revealed that the protein was localized to punctate structures in the cytoplasm, as expected for the yeast Golgi apparatus (Figure 1C). These structures are likely to represent the late Golgi as targeting is perturbed in mutants that affect the late Golgi, and they colocalize with late Golgi markers [11, 23]. To test the importance of the GRIP domain for this targeting, and for the function of Imh1p, a version of the protein was constructed in which tyrosine 870 was mutated to alanine (Y870A). This tyrosine corresponds to the only residue that is conserved between all GRIP domains, and mutation of the corresponding residue in both mammalian and protist GRIP domains has been found to block Golgi targeting [9–12]. Although the Y870A mutation did not affect the steady state levels of either the untagged protein expressed from its native promoter or the GFP fusion, the mutant forms were unable to substitute for the wild-type protein in strains lacking Ypt6p or Ric1p (Figures 1A and 1B). Moreover, the mutation caused the GFP-tagged protein to become delocalized and instead be diffusely distributed throughout the cytosol (Figure 1C),

*Correspondence: sean@mrc-lmb.cam.ac.uk
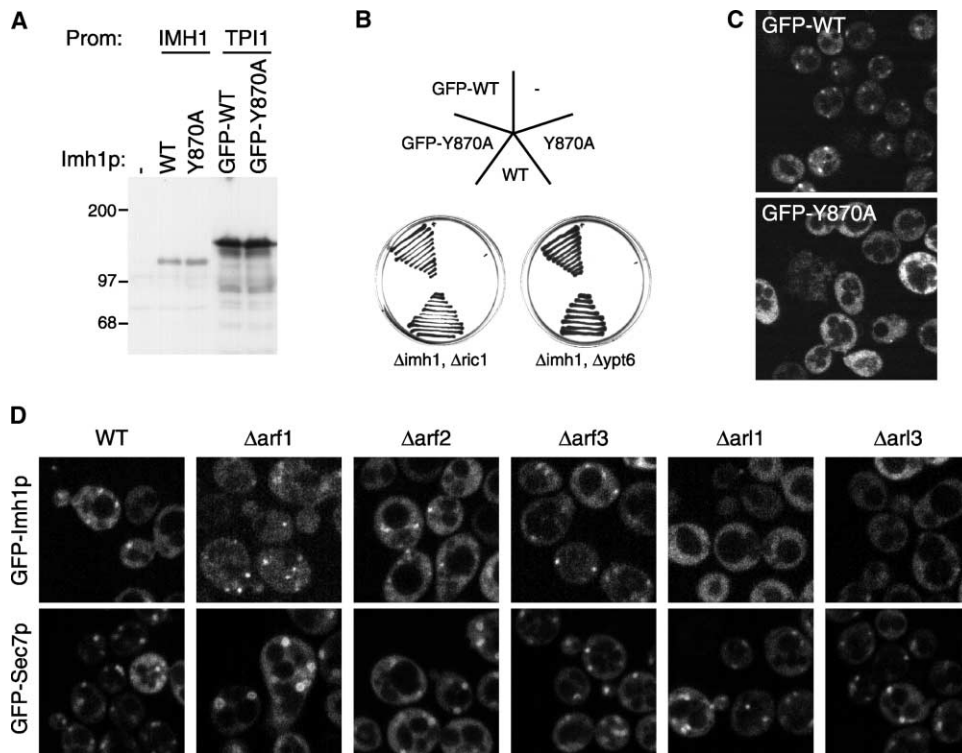[1]These authors contributed equally to this work.

Figure 1. In Vivo Targeting of Imh1p Requires both Its GRIP Domain and Arl1p and Arl3p

(A) Anti-Imh1p protein blot of total proteins from yeast expressing the versions of Imh1p from *TRP1* CEN plasmids under the control of the indicated promoters.

(B) Complementation of Δ*imh1* by the same Imh1p plasmids as in (A). Strains were also lacking *YPT6* or *RIC1* as indicated, and so dependent upon Imh1p activity for normal growth. Plasmids were initially transformed into a strain kept alive by wild-type *IMH1* on a *URA3* CEN plasmid, and then assayed for growth on 5-fluoroorotic acid.

(C) Confocal micrographs of live yeast expressing the same GFP-Imh1p fusions as in (A).

