Supplemental Movie Legends

Movie S1: Sec7-labeled TGN compartments disintegrate into smaller structures (Related to Figure 4A)

Time-lapse movie of a GFP-Sec7 strain (CFY1037). Single focal-plane images were acquired every second. The movie plays at 5 fps, which is 5X real-time.

Movie S2: Sec7 follows Ypt1 and precedes Ypt31 at the Golgi. (Related to Figure 4E)

Two time-lapse movies in series: 1) a Sec7-6xDsRed, GFP-Ypt1 strain (CFY1689+GFP-Ypt1); 2) a Sec7-6xDsRed, GFP-Ypt31 strain (CFY1805). Single focal-plane images were acquired every second. The movies play at 10 fps, which is 10X real-time.

Movie S3: Imaging cargo sorting events relative to Sec7 disappearance (Related to Figure 4D)

Four time-lapse movies in series: 1) a Sec7-6xDsRed, GFP-Sec7 strain (CFY1681+pCF1084) used as a control; 2) a Sec7-6xDsRed, GFP-Snc1 strain (CFY1711) shows coincident disappearance of Snc1 and Sec7; 3) a Sec7-6xDsRed, Kex2-GFP strain (CFY1690) shows Kex2 is sorted before Sec7 disappearance; 4) a Sec7-6xDsRed, GFP-Tlg1 strain (CFY1689+GFP-Tlg1) shows Tlg1 is sorted before Sec7 disappearance. Single focal-plane images were acquired every second. The movies play at 5 fps, which is 5X real-time.

Supplemental Experimental Procedures

Plasmid constructs

Plasmids were constructed using standard techniques and are described below.

Yeast strains and genetic methods

Strains were generated by standard techniques and are described below.

Antibodies

The anti-Ypt1 (Preuss et al., 1992) and anti-Ypt31 (Jedd et al., 1997) antisera used for Figure S3C were gifts from N. Segev. The anti-G6PDH antibody was purchased from Sigma (A9521).

Protein purification

As reported previously, removal of the first 202 residues of Sec7 greatly improved protein expression, and did not compromise the essential function(s) of the protein (Richardson et al., 2012). Purification of $Sec7_f$ and Sec7ΔC+HDS1 constructs were as previously described (Richardson et al., 2012), with the addition of treatment by TEV-protease to remove the 6xHis-tag prior to the final chromatography step. Removal of the tag was confirmed by immunoblot (Figure S1D). The *sec7-4$_f$* and Sec7ΔHDS4 constructs were purified using the same procedure as $Sec7_f$. We were not able to purify a well-behaved construct for the HDS2-3 domain region. In our experience the conserved N-terminal domains of Sec7 appear to be required for expression and purification of truncation constructs. Myristoylated Arf1 was purified as reported (Ha et al., 2005).

C-terminal 7xHis-tagged yeast Rab protein expression constructs were created using the pGEX-6P vector backbone. Constructs were designed so that the C-terminal cysteine residues (prenylated *in vivo*) were replaced with a 7xHis-tag for membrane anchoring. The expression vectors were transformed into Rosetta2 (Novagen) *E. coli* cells and grown in 1-2L TB at 37°C to an OD of ~3.0. The temperature was then decreased to 18°C, expression was induced with 500 μM IPTG, and cells were grown overnight. Cells were lysed by sonication in lysis buffer containing 1xPBS, 2 mM $MgCl_2$, and 5 mM β-Me. The lysate was centrifuged, and protein was purified from the cleared lysate by glutathione resin (G Biosciences) followed by cleavage with PreScission protease overnight at 4°C in a buffer containing 50 mM Tris-HCl pH

7.5, 150 mM NaCl, 1 mM EDTA, 2 mM $MgCl_2$, and 5 mM β-Me. The eluate containing the purified protein was then collected and exchanged into a buffer containing 20 mM Tris-HCl pH 8, 100 nM NaCl, 2 mM $MgCl_2$, and 1 mM DTT before use in GEF activity assays. We note that there are two distinct species in some of the purifications. Analysis by anti-His-tag immunoblot indicated that the faster migrating species are likely N-terminal proteolytic products (Figure S1C). The structure of yeast Ypt31 demonstrated that the N-terminal nine amino acids are disordered (Ignatev et al., 2008).

