GUANINE NUCLEOTIDE EXCHANGE FACTORS (GEFS) HAVE A CRITICAL BUT NOT EXCLUSIVE ROLE IN ORGANELLE LOCALIZATION OF RAB GTPASES

Margarita Cabrera¹,², and Christian Ungermann¹

University of Osnabrück¹, Department of Biology/Chemistry, Biochemistry section, Barbarastrasse 13, 49076 Osnabrück, Germany

Running head: Rab targeting and function at endosomes

Address correspondence to: Margarita Cabrera, Department of Biology/Chemistry, Biochemistry Section, Barbarastrasse 13, 49076 Osnabrück, Germany; Fax: +49-541-969-2884 or -3422; E-mail: Margarita.Cabrera@biologie.uni-osnabrueck.de

Characters (without spaces): 31,478

Keywords: GEF, Rab GTPase, GDF, membrane fusion, Ypt7, GAP

Capsule

Background: Rab localization has been ascribed to the localization of their GEF (Guanine nucleotide exchange factor).

Results: GEF deletions result in mislocalization of Rabs to other membranes, which can be bypassed by a Rab mutant.

Conclusion: GEFs are critical for Rab localization, but Rabs also have a GEF-independent ability to localize correctly.

Significance: Our data reveal that both GEFs and Rabs contribute to Rab localization in cells.

Membrane fusion at eukaryotic organelles is initiated by Rab GTPases and tethering factors. Rabs in their GDP-form are kept soluble in the cytoplasm by the GDI chaperone. Guanine nucleotide exchange factors (GEFs) are found at organelles and are critical for Rab function. Here, we surveyed the overall role of GEFs for Rab localization. We show that GEFs, but none of the proposed GDI displacement factors (GDFs), are essential for the correct membrane localization of yeast Rabs. In the absence of the GEF, Rabs lose their primary localization to the target organelle. Several Rabs like the vacuolar Ypt7 are found at the ER and thus are still membrane-bound. Surprisingly, a Ypt7 mutant, which undergoes facilitated nucleotide exchange, localizes to vacuoles independently of its GEF Mon1-Ccz1 and rescues vacuole morphology. In contrast, wild-type Ypt7 requires its GEF for localization and to counteract the extraction by GDI. Our data agree with the emerging model that GEFs are critical for Rab localization, but raise the possibility that additional factors can contribute to this.

Organelles of the endomembrane system of eukaryotic cells are tightly interconnected. Transport of proteins and lipids between different organelles depends on vesicles that are formed at a donor membrane, released into the cytoplasm and finally consumed by fusion with an acceptor organelle. To be able to fuse with the correct acceptor membrane, vesicles need to carry the right fusion machinery. In principle, three distinct machineries cooperate during fusion. For the initial association of vesicles with the membrane, Rab GTPases localize to vesicles and/or target membranes and recruit tethering factors. At the endosome-vacuole interface, the Rab Ypt7 functions together with the HOPS (homotypic fusion and vacuole protein sorting) tethering complex to promote the initial docking of membranes. The subsequent fusion depends on membrane embedded SNAREs that are present on both membranes. SNAREs fold in a zipper-like manner into tight four-helix complexes. This brings the two membranes into close contact and thus mediates bilayer mixing (1-3). The main regulation of membrane fusion...
appears to occur at the level of Rabs and tethering proteins. Rabs are switch-like proteins that depend on helper proteins for nucleotide exchange and GTP hydrolysis (4-7). GEFs (guanine nucleotide exchange factors) convert the Rab-GDP into the active GTP-bound state. Only in this active conformation Rabs bind to their effectors such as tethering factors. Due to their slow GTP-hydrolysis rate, specific GAPs (GTPase activating proteins) are required for efficient and fast GTP hydrolysis. They complement the active site of the Rab and increase the intrinsic hydrolysis rate by several orders of magnitude. Moreover, reversible attachment of Rabs to membranes requires prenylation of their C-terminal cysteines, which depends on RGGT (Rab geranylgeranyl transferase) and REP (Rab escort protein, MrS6 in yeast). In the cytosol, prenylated Rabs are bound to the chaperone GDI (GDP-dissociation inhibitor, Gdi1 in yeast), which is similar in sequence and structure to REP (8-10). REP and GDI interact with the switch regions, C-terminal residues and the prenyl anchor of the Rab (8, 11), and both show preference for the GDP-form (12, 13). Next, a GDI-displacement factor (GDF) may be required to load a Rab onto membranes (14, 15), followed by activation through a GEF. Yip3/Pra1 has been identified as a Rab9-specific GDF (16, 17). Like Yip3, other Yip family members are integral membrane proteins and interact with multiple Rabs in a prenylation-dependent manner. Yip proteins are considered as good candidates to control specific membrane targeting of Rabs (18). It has been shown, however, that the GEF activity of the Legionella protein DrmA/SidM is both necessary and sufficient to displace GDI and activate the host Rab1 protein independently of an additional GDF, at least in this system (19-21). Within the endomembrane system, different Rabs act in consecutive fusion reactions. For exocytosis, the Ypt31/32 is present on secretory vesicles. Recent data indicate that both the membrane lipid phosphoinositide-4-phosphate and Ypt31-GTP are necessary to recruit the downstream GEF Sec2 (22, 23). Consequently, Sec2 would recruit and activate the exocytic Rab Sec4 onto secretory vesicles (23). A similar scenario is found along the endocytic pathway. It is thought that Rab5-GTP on early endosomes recruits the Mon1-Ccz1 complex (24, 25), which subsequently activates Rab7 (26). These data suggest that an activated upstream Rab, such as Ypt31 or Rab5 in the given examples, is necessary to recruit the GEF of the downstream Rab (6).