(D) Confocal micrographs of live yeast containing CEN plasmids expressing from the *TPI1* promoter GFP fusions to N terminus of Imh1p or Sec7p. The yeast were either wild-type or lacking the indicated genes.

indicating that the GRIP domain is required for both the intracellular targeting of Imh1p and for its function.

We next investigated whether the targeting of GFP-Imh1p requires any of the yeast ARF or ARL GTPases by expressing the fusion in strains lacking each of the five proteins. Figure 1D shows that deletion of any of Arf1p, Arf2p, or Arf3p did not alter the punctate distribution of the protein. However, deletion of either Arl1p or Arl3p resulted in relocation of the GFP-Imh1p into a diffuse distribution. This was apparently due to a defect in targeting rather than a more general disruption of the Golgi as the distribution of a GFP-tagged version of the Golgi-localized protein Sec7p was unaffected in the Δ*arl1* or Δ*arl3* strains.

A requirement for Arl1p and Arl3p for targeting Imh1p could be explained by one or both proteins binding directly to the GRIP domain. To investigate this possibility, Arl1p and Arl3p were expressed in *E. coli* as fusions to GST. In both cases, the first fourteen residues were removed, as the equivalent part of ARF1 forms an amphipathic helix that is involved in membrane association, and its removal has been found to improve solubility without loss of effector interactions [24, 25]. The resulting fusion proteins were bound to beads and loaded with either GDP or the nonhydrolyzable GTP analog

GTP-γ-S. The beads were then exposed to yeast cytosol, and after washing, bound proteins eluted with SDS sample buffer. Figure 2A shows that Imh1p was bound to the GST-Arl1p and that this binding was increased in the GTP form. In contrast, a cytosolic protein showed no detectable binding to the beads, and no binding of Imh1p was seen to GST-Arl3p (Figure 2A and data not shown).

Next, the C-terminal 89 and 109 residues of Imh1p were both expressed in *E. coli* with a His$_6$ tag at the N terminus. Both include the GRIP domain, which by sequence similarity comprises the last ∼50 residues of Imh1p. *E. coli* lysates were then applied to GST-Arl1p immobilized on beads, washed, and eluted as before. Figure 2B shows that the recombinant GRIP domains bound to the GTP form of Arl1p, but not to the GDP form. This binding was efficient enough for the eluted GRIP domain to be easily detectable by Coomassie blue staining. This binding is specific in that no significant binding was observed to GST-Arl3p (Figure 2C). Moreover, when the Y870A mutation was incorporated into the GRIP domain, it did not affect expression in bacteria, but abolished the GTP-specific binding to Arl1p (Figure 2D). Together, these results indicate that the GRIP domain of Imh1p is able to bind directly to Arl1p, but not
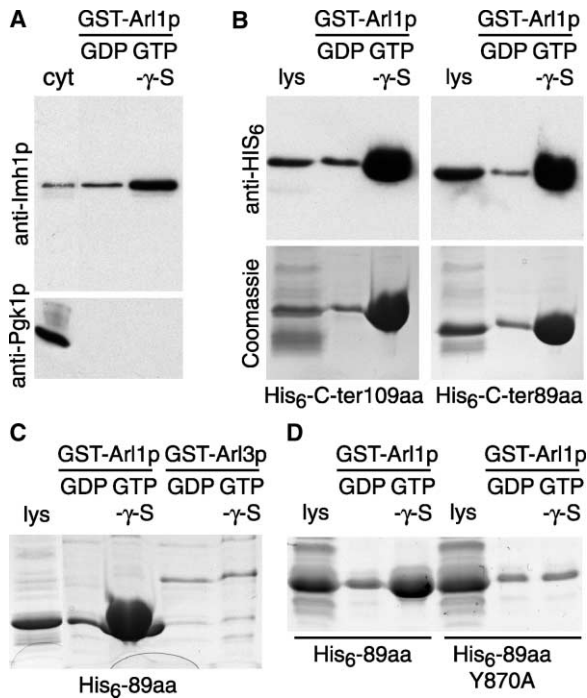
Figure 2. The GRIP Domain of Imh1p Binds Directly to Arl1p

(A) Protein blots of total yeast cytosol (cyt) or of the proteins from the cytosol that bound to GST-Arl1p immobilized on beads and loaded with the indicated nucleotide. The blots were probed with antibodies to either Imh1p or phosphoglycerate kinase (Pgk1p), an abundant cytosolic enzyme.

