Supplementary Material

Golgi Recruitment of GRIP Domain Proteins by Arf-like GTPase 1 Is Regulated by Arf-like GTPase 3

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Supplementary Results and Discussion

Supplementary Material for Figure 1

The DsRedT.4 RFP yeast expression vector was constructed by PCR overlap extension of the PCR-amplified DsRedT.4 gene [S1] and the PCR-amplified yeast *PRC1* promoter (−300 to +3, amplified from genomic yeast DNA). This PCR product was cloned into pRS416 and pRS426. A portion of *IMH1* (encoding amino acids 735–912) was PCR amplified, which placed a BamHI site at the beginning of the fragment, and was then cloned in frame into the pRS426-PRC1-DsRedT.4 plasmid. This plasmid was transformed into strain BY4742 expressing Sec7p-GFP as the only source of Sec7p [S2], or into a strain transformed with *CHS3*-GFP and *CHS7* plasmids [S3]. Cells were visualized with a Nikon Eclipse E800 microscope fitted with a cooled, high-resolution, charge-coupled device camera. All micrographs in Figures 1, 2, 4 and the Supplementary Figures were acquired with Phase 3 Imaging software (Phase 3 Imaging Systems) and were false colored and contrast enhanced with Adobe PhotoShop software.

Supplementary Material for Figure 2

The GRIP domains of the mouse tGolgin-1 GRIP domain (amino acids 2053–2238) or the human Golgin-97 GRIP domain (amino acids 588–767) were amplified by PCR from the corresponding cDNAs and were then cloned into the yeast GFP expression vector pGO-GFP [S4]. Each plasmid was transformed into wild-type, *arl1*Δ, and *arl3*Δ strains and were then visualized by fluorescence microscopy. All deletion mutants were purchased from InVitrogen and are from the *Saccharomyces* Genome Deletion Project Consortium collection [S5]. The BY4742 strain background was used. The relevant genotypes of all yeast strains in which GFP-GRIP proteins were mislocalized were confirmed by PCR using oligonucleotide primers to amplify the relevant locus, including approximately 300 bp upstream of the start codon and 300 bp downstream of the stop codon.

Supplementary Material for Figure 3

For the GST-GRIP binding experiments, a fusion protein consisting of GST coupled to the Imh1p GRIP domain, or GST as a control (approximately 20 μg each), was captured on GSH Sepharose and mixed with 15 μg purified, recombinant wild-type ("WT"), GTPase-deficient ("GTP"), or GTP binding-defective ("GDP") Arl1p or Arl3p. The beads were incubated in a total volume of 150 μl binding buffer (1 mM KH_2PO4, 10 mM Na_2HPO4, 137 mM NaCl, 2.7 mM KCl, 1 mM EDTA, 3 mM $MgCl_2$, 1mM DTT, 0.05% Triton X-100 [pH 7.4]) for 60 min at 22°C with intermittent shaking and were then washed twice with 300 μl binding buffer. Bound material was eluted with SDS sample buffer, and Arl proteins were visualized by immunoblotting with anti-T7 antibodies (Novagen). To produce recombinant Arl1p and Arl3p, each locus was amplified by PCR from yeast genomic DNA with primers to place a BamHI site in front of the start codon and was then cloned into pET28 (Novagen) so that a 6× His tag and a T7 epitope tag were encoded at the amino terminus. The GTPase-deficient (Arl1p, Q72L; Arl3p, Q78L) and GTP binding-defective (Arl1p T32N; Arl3p T31N) mutants were constructed by point mutagenesis of these plasmids by using the Quickchange kit (Stratagene). The DNA sequences of all PCR-derived and mutagenized genes were determined to confirm that only the desired sequence was present. Recombinant GTPases (including control proteins described in the text) were purified by using an Amersham FPLC system from *E. coli* extracts on metal chelating resin columns that had been preloaded with nickel sulfate. Bound proteins were eluted with an imidazole gradient, and peak fractions were pooled

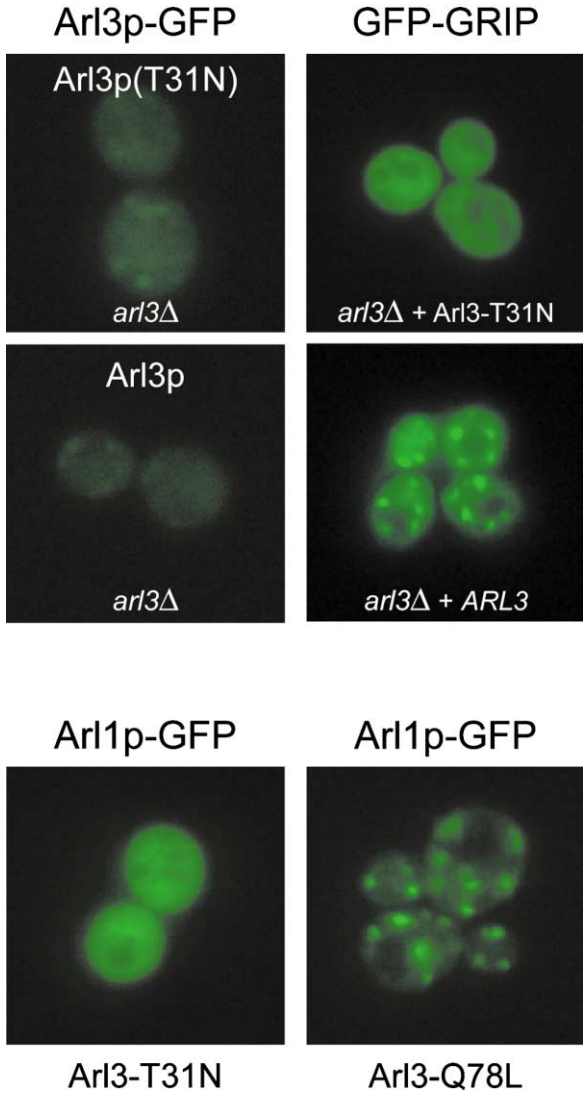

Figure S1. Localization of Arl3p, Arl1p, and Imh1p GRIP Domains in Arl3p GTPase Mutants