Interestingly, if GEFs were targeted to mitochondria, Rabs localized to this organelle as well, suggesting that GEFs determine localization of Rab GTPases to their target organelle (27, 28). This raises the question whether cells require additional factors for correct Rab targeting. Here, we address this question by following the fate of selected Rabs upon manipulation of their respective GEFs.

### Experimental Procedures

**Yeast strains and molecular biology -** Strains and plasmids used are listed in Table S1 and S2 respectively. yip1-1 and sec2-41 temperature-sensitive mutants were kindly provided by Mary Munson (UMASS, Worcester, MA) and Dieter Gallwitz (MPI, Göttingen, Germany), respectively. bet3-1 and trs130(D33) temperature-sensitive mutants were kindly shared by Nava Segev (University of Illinois, Chicago, IL, USA). Deletions and tagging of genes were done using homologous recombination of PCR fragments (29, 30). Ypt7 was cloned into plasmid pRS411-TPIpr-mCherry-V5, which was generated from plasmid pRS414-TPIpr-mCherry-V5 (a gift from Fulvio Reggiori, University Medical Center Utrecht, The Netherlands). pVT100 dslRed plasmid containing marker for mitochondrial matrix was also provided by Fulvio Reggiori. Coding sequences of Rab7 and Sec4 were provided by Francis Barr (Molecular and Clinical Cancer Medicine, University of Liverpool, UK), and inserted into pRS414-PHO5pr-dsRed and pRS415-NOP1pr-yEGFP plasmids, respectively. Point mutations in YPT7 and VPS21 were generated using the QuickChange Site-Directed Mutagenesis Kit from Stratagene (La Jolla, CA, USA).

**Microscopy -** Yeast cells were grown to mid-log phase in YPD, YPG, or synthetic complete (SDC) medium lacking selected amino acids or nucleotides, collected by centrifugation,
washed once with SDC or SGC medium supplemented with all amino acids, and immediately analyzed by fluorescence microscopy. For FM4-64 staining of vacuoles, cells were incubated with 30 μM FM4-64 for 30 min, washed twice with YPD medium and incubated in the same medium without dye for 1 h. Images were acquired with a Leica DM5500 B microscope equipped with a SPOT Pursuit camera equipped with an internal filter wheel (D460sp, BP460-515 and D580lp; Leica Microsystems GmbH), fluorescence filters (49002 ET-GFP (FITC/Cy2): Exc. ET470/40x, Em. ET525/50m; Wide Green: Exc. D535/50, Em. E590lp; 49008 ET-mCherry, Texas Red: Exc. ET560/40x, Em. ET630/75m Chroma Technology Corp.), and Metamorph 7 software (Visitron Systems, Munich, Germany). Images were processed using Image J 1.42 (National Institute of Health) and Autoquant x v1.3.3 (Media Cybernetics, Inc).