(B) Binding of the indicated C-terminal portions of Imh1p to immobilized GST-Arl1p loaded with the indicated nucleotide. Extracts of *E. coli* expressing the His$_6$-tagged proteins (lys) were applied to the beads, which were washed, and the bound proteins eluted with SDS buffer and analyzed by gel electrophoresis followed by blotting with anti-His$_6$ or by staining with Coomassie blue.

(C) As (B) except that binding was to GST fusions to Arl1p or Arl3p as indicated.

(D) As (B) except that binding of the Imh1p C-terminal 89 residues was compared to that of a version in which the tyrosine corresponding to residue 870 in the intact protein had been mutated to alanine.

Arl3p, and that the binding is preferentially to the GTP form of Arl1p. This conclusion is consistent with the previously reported yeast two-hybrid interaction between mammalian ARL1 and the GRIP domain of golgin-245, although this interaction was proposed to serve to recruit ARL1 to the Golgi [1]. Our in vivo results suggest that the converse is true, as Imh1p is mislocalized when Arl1p is missing, and indeed the localization of Arl1p is unaffected by deletion of *IMH1* (our unpublished observations).

The previous yeast two-hybrid screen for effectors for mammalian ARL1 identified a number of putative effectors in addition to golgin-245 [1]. These include Arfaptin2 (POR1), phosphodiesterase (PDE) δ and its relative Unc-119, mitotic kinesin-like protein 1 (MKLP1), and a protein of unknown function called SCOCO (short coiled-coil). None of these proteins has an obvious homolog among the currently described proteins of *S. cerevisiae*. However, human SCOCO comprises only 81 amino acids, which is less than the 100 residue lower limit for open reading frame (ORF) size that was used

for the annotation of the *S. cerevisiae* genome. Therefore, we used tBLASTn to search the genome with the human SCOCO sequence and found a small ORF between genes YER180c and YER181c that encodes an 85 residue protein related to SCOCO (Figures 3A). Moreover, a very closely related ORF is present in the genomes of other budding yeasts, including *S. mikitae*, *S. paradoxus*, and *S. castellii* (Figure 3A). This conservation strongly suggests that the ORF is a bona fide gene, and to confirm that it is transcribed and translated, a GFP tag was inserted at the C terminus of the ORF by homologous recombination. Figure 3B shows that this resulted in the appearance of a GFP-tagged protein with a mobility corresponding to the predicted size of the fusion protein. A similar sized band was also observed when the ORF was expressed from a constitutive promoter with a GFP tag attached to the putative initiator methionine. These results indicate that this ORF is expressed, and we shall refer to it as *SLO1* (for *SCOCO-like ORF*).

To examine a possible interaction with Arl1p, Slo1p was expressed in *E. coli* with an N-terminal His$_6$ tag and applied to the immobilized GST forms of the GTPases as above. Figure 3C shows that Slo1p did not exhibit the Arl1p-GTP-specific binding seen with the GRIP domain. However, the protein did bind to immobilized Arl3p, although this was apparently independent of nucleotide state. The sequence of SCOCO shows that a tyrosine residue near the C terminus is invariant in all species so far examined (Figure 3A). Mutation of this residue to alanine (Y72A) did not affect the solubility of Slo1p, but all but abolished binding to Arl3p, suggesting that the interaction is specific (Figure 3D). It is perhaps intriguing that the sequence following this tyrosine (YIxNLM) is similar to that following the conserved tyrosine in the GRIP domain (YLxNhh, where h is hydrophobic), although the rest of Slo1p does not resemble the GRIP domain. While human SCOCO was found to be Golgi localized [1], GFP-Slo1p is diffusely distributed through the cytoplasm (our unpublished observations), although it is quite possible that the large tag could affect the distribution of such a small protein. Finally, deletion of *SLO1* had no effect on viability and, unlike deletion of *ARL3*, it did not affect the distribution of GFP-Imh1p, suggesting that Arl3p may have additional functions that are mediated by Slo1p (Figure 3E).