For purification of myristoylated Arl1, a plasmid encoding full-length yeast Arl1 was introduced into BL21(DE3) *E. coli* cells together with the Nmt1 plasmid encoding the N-myristoyl transferase enzyme. Growth and expression was the same as for Arf1. Following cell lysis (in 15 mL lysis buffer per 1 liter cell pellet: 25 mM Tris pH 7.5, 100 mM NaCl, 1 mM $MgCl_2$, 10 mM ßME, 1 mM PMSF) by sonication, the lysate was clarified by centrifugation. The supernatant was incubated with SP-sepharose (GE Healthcare) resin (1 ml resin per 7 mg protein in lysate). The unbound fraction was applied to a MonoQ column (GE Healthcare), and a 100 mM to 1 M NaCl gradient (25 mM Tris pH 8.0, 2 mM $MgCl_2$, 1 mM DTT) was run. Peak fractions were pooled and run on a Superdex 200 column (GE Healthcare). Peak fractions were then run on a HiTrap Phenyl column (GE Healthcare), using a 3 M to 0 M NaCl gradient (20 mM Tris pH 8.0, 2 mM $MgCl_2$, 1 mM DTT). We note that the purified protein runs as three species on an SDS-PAGE gel.

Liposome flotation (binding) assays

Flotation assays were prepared as described (Richardson et al., 2012), using 4 ug of each protein and 0.3 mM of liposomes per 75 ul binding reaction. The His-tags at the C-termini of the Rab proteins allow these proteins to be bound to the liposomes regardless of their activation state. In contrast, the Arf1 and Arl1 proteins only bind to liposomes when activated, due to a conformational change that exposes an N-terminal membrane-inserting helix. 15% PAGE gels were stained with IRDye (Li-Cor) and imaged on a Li-Cor Odyssey instrument. Gel images were processed with the ImageJ despeckle filter and min/max levels were adjusted in Photoshop for clarity.

GEF activity assays

Tryptophan fluorescence GEF assays were performed at 30°C as described (Richardson et al., 2012). See Figure S2A for an example of a single full experiment. Unless otherwise indicated, most assays were performed with 500 nM GTPase activators, 30 nM GEF construct, and 400 nM myrArf1-GDP substrate. Assays in Figure 2C,D used 100nM GEF construct. Assays in Figures 3C and S2B,D,E used either 100 nM GTPase activator or the concentration specified in the figure and 50 nM GEF construct. Liposomes batches were pooled, and the same pool was used for all GEF assays, except for those in Figures 3C and S2B,D,E, which were performed with a separate pool of liposomes.

Microscopy

See below for which strains and plasmids were used for each experiment.

