Supplemental Materials

Molecular Biology of the Cell

Gustafson and Fromme

Supplemental Table 1. Yeast strains used in this study

Name	Description	Source
SEY6210	*MATα suc2-Δ9 ura3-52 his3-Δ200 leu2-3,112 lys2-801 trp1-Δ901*	Robinson 1988[1]
SEY6210.1	*MATa suc2-Δ9 ura3-52 his3-Δ200 leu2-3,112 lys2-801 trp1-Δ901*	Robinson 1988[1]
BY4741α	*MATα ura3-Δ0 his3-Δ1 leu2-Δ0 lys2-Δ0*	Brachmann 1998[2]
CFY578	SEY6210*Sec7-Mars::TRP1*	Richardson 2016[3]
CFY2376	SEY6210 *Sec7-Mars::TRP1 Gea1-mNeonGreen::HIS3*	This study
CFY2378	SEY6210 *Sec7-Mars::TRP1 Gea2-mNeonGreen::HIS3*	This study
CFY2490	SEY6210.1 *Gea1-3xMars::TRP1 GFP-Vrg4*	This study
CFY2503	SEY6210.1 *Gea2-3xMars::TRP1 GFP-Vrg4*	This study
CFY2872	*BY4741α gea1Δ::KanMX gea2Δ::HIS3* +pCF1248	This study
CFY2873	*BY4741α gea1Δ::NatMX gea2Δ::HIS3 arf1Δ::KanMX* +pCF1248	This study

Name	Description	Vector	Source

Supplemental Table 2. Plasmids used in this study

pBCR314	Sec7(203-2009) with a cleavable His_6 tag	pFastBacHT	Richardson 2012[4]
pMG005	Gea2 FL (1-1459) with a cleavable His_6 tag	pET28	Richardson 2016[3]
pMG020	Gea2ΔHDS3 (1-1196) with a cleavable His_6 tag	pET28	Richardson 2016[3]
pCF1299	Gea2ΔC (1-782) with a cleavable His_6 tag	pET28	Richardson 2016[3]
pMG036	Gea2GEF (558-782) with a cleavable His_6 tag	pET28	This study
pCF1163	Gea1 FL (1-1408) with a cleavable His_6 tag	pET28	Richardson 2012[4]
pArf1	Arf1	pET3	Weiss 1989[5]
pCF1053	Arf1ΔN17	pET28	Richardson 2012[4]
pCF1184	Arl1	pET23	McDonold 2014[6]
pNMT1	Nmt1	pCYC	Duronio 1990[7]
Ypt1-His_7	Ypt1 with C-terminal His_7 tag and cleavable N-terminal GST tag	pGEX-6P	Gift from T. Bretscher
pCM14	Ypt6 with C-terminal His_7 tag and cleavable N-terminal GST tag	pGEX-6P	McDonold 2014[6]
pLT50	Full-length Ypt1 with cleavable N-terminal GST tag	pGEX-6P	Thomas 2016[8]
pLT40	Gdi1 with cleavable N-terminal GST tag	pGEX-6P	Thomas 2016[8]
pLT35	Mrs6 with cleavable N-terminal His_6 tag	pET28	Thomas 2016[8]
pLT41	Bet2 with cleavable N-terminal His_6 tag and Bet4	pCDF-Duet-1	Thomas 2016[8]
pCM10	Gea1 FL (1-1408)-GFP driven by P_{GEA1}	pRS415	This study
pCM09	Gea1ΔHDS3 (1-1225)-GFP driven by P_{GEA1}	pRS415	This study
pCF1312	Gea1ΔC (1-761)-GFP driven by P_{GEA1}	pRS415	This study
pMG001	Gea2 FL (1-1459)-GFP driven by P_{GEA2}	pRS415	This study
pMG002	Gea2ΔHDS3 (1-1196)-GFP driven by P_{GEA2}	pRS415	This study
pCF1313	Gea2ΔC (1-766)-GFP driven by P_{GEA2}	pRS415	This study
pCF1301	Gea1-GFP driven by P_{GEA2}	pRS415	This study
pCF1302	Gea2-GFP driven by P_{GEA1}	pRS415	This study
pCF1248	Gea2 driven by P_{GEA2} (URA3 maintenance plasmid for shuffling strains)	pRS416	This study
pRC2100	GFP-Ypt1 driven by P_{YOP1}	pRS415	Gift from R. Collins
YIplac211-iGFP-VRG4	Integrating plasmid for GFP-VRG4 by two-step gene replacement	YIplac211	Gift from B. Glick

Supplemental Table 3. Composition of liposomes used in this study

	PC	PC-Ni^{2+}	TGN	TGN-Ni^{2+}
Lipid		*Amount (Molar %)*		
DOPC	99	94	24	24
POPC			6	6
DOPE			7	7
POPE			3	3
DOPS			1	1
POPS			2	2
DOPA			1	1
POPA			2	2
PI			29	24
PI(4)P			1	1
CDP-DAG			2	2
PO-DAG			4	4
DO-DAG			2	2
Ceramide (C18)			5	5
Nickel-DOGS		5		5
Cholesterol			10	10
DiR	1	1	1	1