The above results indicate that Arl1p recruits Imh1p to membranes. This raises the question of whether this is the sole function of Arl1p in yeast. To address this we scaled up the affinity chromatography of yeast cytosol with immobilized GST-Arl1p that we had used to detect the interaction with Imh1p. Figure 4A shows that most of the proteins that bound to the immobilized Arl1p were common between GTP and GDP forms of Arl1p, and are thus likely to represent nonspecific background binding. However, a couple of ~90 kDa bands were specific to the eluate from the GTP form of Arl1p. Mass spectrometry of tryptic peptides from these proteins identified them as Vps54p and Vps53p, components of the GARP/VFT complex, a tetrameric complex that is proposed to tether endosome-derived vesicles to the late Golgi and has been shown to interact with the GTPase Ypt6p and the Golgi SNARE Tlg1p [2, 3, 26].

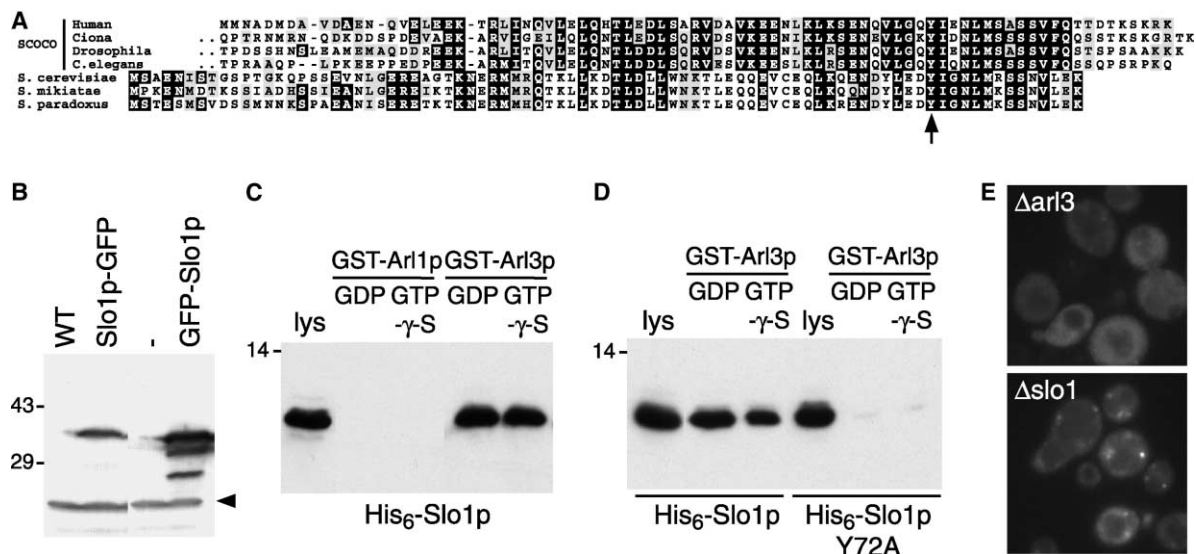To confirm the identification of GARP/VFT subunits,

Figure 3. Slo1p, a Yeast Relative of the Mammalian Protein SCOCO

(A) Alignment of SCOCO and its relatives from *Drosophila* (CG5934), *C. elegans* (T07C4.10b), and *Ciona intestinalis* (from ESTs), along with proteins encoded by short ORFs from the genomes of the indicated yeast species. Sequences were aligned with CLUSTAL W and shaded where three or more residues are related (gray) or identical (black). An arrow indicates the invariant tyrosine.

(B) Anti-GFP protein blot of total cellular protein from either wild-type cells (WT), or the same with GFP integrated at the C terminus of the Slo1p ORF, or wild-type cells containing a *URA3* CEN plasmid expressing either nothing (−) or GFP-Slo1p from the *TPI1* promoter. The antibody detects a background band (arrow) in total yeast extract.

(C) Anti-His$_6$ protein blot of total lysate from *E. coli* expressing His$_6$-Slo1p, or of the proteins from the lysate that bound to GST-Arl1p or GST-Arl3p beads that had been pre-loaded with the indicated nucleotide.

(D) As (C) except that only GST-Arl3p was used, and binding of Slo1p was compared to a version in which tyrosine 72 (indicated in [A]) had been mutated to alanine.