Plasmids used in this study

Name	Description	Source
pNmt1	Nmt1 (S. ce.) in pCYC plasmid	(Duronio et al., 1990)
pArf1	Arf1 (S. ce.) in pET3c	(Weiss et al., 1989)
pCF1184	Arl1 (S. ce.) in pET23	this study
Ypt1-7His	Ypt1 with C-terminal his-tag and cleavable GST-fusion in pGEX-6P	T. Bretscher
pCM14	Ypt6 with C-terminal his-tag and cleavable GST-fusion in pGEX-6P	this study
pCM15	Ypt31 with C-terminal his-tag and cleavable GST-fusion in pGEX-6P	this study
pCM16	Ypt31 “soluble” (no his-tag) with cleavable GST-fusion in pGEX-6P	this study
pCM17	*ypt31-101* with C-terminal his-tag and cleavable GST-fusion in pGEX-6P	this study*
Ypt32-7His	Ypt32 with C-terminal his-tag and cleavable GST-fusion in pGEX-6P	T. Bretscher
pBCR314	6xHis-Sec7$_f$ (residues 203-2009) in pFastBacHT	(Richardson et al., 2012)
pBCR389	6xHis-Sec7ΔC+HDS1 (residues 203-1220) in pET28	(Richardson et al., 2012)
pCM18	6xHis-Sec7ΔHDS4 (residues 203-1799) in pFastBacHT	this study
pCF1257	6xHis-*sec7-4*$_f$ (residues 203-2009, G883D) in pFastBacHT	this study
pRS304	yeast integration vector with *TRP1* marker	(Sikorski and Hieter, 1989)
pRS305	yeast integration vector with *LEU2* marker	(Sikorski and Hieter, 1989)
pRS415	yeast centromeric plasmid with *LEU2* marker	(Sikorski and Hieter, 1989)
pRS416	yeast centromeric plasmid with *URA3* marker	(Sikorski and Hieter, 1989)
pRS426	yeast 2-micron (high copy) vector with *URA3* marker	(Sikorski and Hieter, 1989)
pCF1043	*SEC7* (includes ~1 kB of 5’UTR) in pRS416	(Richardson et al., 2012)
pCF1084	GFP-Sec7 driven by P_{SEC7} in pRS415	(Richardson et al., 2012)
pCF1105	GFP-sec7-1 driven by P_{SEC7} in pRS415	this study
pCF1106	GFP-sec7-4 driven by P_{SEC7} in pRS415	this study
pCF1191	GFP-Sec7 (driven by P_{SEC7}) integration plasmid in pRS305	this study
pCF1197	GFP-sec7-1 (driven by P_{SEC7}) integration plasmid in pRS305	this study
pCF1246	RFP^{MARS}-sec7-4 (driven by P_{SEC7}) integration plasmid in pRS304	this study
pCF1258	*SEC7* gene in pRS426	this study
VSB284	*YPT31* gene in pRS426	(Sciorra et al., 2005)
pCF1259	*ARL1* gene in pRS426	this study
pCF1261	*ARF1* gene in pRS426	this study
pCF1263	*YPT1* gene in pRS426	this study
GFP-Tlg1	GFP-Tlg1 in pRS416	(Xu et al., 2013)
GFP-Ypt1	GFP-Ypt1 in pRS415	(Buvelot Frei et al., 2006)
VSB311	GFP-Ypt31 in pRS415	(Sciorra et al., 2005)
GFP-Snc1	GFP-Snc1 integration plasmid in pRS306	(Lewis et al., 2000)
pRC678	GFP-Ypt31 integration plasmid in pRS306	(Buvelot Frei et al., 2006)
pRC679	GFP-Ypt32 integration plasmid in pRS306	(Buvelot Frei et al., 2006)
Sec7-6xDsRed	Sec7-6xDsRed integration plasmid (URA3)	(Losev et al., 2006)
Sys1-DsRed	Sys1-DsRed in pRS416	T. Graham

*The *ypt31-101* mutation was originally reported in (Sciorra et al., 2005). This allele has the following mutations: K43R, K127N