Supplemental References

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4. Richardson, B. C., McDonold, C. M. & Fromme, J. C. The Sec7 Arf-GEF Is Recruited to the trans-Golgi Network by Positive Feedback. *Dev. Cell***22,** 799–810 (2012).
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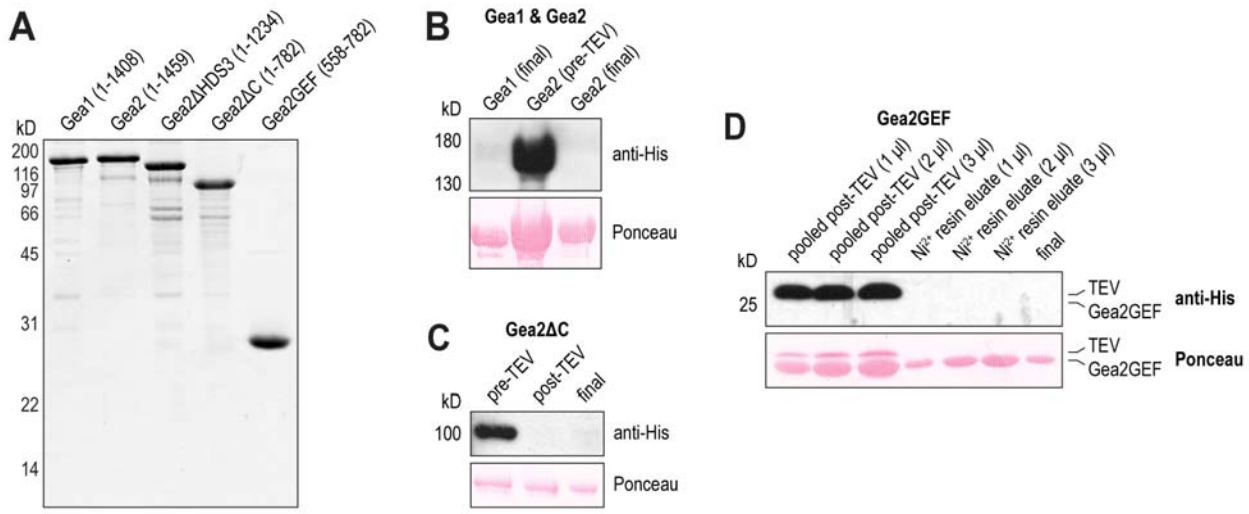
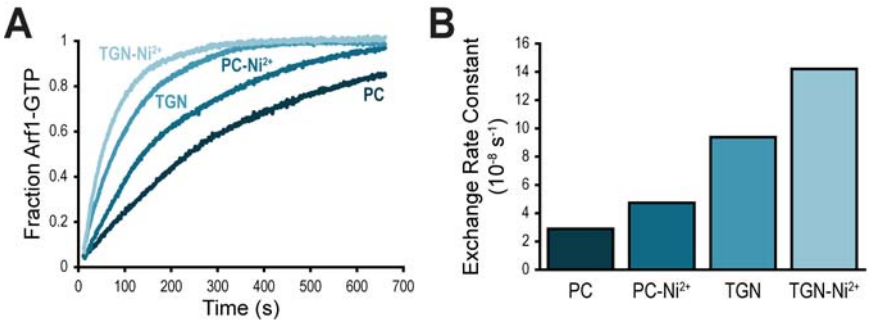
6. McDonold, C. M. & Fromme, J. C. Four GTPases Differentially Regulate the Sec7 Arf-GEF to Direct Traffic at the trans-Golgi Network. *Dev. Cell***30,** 759–767 (2014).
7. Duronio, R. J. *et al.* Protein N-myristoylation in Escherichia coli: Reconstitution of a eukaryotic protein modification in bacteria. *Proc. Natl. Acad. Sci. U. S. A.***87,** 1506–1510 (1990).
8. Thomas, L. L. & Fromme, J. C. GTPase cross talk regulates TRAPPII activation of Rab11 homologues during vesicle biogenesis. *J. Cell Biol*.**215,** 499–513 (2016).



Supplemental Figure 1. GEF constructs used for biochemistry are pure and His_6 tags were successfully cleaved. (A) Equal masses of full length Gea1 and Gea2, Gea2ΔHDS3, Gea2ΔC, and Gea2GEF were separated on an 8% polyacrylamide gel and visualized by Coomassie staining. Western blots with anti-His antibody against (B) Gea2 before TEV cleavage of the His_6 tag and final reagent samples of Gea1 and Gea2, (C) Gea2ΔC before and after TEV cleavage of the His_6 tag, as well as the final Gea2ΔC reagent, and (D) dilutions of fractions of Gea2GEF pooled after TEV cleavage, after incubation with and elution from Ni-NTA resin, and the final Gea2GEF reagent. For all western blots, Ponceau stains reveal protein on the membrane. Note that TEV protease is His_6-tagged.



Supplemental Figure 2. EDTA-induced Arf1 nucleotide exchange favors TGN over PC liposomes *in vitro*. (A) Normalized traces showing EDTA exchange of Arf1 on PC, PC-Ni^{2+}, TGN, and TGN-Ni^{2+} liposomes. (B) Rates of Arf1 exchange determined from traces in (A).