Cell lysis and membrane fractionation. Yeast cells grown in YPG to OD\textsubscript{600} ~1 were collected by centrifugation and treated with 10 mM DTT followed by incubation with lyticase for 20 min at 30°C. Spheroplasts were then resuspended in lysis buffer [200 mM sorbitol, 50 mM KOAc, 20 mM Hepes-KOH, pH 6.8, 1x PIC (protease inhibitor cocktail; 0.1 μg/ml leupeptin, 1 mM o-phenanthroline, 0.5 μg/ml pepstatin A, 0.1 mM pefablock), 1 mM PMSF, 1 mM DTT] containing 2 μg/ml DEAE-dextran and incubated on ice for 5 min, followed by a 2 min incubation at 30°C. Lysates were centrifuged at 300 g for 3 min at 4°C, and then the supernatant was centrifuged at 13,000 x g for 15 min to generate P13 (pellet) and S13 (supernatant) fractions. The S13 fraction was then centrifuged at 100,000 g for 30 min to obtain P100 (pellet) and S100 (supernatant) fractions. TCA-precipitated supernatants and pellets were resuspended in SDS sample buffer and proteins separated by SDS-PAGE, followed by Western blot and detection using antibodies against Ypt7 and Vac8.

RESULTS

Rab GTPases are mislocalized in the absence of their GEF or upon GEF inactivation – Relocalization experiments have shown that GEFs can determine the localization of Rab GTPases to their target membrane (27, 28). Using the same strategy we observed that the Rab Ypt7 remains associated with the vacuole membranes when its GEF Mon1-Ccz1 is targeted to mitochondria via the C-terminal transmembrane domain of the mitochondrial Fis1 protein (Fig. 1A). This suggests that Ypt7 either gets activated by the mitochondrial GEF or by small pool of endosome-localized GEF, which was not sorted to the mitochondria. As this approach was thus unsuccessful in yeast, we decided then to use an alternative assay and followed the fate of the Rab closely in GEF deletion strains. We have previously used subcellular fractionation to analyze the distribution of Ypt7 in cells lacking its GEF but did not observe any significant change compared to wild-type (Fig. 1B) (26). In contrast, when we examined GFP-tagged Ypt7 by fluorescence microscopy, we noticed that Ypt7 is not exclusively found at the vacuoles in mon1Δ and ccz1Δ cells and accumulated at different membrane structures. This distribution pattern is also distinct from the multilobed vacuoles observed upon loss of the HOPS subunit Vps39 (Fig. 1B). This confirms that the Rab effectors like Vps39 are downstream of the GEF. Similar membrane redistribution was also observed for the monomeric Cherry-tagged Ypt7 in GEF deletion strains (Fig. S1A). It is thus unlikely that a potential GFP-induced oligomerization of GFP-Ypt7 affects the subcellular distribution of Ypt7. Human Rab7, which requires human Mon1-Ccz1 complex for localization (Cabrera, Barr et al., in preparation), behaved like Ypt7 in GEF deletion strains (Fig. S1B).

To identify the membranes where Ypt7 is targeted to in the absence of the Mon1 and Ccz1 subunits, we followed several membrane markers, and observed the best co-localization with the nuclear ER (Figure 1C, S1C). We wondered whether this mislocation was typical for Ypt7 or applied to other Rabs as well. For Ypt6, which operates between endosome and Golgi, and the endosomal Rab Vps21, we observed similar redistribution upon GEF deletions. Ypt6 requires the two GEF subunits Ric1 and Rgp1 (31, 32), and loss of either subunit revealed partial mislocation of Ypt6.
to ER as observed for Ypt7 (Fig. 2A). For Vps21, it was shown recently that two GEFs, Vps9 and Muk1, act redundantly (33, 34). In agreement with this, only the dual deletion of both GEFs resulted in redistribution of Vps21 to ER among other membranes (Fig. 2B). As shown in Fig. 2C, both Rabs remained membrane-associated in the respective GEF deletion strain. We also tested the fate of exocytic Rabs such as Ypt1, Ypt31 and Sec4 upon inactivation of their GEF. Strikingly, the Golgi and polarized localization of Ypt31 and Ypt32 was reduced upon inactivation of the GEF complexes TRAPPI and TRAPPII by usage of the temperature-sensitive (ts) subunits bet3ts and trs130ts, respectively (Fig. 2D) (35). In contrast, Ypt1 Golgi distribution was only affected by inactivation of TRAPPI complex (via the bet3 ts-mutant), even at permissive temperature (35)(Fig. 2D). For Sec4, a redistribution from a polarized localization to a more diffuse pattern was observed in sec2 ts-mutant at different cell-cycle stages, in agreement with previous findings (36)(Fig. 2E). In summary, these data indicate that Rabs may remain membrane-bound even in the absence of their GEF, but the targeting to the correct organelle requires the activity of the corresponding GEF.