(E) Fluorescent micrographs of the indicated yeast strains expressing GFP-Imh1p from the plasmid used in Figure 1.

cytosols from yeast expressing GFP-tagged forms of Vps53p and Vps54p were applied to the GST-Arl1p beads, and in both cases, the tagged protein was found to associate with the beads in a GTP-dependent manner (Figure 4B). The GARP/VFT complex has four subunits, and to investigate which subunit is responsible for the interaction with Arl1p, we took advantage of the observation that the tetramer disassembles when one subunit is missing, allowing a different subunit to be overexpressed without interacting with the other components [2, 27]. This approach has been used to show that the subunit Vps52p interacts with Ypt6p, and we found that overexpressed Vps53p is preferentially bound to the immobilized Arl1p, although the interaction is no longer nucleotide dependent (Figure 4C). A similar phenomenon was observed for the association between Vps52p and Ypt6p [27], and perhaps reflects the free subunits having slightly altered conformations compared to those adopted in the assembled complex. So far it has not proven possible to express and assemble the GARP/VFT tetramer in *E. coli* to demonstrate a direct interaction with Arl1p (S. Siniossoglou, personal communication). However, the binding of GARP/VFT from yeast cytosol to immobilized GST-Arl1p, as described above, is at least unaffected by deletion of Imh1p (data not shown). Finally, when Vps54-GFP was coexpressed with Arl1p that had been tagged with a nonmultimerizing red fluorescent protein [28], the two proteins were found to be colocalized in living cells, strengthening the possibil-

ity that they interact in vivo. Together, these results indicate that Arl1p not only recruits Imh1p, but also interacts, either directly or indirectly, with the GARP/VFT vesicle-tethering complex.

Although GARP/VFT has already been found to be an effector for the GTPase Ypt6p, it is distantly related to the exocyst and COG "quatrefoil" vesicle-tethering complexes, of which the exocyst has been found to be bound by multiple GTPases, including Sec4p, Rho1p, and Cdc42p [3, 29, 30]. When GFP-tagged subunits of GARP/VFT were expressed in cells lacking Ypt6p, they were found to be much more dispersed, but not completely delocalized [3]. Moreover, deletion of GARP/VFT subunits has a more severe effect on vacuolar sorting than loss of Ypt6p [2, 21]. However, deletion of Arl1p did not substantially alter the punctate distribution of Vps54p-GFP (our unpublished observations), suggesting that the major determinant of GARP/VFT complex localization is Ypt6p. It is possible that the interaction with Arl1p serves some other perhaps regulatory role, or is required for attachment to other membranes.

The direct binding between Arl1p and the Imh1p GRIP domain provides a clear rationale for the requirement for Arl1p for the normal localization of Imh1p, but does not account for Arl3p also being required for Imh1p targeting. However, it seems likely that mislocalization of Imh1p could also result from perturbation of the targeting of Arl1p. Therefore, we examined the localization of Arl1p tagged at its C terminus with GFP. Figure 5A
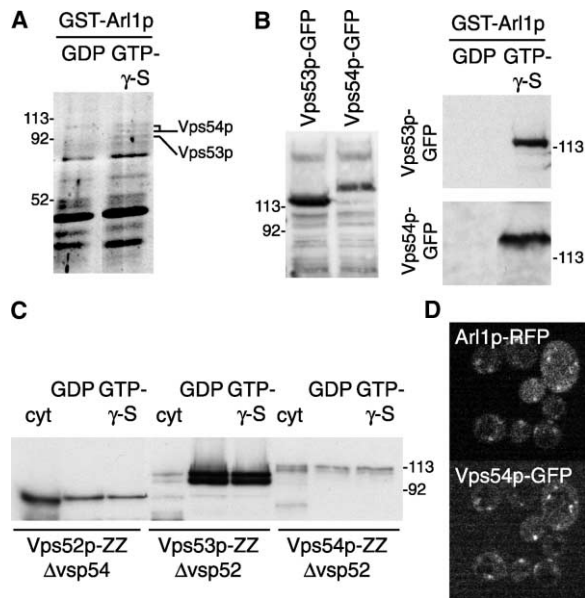
Figure 4. The GARP/VFT Complex Binds to Immobilized GST-Arl1p

(A) Coomassie blue-stained gel of proteins isolated from yeast lysate by affinity chromatography with immobilized GST-Arl1p loaded with the indicated nucleotides. Proteins specific to the GST-γ-S lane were identified by mass spectrometry as indicated. Vps54p peptides were found in two bands, the upper containing in addition Eft2p, an abundant cytosolic protein also found in the GDP lane.