GFP-tagged wild-type Arl3p and Arl3p(T31N) were expressed in *arl3*Δ cells from single copy vectors and were visualized by fluorescence microscopy (top, left panels). Localization of GFP-Imh1p-GRIP in Arl3p(T31N) and plasmid-complemented *ARL3* cells was done by cotransforming the *arl3*Δ strain with plasmids to express (nontagged) Arl3p(T31N) or wild-type *ARL3* and GFP-Imh1p-GRIP (right). To determine the localization of Arl1p in *arl3* mutants, a single copy Arl1-GFP vector was cotransformed into an *arl1*Δ *arl3*Δ double mutant strain along with the indicated single copy *arl3* mutant vectors (lower panels).

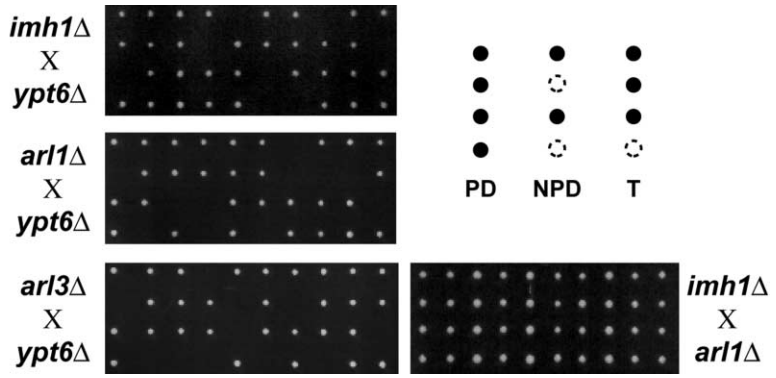

Figure S2. Deletion of *ARL1*, *ARL3*, or *IMH1* Is Synthetically Lethal with a Deletion of *YPT6*

The deletion mutants indicated to the left and right were crossed, and meiotic progeny (i.e., tetrads) were dissected on YPD plates. The plates were photographed after 3 days of incubation at 30°C. All deletion strains were from the *Saccharomyces* Genome Deletion Project Consortium collection. The deleted genes are marked with the kanMX cassette (which confers resistance to G418), which allowed identification of colonies derived from spores with the deletion alleles. In all cases where no growth was observed, the genotype of the spore was predicted to be the double deletion mutant, indicating synthetic lethality between the two deletion alleles. Synthetic lethality has been previously reported between mutations in *imh1* and *ypt6* [S7], and this finding is confirmed in the strain background used for these studies (upper left). These results indicate synthetic lethality between *ypt6*Δ and *arl1*Δ and *ypt6*Δ and *arl3*Δ. We have also found that a *ypt6*Δ *mon2*Δ double mutant is also inviable (data not shown). In crosses between *imh1*Δ and *arl1*Δ and *imh1*Δ and *arl3*Δ, double mutants were recovered (the results of the *imh1*Δ × *arl1*Δ cross is shown, lower right), consistent with the idea that these two genes function on the same pathway. A key is shown in the upper-right corner. NPD = nonparental ditype, PD = parental ditype, T= tetratype. The hollow, dotted circles indicate the number of double mutant (i.e., dead) spores.

and desalted by gel filtration. Proteins were stored in phosphate-buffered saline (1 mM KH_2PO4, 10 mM Na_2HPO4, 137 mM NaCl, 2.7 mM KCl [pH 7.4]) and were used immediately or stored at −80°C until use.

Supplementary Material for Figure 4

Homologous recombination-mediated integrative tagging was used to extend the open reading frames of chromosomal and plasmid-borne *ARL1* and *ARL3* loci with the gene encoding GFP [S6]. For (A)–(C), the Arl1-GFP strain was transformed with the RFP-Imh1p-GRIP plasmid, each tagged protein was visualized by fluorescence microscopy individually (A and B), and then the two micrographs were overlayed (C). The signal for chromosomal Arl3p-GFP was too low to definitively determine localization; however, we found that when Arl3p-GFP was expressed (in *arl3*Δ cells) from a single copy vector (pRS416), the GFP signal was clearly evident. Thus, to visualize Arl3p, the genes encoding wild-type, inactive Arl3p(T31N) or constitutively active Arl3p(Q78L) were cloned into a single copy vector (pRS416) as carboxy-terminal fusions to GFP and were transformed into the *arl3*Δ strain. The constitutively active Arl3p(Q78L) showed a clear punctate signal consistent with Golgi localization (D), while the other forms of Arl3p were localized to the cytosol (see Figure S1). Localization of Arl1p in *arl3*Δ mutant cells was determined by expressing GFP-tagged wild-type Arl1p from a single copy vector that had been transformed into the *arl3*Δ strain (E). To determine if the GTPase cycle of Arl3p regulates Arl1p-dependent Golgi localization of the Imh1p GRIP domain, the *arl3*Δ strain was cotransformed with single copy vectors expressing wild-type or mutant *arl3* genes and the GFP-Imh1p-GRIP plasmid. Golgi localization of GFP-Imh1p-GRIP was observed in wild-type and Arl3p(Q78L) cells (F), but not in Arl3p(T31N) cells (see Figure S1).

Supplementary References

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