Yeast strains used in this study

Name	Description	(Strain construction method)	Source
SEY6210	*MATa his3-Δ200 leu2-3,112 lys2-801 trp1-Δ901 ura3-52 suc2-Δ9*		(Robinson et al., 1988)
SEY6210.1	*MATa his3-Δ200 leu2-3,112 lys2-801 trp1-Δ901 ura3-52 suc2-Δ9*		(Robinson et al., 1988)
BY4741	*MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0*		(Brachmann et al., 1998)
BY4742	*MATa his3-Δ1 leu2-Δ0 lys2-Δ0 ura3-Δ0*		(Brachmann et al., 1998)
BYgeneΔ	*BY4741 geneΔ::KanMX*		(Giaever et al., 2002)
VSY468	*SEY6210 ypt31-101::URA3 ypt32Δ::TRP1*		(Sciorra et al., 2005)
CFY409	BY4742 *sec7Δ::KANMX* +pCF1043		(Richardson et al., 2012)
CBY474	*MATa ade2-1 can1-100 leu2-3,112 trp1-1 ura3-1 ypt1-3*		(Cao et al., 1998)
CFY1037	*SEY6210 GFP-Sec7::LEU2*	(integration of pCF1191 into SEY6210)	this study
CFY1111	*SEY6210.1 GFP-sec7-1::LEU2*	(integration of pCF1197 into SEY6210.1)	this study
CFY1319	*SEY6210 ypt31-101::URA3::ura3 ypt32Δ::TRP1*	(5-FOA selection of VSY468)	this study
CFY1499	*SEY6210.1 RFP^{MARS}-sec7-4:TRP1*	(integration of pCF1246 into SEY6210.1)	this study
CFY1681	*SEY6210.1 Sec7-6xDsRed::URA3*	(integration of Sec7-6xDsRed into SEY6210.1)	this study
CFY1689	*SEY6210.1 Sec7-6xDsRed::URA3::ura3*	(5-FOA selection of CFY1681)	this study
CFY1690	*SEY6210.1 Sec7-6xDsRed::URA3 Kex2-GFP::HIS3*	(PCR integration into CFY1681)	this study
CFY1711	*SEY6210.1 Sec7-6xDsRed::URA3::ura3 ura3::GFP-Snc1::URA3*	(GFP-Snc1 plasmid integration into CFY1689)	this study
CFY1752	*SEY6210.1 Sec7-6xDsRed::URA3 Kex2-GFP::HIS3 arf1Δ::KanMX*	(PCR integration into CFY1690)	this study
CFY1754	*SEY6210.1 Sec7-6xDsRed::URA3::ura3 ura3::GFP-Snc1::URA3 arf1Δ::KanMX*	(PCR integration into CFY1711)	this study
CFY1764	*SEY6210.1 Sec7-6xDsRed::URA3::ura3 arf1Δ::KanMX*	(PCR integration into CFY1689)	this study
CFY1780	*SEY6210 ypt31-101::URA3::ura3 ypt32Δ::TRP1 Sec7-6xDsRed::URA3*	(integration of Sec7-6xDsRed into CFY1319)	this study
CFY1784	*SEY6210 GFP-Ypt31::URA3*	(GFP-Ypt31 plasmid integration into SEY6210)	this study
CFY1786	*SEY6210 ura3::GFP-Ypt32::URA3*	(GFP-Ypt32 plasmid integration into SEY6210)	this study
CFY1805	*SEY6210 GFP-Ypt31::URA3 Sec7-6xDsRed::URA3::ura3*	(made by mating/sporulation CFY1689 x CFY1784)	this study
CFY1806	*SEY6210 ura3::GFP-Ypt1::URA3 Sec7-6xDsRed::URA3::ura3*	(made by mating/sporulation CFY1689 x CFY1786)	this study
CFY1811	*SEY6210 ypt31-101::URA3::ura3 ypt32Δ::TRP1 Sec7-6xDsRed::URA3*::ura3	(5-FOA selection of CFY1780)	this study
CFY1817	*SEY6210 ypt31-101::URA3::ura3 ypt32Δ::TRP1 Sec7-6xDsRed::URA3 Kex2-GFP::HIS3*	(PCR integration into CFY1780)	this study
CFY1819	*SEY6210 ypt31-101::URA3::ura3 ypt32Δ::TRP1 Sec7-6xDsRed::URA3*::ura3 *ura3::GFP-Snc1::URA3*	(GFP-Snc1 plasmid integration into CFY1811)	this study

Strains and plasmids used in microscopy and growth experiments.

Experiment (Figure #)	Strain(s) and plasmid(s) used
Figure 1C	BY4741, BYarf1Δ, BYarl1Δ, SEY6210, VSY468, CBY474, each with pCF1084
Figure 1D	CFY1111 with pRS426, pCF1258, pCF1259, pCF1261, pCF1263, or VSB284
Figure 1E	CFY1499 with pRS426, pCF1258, pCF1259, pCF1261, pCF1263, or VSB284
Figure 2A	CFY409 with shuffled pCF1084, pCF1105, or pCF1106
Figure 2B	CFY1111 with pRS426 or pCF1263
Figure 4A-C	CFY1037
Figure 4D	CFY1681+pCF1084, CFY1711, CFY1690, CFY1689+GFP-Tlg1
Figure 4E	CFY1689+GFP-Ypt1, CFY1805
Figure S1E	CBY474, VSY468, BY4171, BYgeneΔ, each with pCF1084
Figure S1F	BY4741, BYarf1Δ, BYarl1Δ, SEY6210, VSY468, CBY474, each with pCF1084 and Sys1-DsRed
Figure S1G	CFY409 with shuffled pCF1084, pCF1105, or pCF1106, each with Sys1-DsRed
Figure S3C,D	CFY1689+GFP-Ypt1, CFY1689+GFP-Ypt31, CFY1805, CFY1806
Figure S4A-D	CFY1690, CFY1689+GFP-TLG1, CFY1711, CFY1752, CFY1754, CFY1764+GFP-Tlg1, CFY1811+GFP-Tlg1, CFY1817, CFY1819

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