(B) Anti-GFP proteins blots of total yeast lysate from cells expressing GFP-tagged forms of Vps53p or Vps54p as indicated, or of proteins isolated from these lysates by binding to immobilized and nucleotide-loaded GST-Arl1p. The GFP-tagged proteins were expressed from their own promoters in *LEU2* CEN plasmids [3].

(C) Anti-protein A blots of total lysates prepared from the indicated strains (cyt) or of proteins isolated from these lysates by binding to GST-Arl1p as in (B). The strains lacked the indicated subunits of the GARP/VFT complex and expressed protein A (ZZ) tagged versions of different subunits from their own promoters in 2 μ plasmids [27]. Vps54p-ZZ migrates as a doublet, as previously described [3, 27].

(D) Confocal micrographs of live yeast expressing both Arl1p tagged at the C terminus with an intrachain dimer of dsRed (tdimer2(12), [28]) and Vps54p-GFP.

shows that this fusion has a similar punctate distribution to that seen with GFP-Imh1p. However, in a Δarl3 strain, the distribution of Arl1p-GFP is altered and the protein is primarily cytosolic. In such cells, the Golgi itself is not disturbed as judged by an unaltered distribution of GFP-Sec7p (Figure 1D). We also examined the distribution of a GFP fusion to Arl3p itself and found it to be mostly in a diffuse distribution, although faint punctate staining could be seen in some cells, suggesting that the protein is not predominantly Golgi associated (Figure 5B). Thus, it appears that Arl3p is required for the targeting of Imh1p because it acts in a pathway that recruits Arl1p to Golgi membranes.

The membrane association of Arl1p, like other GTPases of the ARF/Sar family, would be expected to be linked to nucleotide state, as GTP binding will cause exposure of the N-terminal amphipathic helix that mediates tight membrane association [4, 5]. Thus, one function of Arl3p may be to specify the location of a GTP-exchange factor (GEF) for Arl1p. No specific GEF has so far been identified for any of the ARL GTPases, al-
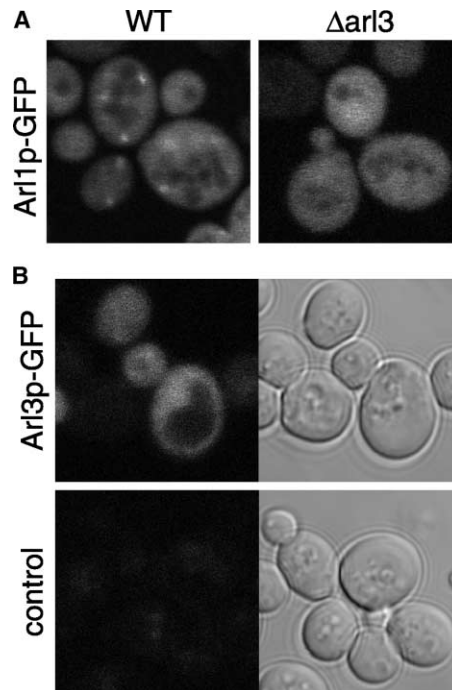

Figure 5. Arl3p Is Required for Normal Targeting of Arl1p

(A) Confocal micrographs of live yeast expressing Arl1p-GFP on a CEN vector with a constitutive *PHO5* promoter. The cells were either wild-type or lacking *ARL3* as indicated.

(B) Confocal micrographs of live yeast containing either a plasmid expressing Arl3p-GFP as in (A) or an empty vector, as indicated.

though it has recently been suggested that the protein Mon2p (Ysl2p) could be a GEF for Arl1p, based on it being distantly related to the ARF GEF Sec7p [31]. However, this sequence homology is outside of the Sec7 domain that is responsible for exchange activity in Sec7p, and moreover, we find the distribution of Arl1p-GFP to be apparently unaffected in a strain lacking *MON2* (our unpublished observations). Resolution of the role of Arl3p will require identification of further effectors and GEFs for both Arl1p and Arl3p. Nonetheless, it seems clear that the two proteins function in a pathway that results in Arl1p-dependent binding of proteins on Golgi membranes. Recently, two Ypt/Rab proteins have been proposed to form a pathway [32], and it may be that such complexity of GTPase function is required to ensure that GTPases are activated with a high degree of spatial accuracy so that vesicle coats and tethers, and hence membrane traffic itself, can be organized with the required precision [33].