Localization of Ypt7 to vacuoles in the absence of the GEF - Elegant genetic studies revealed that the deletion of Ccz1 can be bypassed by a point-mutant in Ypt7(37). Lysine 127 resides in the highly conserved GNKID (G4) motif, which recognizes the guanine base (Fig. 3A). The mutation K127E weakens the affinity for the nucleotide, and may thus facilitate nucleotide exchange. We asked if this mutant would compensate for both GEF subunits or could rely on a partial GEF complex for function, and thus followed morphology of FM4-64 stained vacuoles as a read-out (Fig. 3B). Expression of Ypt7 K127E, but not wild-type Ypt7, could rescue the morphology of mon1Δ and ccz1Δ cells. Importantly, even the double mutant was rescued, indicating that the entire GEF complex is dispensable in this mutant. To follow the localization of Ypt7 K127E, we GFP-tagged the protein in wild-type and mon1Δ, ccz1Δ, and double deletion cells (Fig. 3C). Interestingly, Ypt7 was localized only to vacuoles and the cytosol. The additional fragmentation is likely due to the GFP-tag on Ypt7, which impairs activity partially (38). In contrast, Ypt7 GTP-locked mutant (Q68L) requires a GEF for proper localization to the vacuoles (Fig. 3D). Our data indicate that localization of Ypt7 to the vacuole membrane can be achieved also in the absence of its GEF, indicating that additional factors may support this process.

Effect of interactors and modifiers on the Rab localization - We then asked if any of the previously identified Rab interacting proteins might affect Ypt7 localization. The Yip-family encodes Rab interacting proteins, which localize to ER, Golgi and endosomes and might contribute to the specific distribution of Rabs. They have strong homology to the mammalian Yip3/Pra1 (16), and have been postulated to act as GDFs at different organelles. Yip proteins might work in concert with other ER components like the reticulon Rtn1 and SNAREs (18, 39). Among the Yip family, Yip1 is the only essential member due to its role in COPII vesicle formation (40). We thus analyzed the localization of Ypt7 to vacuoles, but did not observe any change in either the yip1-1 mutant, or any other yip or rtn1 deletion, including a quadruple mutant of yip1, yip2,3,4 and 5 (Fig. 4A, B). We then asked if methylation of the C-terminal prenylation sequence might affect Rab localization. However, deletion of the prenylcysteine-carboxy1 methyltransferase Ste14 (41) did not interfere with localization of Ypt7, Vps21 or Ypt6 to their target organelle (Fig. 4C). Because all these Rabs have a Cys-X-Cys tripeptide at their C-terminus, we wondered if the replacement by a Cys-Cys sequence, which is not subjected to methylation (42), affect their distribution, but did not observe any defect for Vps21 and Ypt7 (Fig. 4D). We thus consider it unlikely that Yip proteins or the kind of prenylation play a critical role in Rab localization.

