A Structural Model of the GDP Dissociation Inhibitor Rab Membrane Extraction Mechanism*

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Rab GDP dissociation inhibitors (GDI)-facilitated extraction of prenylated Rab proteins from membranes plays an important role in vesicular membrane trafficking. The investigated thermodynamic properties of yeast Rab-GDI and Rab-MRS6 complexes demonstrated differences in the Rab binding properties of the closely related Rab GDI and MRS6 proteins, consistent with their functional diversity. The importance of the Rab C terminus and its prenylation for GDI/MRS6 binding was demonstrated using both biochemical and structural data. The presented structures of the apo-form yeast Rab GDI and its two complexes with unprenylated Rab proteins, together with the earlier published structures of the prenylated Ypt1-GDI, provide evidence of allosteric regulation of the GDI lipid binding site opening, which plays a key role in the proposed mechanism of GDI-mediated Rab extraction. We suggest a model for the interaction of GDI with prenylated Rab proteins that incorporates a stepwise increase in affinity as the three different partial interactions are successively formed.

Rab/Ypt proteins present the largest group of small GTPases within the Ras superfamily and play a key role in membrane trafficking in eukaryotic cells. More than 60 members of the Rab-GTPase family have been identified (1). All Rabs undergo posttranslational modification by prenylation, where in most cases two C-terminal cysteines are modified by geranylgeranyl lipids (1, 2). The geranylgeranylation is catalyzed by Rab geranylgeranyl transferase acting in concert with Rab escort protein (REP).3 Prenylation is essential for Rab membrane anchoring and functioning (3–5).

Rab-GTPases can exist in two different conformations: GTP bound (active) and GDP bound (inactive). The process of switching between the active and inactive conformations is controlled by the interaction between Rab-GTPases and regulatory proteins. The active form of Rab proteins interacts with Rab effectors and GTPase-activating proteins, whereas the inactive conformation is recognized by guanine nucleotide exchange factors and by two closely related molecular chaperones, REPs and Rab-GDP dissociation inhibitors (GDIs). REP (Msr6 in yeast) acts as a type of chaperone, bringing the newly synthesized Rab to the geranylgeranyl transferase for prenylation and subsequently delivering it to a specific membrane. GDI does not facilitate Rab prenylation but serves as a generic regulator for recycling of Rab-GTPases (6) for use in multiple rounds of membrane transport. It retrieves Rab in the GDP-bound form from the membrane and delivers it to the cytosol, controlling the distribution of Rabs between membranes and cytosol (7). GDI is believed to be stably associated only with GDP-loaded and prenylated Rab/Ypt proteins, ensuring retrieval of inactivated GTPases from the membrane at the end of their functional cycle (8). Rab-GDI is critically important for the proper functioning of the vesicular transport machinery, and its deletion is lethal in yeast (9).

The two related Rab molecular chaperones REP and GDI have similar structural organization, and their Rab binding interface is well conserved (5, 10, 11). This finding is consistent with the common function of REP/GDI (i.e. Rab delivery to specific membranes). GDI lacks the geranylgeranyl transferase binding site, making it unable to assist prenylation, whereas the structural determinants of GDI competence in Rab membrane extraction remain unclear.

EXPERIMENTAL PROCEDURES

Protein and Protein Complex Production—Yeast RabGDI and Ypt31Δ2 were produced, and the RabGDI-Ypt31Δ2 complex was purified as described elsewhere (12). Yeast Rabs and their C-terminally truncated mutants (Ypt1Δ2, Ypt6, Sec4Δ2, Ypt31, Ypt32, Ypt51, Ypt52, Ypt7Δ2, Ypt7Δ14, Sec4Δ29, Ypt7Δ26, and Ypt7Δ10) and MRS6 were produced as N-terminally His6-tagged proteins in Escherichia coli BL21(DE3) strain using a pET19 expression vector. Cells were grown in LB medium containing 125 μg/ml of ampicillin at 37 °C until they reached an A600 nm of 0.6, cooled on ice for 10 min, induced with 0.7 mM isopropyl 1-thio-β-D-galactopyranoside, and then grown at 18 °C overnight. Cells were harvested, washed with phosphate-buffered saline, resuspended in lysis buffer (50 mM Hepes, pH 7.5, 300 mM NaCl, 1 mM protease inhibitor phenyl-