References

1. Van Valkenburgh, H., Shern, J.F., Sharer, J.D., Zhu, X., and Kahn, R.A. (2001). ADP-ribosylation factors (ARFs) and ARF-like 1 (ARL1) have both specific and shared effectors: characterizing ARL1-binding proteins. J. Biol. Chem. *276*, 22826–22837.

2. Conibear, E., and Stevens, T.H. (2000). Vps52p, Vps53p, and Vps54p form a novel multisubunit complex required for protein sorting at the yeast late Golgi. Mol. Biol. Cell *11*, 305–323.

3. Siniossoglou, S., and Pelham, H.R. (2001). An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes. EMBO J. *20*, 5991–5998.

4. Pasqualato, S., Renault, L., and Cherfils, J. (2002). Arf, Arl, Arp and Sar proteins: a family of GTP-binding proteins with a structural device for 'front-back' communication. EMBO Rep. *3*, 1035–1041.

5. Antonny, B., Beraud-Dufour, S., Chardin, P., and Chabre, M. (1997). N-terminal hydrophobic residues of the G-protein ADP-ribosylation factor-1 insert into membrane phospholipids upon GDP to GTP exchange. Biochemistry *36*, 4675–4684.

6. Lowe, S.L., Wong, S.H., and Hong, W. (1996). The mammalian ARF-like protein 1 (Arl1) is associated with the Golgi complex. J. Cell Sci. *109*, 209–220.

7. Tamkun, J.W., Kahn, R.A., Kissinger, M., Brizuela, B.J., Rulka, C., Scott, M.P., and Kennison, J.A. (1991). The arflike gene encodes an essential GTP-binding protein in *Drosophila*. Proc. Natl. Acad. Sci. USA *88*, 3120–3124.

8. Lu, L., Horstmann, H., Ng, C., and Hong, W. (2001). Regulation of Golgi structure and function by ARF-like protein 1 (Arl1). J. Cell Sci. *114*, 4543–4555.

9. Barr, F.A. (1999). A novel Rab6-interacting domain defines a family of Golgi-targeted coiled-coil proteins. Curr. Biol. *9*, 381–384.

10. Kjer-Nielsen, L., Teasdale, R.D., van Vliet, C., and Gleeson, P.A. (1999). A novel Golgi-localisation domain shared by a class of coiled-coil peripheral membrane proteins. Curr. Biol. *9*, 385–388.

11. Munro, S., and Nichols, B.J. (1999). The GRIP domain—a novel Golgi-targeting domain found in several coiled-coil proteins. Curr. Biol. *9*, 377–380.

12. McConville, M.J., Ilgoutz, S.C., Teasdale, R.D., Foth, B.J., Matthews, A., Mullin, K.A., and Gleeson, P.A. (2002). Targeting of the GRIP domain to the trans-Golgi network is conserved from protists to animals. Eur. J. Cell Biol. *81*, 485–495.

13. Gaynor, E.C., Chen, C.Y., Emr, S.D., and Graham, T.R. (1998). ARF is required for maintenance of yeast Golgi and endosome structure and function. Mol. Biol. Cell *9*, 653–670.

14. Peyroche, A., Courbeyrette, R., Rambourg, A., and Jackson, C.L. (2001). The ARF exchange factors Gea1p and Gea2p regulate Golgi structure and function in yeast. J. Cell Sci. *114*, 2241–2253.

15. Lee, F.J., Stevens, L.A., Kao, Y.L., Moss, J., and Vaughan, M. (1994). Characterization of a glucose-repressible ADP-ribosylation factor 3 (*ARF3*) from *Saccharomyces cerevisiae*. J. Biol. Chem. *269*, 20931–20937.

16. Lee, F.J.S., Huang, C.F., Yu, W.L., Buu, L.M., Lin, C.Y., Huang, M.C., Moss, J., and Vaughan, M. (1997). Characterization of an ADP-ribosylation factor-like 1 protein in *Saccharomyces cerevisiae*. J. Biol. Chem. *272*, 30998–31005.