The Mon1-Ccz1 GEF drives Gdi1-bound Ypt7 to the vacuole - Our data so far indicate that GEFs are critical for correct organelle localization of the tested Rabs, but that additional factors might contribute to Rab localization that only become apparent in Rab mutants. To monitor the
crosstalk of Rabs with GEFs, GDI and GAPs, we decided to focus on the membrane localization of Ypt7 in vivo. As mentioned above, Rabs cycle between the membrane-bound and active pool, and the inactive GDP-bound fraction. Within the cytosol, newly synthesized Rabs are chaperoned by the Rab escort protein (REP), which binds unprenylated and prenylated Rabs, and later on by Gdi1, which is the general Rab chaperone of the cytosol. We reasoned that overproduction of any of the two should increase the cytosolic pool of Rabs, and thus followed Ypt7 as an example. Only Gdi1 overproduction, but not that of the REP Mrs6, resulted in an increased pool of cytosolic Ypt7 (Figure 5A-C). Similarly to the deletion of its GEF, Ypt7 was partially extracted from vacuole membranes and accumulated in the ER if any of the two known GAPs of Ypt7, Gyp7 and Msb3 (43-45), was overexpressed (Fig. 5C). This ER accumulation was not visible if both GAP and Gdi1 were co-overexpressed, suggesting that that ER pool is only observed if the binding capacity of Gdi1 is exceeded. In contrast, simultaneous overproduction of the GEF complex efficiently counteracted the excess of Gdi1 and deposited Ypt7 on the vacuole (Figure 5C). This indicates that the GEF is the limiting factor needed to dissociate the Rab from Gdi1 and convert it to the membrane- and GTP-bound active form.

**DISCUSSION**

Our data reveal that GEFs are critical for localization of the Rab GTPases. In their absence or inactivation of the respective GEFs, Rabs loose their characteristic distribution pattern. For instance, Ypt7, Ypt6 and Vps21 prominently mislocalize to the ER. This indicates that Rabs might not become cytosolic if they cannot be localized to the right organelle. Our data indicate that Rabs have additional possibilities to target to the appropriate organelle membrane even in the absence of the GEF. For Ypt7, we show here that a mutation in the guanine base-binding motif not only rescues Ypt7 function in the absence of the entire GEF complex, but also results in localization to vacuoles. Importantly, the wild-type protein requires the GEF for vacuole localization.

Recently, a couple of studies employed the mistargeting of GEFs to the mitochondrial outer membrane to demonstrate that GEFs are an important determinant for Rab localization (27, 28). These observations are in agreement with findings on the Legionella DrrA protein, which has strong GEF activity for Rab1. DrrA can displace Rab1 from the GDI-Rab1 complex due to its GEF activity and thus redirect ER membrane traffic during pathogen infection in cells (19-21). It is thus likely that GEFs have a critical role for GDI displacement and could thus drive Rab recruitment. However, one should keep in mind that the relocalization is likely also promoted by the large excess of the GEF on the mitochondrial surface, which could bypass any supporting factor on the original membrane. In this context, it was surprising that correct targeting of Ypt7 was possible even if the entire GEF complex was lacking. This suggests that the GEF complex is not the sole determinant and additional factors contribute to Rab targeting. As the mutant Ypt7 K127E is a fast cycling mutant, it is possible that the apparent targeting is partially driven by binding to effectors such as the HOPS complex. Alternatively, additional cofactors may support the GEF also in wild-type cells. We can at this point exclude that the proposed GDF proteins of the Yip family contribute to this targeting (Fig. 4), though it is possible that they act redundantly with other factors. At least the yip2-5 quadruple mutant did not affect Ypt7 targeting. Considering that the wild-type Ypt7 relies, however, strongly on the Mon1-Ccz1 complex for its targeting to vacuoles and that this complex can compensate for the Gdi1 overexpression, we believe that the GEF is the most critical factor for organelle targeting of any Rab.

To test mistargeting of Rabs to mitochondria in vivo, we also relocalized Mon1-Ccz1, but did not observe any relocalization of the Rab. We speculate that the possible contacts between endosomes/vacuoles and mitochondria in yeast may allow Ypt7 targeting to vacuoles, and cannot exclude that a minor portion of Mon1-Ccz1 escapes mitochondrial import and is still found on endosomes. It will therefore be important to dissect the precise GEF function of Mon1-Ccz1 in future assays to clarify if
additional factors are important to support the GEF complex.

In the light of our findings, how do we imagine the Rab cycle? Once a Rab is synthesized, it will be deposited by the REP to the target organelle, and then cycles in its GDP-form between the membrane and the cytosol (GDI-bound). In agreement with this, overexpression of the REP Mrs6 did not affect Ypt7 localization, whereas Gdi1 did (Fig. 5A). Only after the appropriate GEF is recruited to the organelle membrane, presumably via the preceding Rab-GTP, the Rab will be displaced from GDI and activated. This may be facilitated by additional factors though we did not observe any effect by deleting the Rab-interacting Yip or Rtn proteins. They could alternatively control Rab prenylation or GDI loading, which would explain their strong interaction with many different Rabs and GDI (16, 46-49). Several Rabs were found at the ER if they cannot be delivered to the appropriate membrane. Interestingly, the ER also constitutes the target organelle for mono-prenylated Rabs (47, 50), and chimeric Rab proteins (51, 52). We do not yet know if the ER localization is also important during the normal life-time of Rabs, though it shows that a large fraction of Rabs does not remain cytoplasmic if the GEF is missing. It is possible that under conditions when Gdi1 becomes limiting, during cell cycle transitions or stress, the ER serves as a default buffer to maintain the remaining Rab pool functional in cell. According to our data, Rab localization to the right organelle can be explained by the interplay of GDI, GEF and GAP. With excess GDI, most of the Rab likely cycles continuously between cytosol and organelle membrane if the GEF is present. With excess GAP and normal levels of GEF and GDI, the large Rab-GDP pool is transferred by GDI to the ER. Only sufficient levels of the GEF can then shift the Rab quantitatively to the correct organelle.

On the membrane, the active Rab will recruit effector proteins and promote membrane fusion. For the endosome and vacuole membrane, Mon1-Ccz1 likely activates Ypt7 at the late endosome, which in turn interacts with the vacuole-localized HOPS (53). The GAP would then be required to sharpen the activity of the Rab along one pathway, which could be further controlled by the active downstream Rab. How activation and turn-over of Rabs are then controlled, will require further studies.

FOOTNOTES

We thank Francis Barr, Nava Segev, Sean Munro, Dieter Gallwitz, Hans-Dieter Schmitt, Fulvio Reggiori, and Mary Munson for reagents and strains, and Siegfried Engelbrecht-Vandré and Jens Lachmann for feedback on the manuscript. This work was supported by the SFB 944 (project P11), and by the Hans-Mühlenhoff foundation (to C.U.).

REFERENCES


26. Nordmann, M., Cabrera, M., Perz, A., Bröcker, C., Ostrowicz, C. W., Engelbrecht-Vandér, S., and


**FIGURE LEGENDS**

**Fig. 1.** Loss of Mon1-Ccz1 complex alters Rab Ypt7 distribution. *A.* Localization of Ypt7 upon relocation of Mon1-Ccz1 complex to the mitochondria. *B.* Subcellular fractionation of cells expressing GFP-tagged Ypt7 in wild-type (wt) and ccz1Δ cells. P13, pellet after 13,000 g (15 min/4°C) centrifugation; P100, S100, pellet and supernatant after 100,000 g (30 min/4°C) centrifugation. Protein distribution was analyzed by Western blot and antibody decoration. Note that Vac8 was used as a vacuole marker. *C.* Localization of GFP-tagged Ypt7 in wild-type (wt) and mon1Δ, ccz1Δ and vps39Δ strains. *D.* Co-localization of Ypt7 and C-terminally MARS-tagged Sec63 in wild-type and GEF deletion strains. Size bar, 5 µm.

**Fig. 2.** GEF activity determines right localization of Rabs. *A.* GFP-tagged Ypt6 distribution in wild-type (wt) and GEF deletion strains. Sec63 was used as a ER marker. *B.* Analysis of Vps21 co-localization with Sec63 in wild-type (wt) and the indicated deletion strains. *B.* GFP-tagged Ypt6 (left panel) and Vps21 (right panel) remained membrane-bound in the absence of their corresponding GEF. Subcellular fractionation was performed as in Fig. 1B. Arc1 was used as a cytosolic marker. *C.* Subcellular targeting of Ypt1, Ypt31 and Ypt32 in wild-type (wt), bet3, and trs130 temperature sensitive (ts) mutants grown at 26°C and 37°C for 1.5 h. *D.* Localization of GFP-Sec4 was examined in sec2 temperature sensitive (ts) mutant grown at 25°C and 37°C for 1 h, followed by fluorescence microscopy. Size bar, 5 µm.
Fig. 3. Mutation K127E in Ypt7 bypasses the need of GEF activity. A. Representation of Ypt7-GPPNHP (pdb:1ky2, generated with Yasara, www.yasara.org) with p-loop and switch regions indicated in yellow and green, respectively. The surface of K127 residue is shown in grey. B. Analysis of vacuole morphology via the lipophlic dye FM4-64 in wild-type (wt) and GEF deletion strains expressing Ypt7 or Ypt7 K127E mutant from a centromeric (CEN) plasmid. C. Localization of Ypt7 K127E mutant in wild-type (wt) and cells lacking Ypt7 GEF complex. D. Microscopy of GFP-tagged Ypt7 Q68L mutant in wild-type (wt) and ccz1Δ strain. Size bar, 5 μm.