17. Huang, C.F., Buu, L.M., Yu, W.L., and Lee, F.J. (1999). Characterization of a novel ADP-ribosylation factor-like protein (yARL3) in *Saccharomyces cerevisiae*. J. Biol. Chem. *274*, 3819–3827.

18. Rosenwald, A.G., Rhodes, M.A., Van Valkenburgh, H., Palanivel, V., Chapman, G., Boman, A., Zhang, C.J., and Kahn, R.A. (2002). *ARL1* and membrane traffic in *Saccharomyces cerevisiae*. Yeast *19*, 1039–1056.

19. Bonangelino, C.J., Chavez, E.M., and Bonifacino, J.S. (2002). Genomic screen for vacuolar protein sorting genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell *13*, 2486–2501.

20. Tsukada, M., Will, E., and Gallwitz, D. (1999). Structural and functional analysis of a novel coiled-coil protein involved in Ypt6 GTPase-regulated protein transport in yeast. Mol. Biol. Cell *10*, 63–75.

21. Li, B., and Warner, J.R. (1996). Mutation of the Rab6 homologue of *Saccharomyces cerevisiae*, *YPT6*, inhibits both early Golgi function and ribosome biosynthesis. J. Biol. Chem. *271*, 16813–16819.

22. Siniossoglou, S., Peak-Chew, S.Y., and Pelham, H.R. (2000). Ric1p and Rgp1p form a complex that catalyses nucleotide exchange on Ypt6p. EMBO J. *19*, 4885–4894.

23. Gangi Setty, S.R., Shin, M.E., Yoshino, A., Marks, M.S., and Burd, C.G. (2003). Golgi recruitment of GRIP domain proteins by Arf-like GTPase 1 (Arl1p) is regulated by the Arf-like GTPase 3 (Arl3p). Curr. Biol. *13*, this issue, 401–404.

24. Goldberg, J. (1999). Structural and functional analysis of the ARF1-ARFGAP complex reveals a role for coatomer in GTP hydrolysis. Cell *96*, 893–902.

25. Paris, S., Beraud-Dufour, S., Robineau, S., Bigay, J., Antonny, B., Chabre, M., and Chardin, P. (1997). Role of protein-phospholipid interactions in the activation of ARF1 by the guanine nucleotide exchange factor Arno. J. Biol. Chem. *272*, 22221–22226.

26. Conibear, E., Cleck, J.N., and Stevens, T.H. (2003). Vps51p mediates the association of the GARP (Vps52/53/54) complex with the late Golgi t-SNARE Tlg1p. Mol. Biol. Cell, in press. Published online December 2002. 10.1091/mbc.E02-10-0654.

27. Siniossoglou, S., and Pelham, H.R. (2002). Vps51p links the VFT complex to the SNARE Tlg1p. J. Biol. Chem. *277*, 48318–48324.

28. Campbell, R.E., Tour, O., Palmer, A.E., Steinbach, P.A., Baird, G.S., Zacharias, D.A., and Tsien, R.Y. (2002). A monomeric red fluorescent protein. Proc. Natl. Acad. Sci. USA *99*, 7877–7882.

29. Whyte, J.R., and Munro, S. (2001). The Sec34/35 Golgi transport complex is related to the exocyst, defining a family of complexes involved in multiple steps of membrane traffic. Dev. Cell *1*, 527–537.

30. Novick, P., and Guo, W. (2002). Ras family therapy: Rab, Rho and Ral talk to the exocyst. Trends Cell Biol. *12*, 247–249.

31. Jochum, A., Jackson, D., Schwarz, H., Pipkorn, R., and Singer-Kruger, B. (2002). Yeast Ysl2p, homologous to Sec7 domain guanine nucleotide exchange factors, functions in endocytosis and maintenance of vacuole integrity and interacts with the Arf-Like small GTPase Arl1p. Mol. Cell. Biol. *22*, 4914–4928.

32. Ortiz, D., Medkova, M., Walch-Solimena, C., and Novick, P. (2002). Ypt32 recruits the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles; evidence for a Rab cascade in yeast. J. Cell Biol. *157*, 1005–1015.

33. Munro, S. (2002). Organelle identity and the targeting of peripheral membrane proteins. Curr. Opin. Cell Biol. *14*, 506–514.