Fig. 4. Contribution of Yip proteins and methylation to Rab localization. A. yip1-1 mutant carrying GFP-Ypt7 was grown at 25°C or 37°C for 1 h and analyzed by fluorescence microscopy. B. Subcellular distribution of GFP-tagged Ypt7 in the indicated single and multiple deletion strains. C. Microscopy of GFP-tagged Ypt7, Ypt6, and Vps21 was performed in wild-type (wt) and ste14Δ strains. D. Localization of Vps21 and Ypt7 mutants containing C-terminal Cys-Cys prenylation motif. Size bar, 5 μm.

Fig. 5. Mon1-Ccz1 GEF activity towards Ypt7 counteracts GDI. A. Membrane targeting of GFP-tagged Ypt7 was monitored in wild-type (wt) and cells expressing Gdi1 or Mrs6 under the control of the GAL1 promoter. B. Subcellular fractionation of cells expressing GFP-Ypt7 and Gdi1 under the control of the GAL1 promoter. Assay was performed as in Fig. 1B. Vac8 was used as a vacuole marker. C. Localization of GFP-Ypt7 in diploids cells overexpressing Gdi1 together with the Ypt7 GAPs (Gyp7, Msb3) or Mon1 and Ccz1 was analyzed by fluorescence microscopy. ER labeling is indicated by white arrows in cells overexpressing Ypt7 GAPs. Size bar, 5 μm.
Figure 1, Cabrera et al.

A

DIC  GFP-Ypt7  tomato-FIS1  Merge

GAL1-MON1
GAL1-CCZ1

GAL1-MON1-tomato-FIS1
GAL1-CCZ1

GAL1-MON1
GAL1-CCZ1-tomato-FIS1

B

wt  ccz1Δ

GFP-Ypt7
Vac8

55kDa

C

DIC  GFP-Ypt7

wt

mon1Δ

ccz1Δ

vps39Δ

D

GFP-Ypt7  Sec63-MARS  Merge

Ypt7  Sec63

wt

mon1Δ

ccz1Δ

by guest on October 5, 2017 http://www.jbc.org/ Downloaded from
Figure 2, Cabrera et al.
Figure 3, Cabrera et al.

A) Structure of Ypt7 with labeled regions: Switch I, Switch II, p-loop, K127.

B) Western blot analysis of Ypt7 and Ypt7$^{K127E}$ CEN, with lanes for wild type (wt), mon1Δ, ccz1Δ, and mon1Δ ccz1Δ.

C) GFP-Ypt7 K127E expression in wild type (wt), mon1Δ, ccz1Δ, and mon1Δ ccz1Δ.

D) DIC imaging of GFP-Ypt7 Q68L with wild type (wt) and ccz1Δ.
Figure 5, Cabrera et al.

A

DIC       GFP-Ypt7

wt

GAL1-GDI1

GAL1-MRS6

B

wt           GAL1-GDI1

Vac8       GFP-Ypt7

GAL1-GDI1

GAL1-GYP7

GAL1-MSB3

GAL1-MON1

GAL1-CCZ1
Guanine nucleotide exchange factors (GEFs) have a critical but not exclusive role in organelle localization of Rab GTPases
Margarita Cabrera and Christian Ungermann

J. Biol. Chem. published online August 26, 2013

Access the most updated version of this article at doi: 10.1074/jbc.M113.488213

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2013/08/26/M113.488213.DC1

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/early/2013/08/26/jbc.M113.488213.full.html#ref-list-1