1 The abbreviations used are: REP, Rab escort protein; GDI, GDP dissociation inhibitor; ITC, isothermal titration calorimetry; MES, 4-morpholineethanesulfonic acid; RBP, Rab binding platform; CCR, C terminus coordinating region; r.m.s., root mean square.
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5 The atomic coordinates and structure factors (code 3cpg, 3cpn, and 3pi) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

JUNE 27, 2008•VOLUME 283•NUMBER 26
JOURNAL OF BIOLOGICAL CHEMISTRY 18377
methylsulfonyl fluoride, 10% (v/v) glycerol, 5 mM β-mercaptoethanol), and disrupted using a microfluidizer. The lysate was cleared by centrifugation (35,000 × g), and the supernatant was loaded onto a HiTrap Chelating HP column (GE Healthcare) equilibrated with buffer A (50 mM Hepes, pH 7.5, 300 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol). After elution with a linear imidazole gradient (0–500 mM imidazole), the sample was dialyzed against gel filtration buffer (50 mM Hepes, pH 7.5, 100 mM NaCl, 2 mM MgCl₂, 10 mM GDP, 5 mM β-mercaptoethanol) at 4 °C. The concentrated sample was applied to a Superdex200 (26/60) column equilibrated with gel filtration buffer. The fractions corresponding to the protein molecular weight were collected and the proteins were concentrated, flash-frozen in liquid nitrogen, and stored at −80 °C.

Ypt1 C-terminal mutagenesis was performed on a plasmid containing the cDNA fragment Ypt1Δ2 using the QuickChange kit (Stratagene). For the Ypt1-CAAX mutant, the nucleotide sequence coding the CIIIM amino acid residues was incorporated at the 3′-end of the Ypt1Δ2 coding gene. Further mutations (Ypt1-V191A-CAAX, Ypt1-L193A-CAAX, Ypt1-V191H-CAAX, and Ypt1-L193H-CAAX) were performed on a plasmid containing Ypt1-CAAX cDNA. The mutants were purified as described above.

The GDI-Sec4 complex was obtained by mixing the proteins in gel filtration buffer using a 2-fold molar excess of Sec4 and subsequent purification by gel filtration on Superdex200 (26/60). The eluted fractions containing the complex protein, as detected by SDS-PAGE, were collected, concentrated, and flash-frozen and stored at −80 °C.

**Enzymatic Farnesylation of Ypt1-CAAX Mutants**—Purified recombinant Ypt1 mutants containing a C-terminal CAAX box sequence were enzymatically farnesylated in vitro. The reaction mixture contained 75 mM potassium phosphate, pH 7.5, 5 mM MgCl₂, 30 μM Ypt1-CAAX, 50 μM farnesyl pyrophosphate (Sigma), and 50 mM of yeast recombinant farnesyl transferase (Sigma). After incubation for 1 h at 4 °C, the farnesylated proteins were purified by gel filtration as described before. Incorporation of the farnesyl moiety was monitored by the molecular weight change using matrix-assisted laser desorption ionization-mass spectrometry.

**Isothermal Titration Calorimetry (ITC) Measurements**—Binding affinities of yeast Rab and their mutants to GDI and MRS6 were determined by ITC (MicroCal). All proteins were kept in buffer containing 50 mM Hepes, pH 7.5, 100 mM NaCl, 2 mM MgCl₂, 2 mM β-mercaptoethanol, 10 mM GDP. The concentration of the Rab proteins in the syringe was 10-fold higher (100 μM) than the GDI/MRS6 concentration (10 μM) in the cell. In blank experiments, the respective protein was injected from the syringe into the buffer solution, yielding small background heats that were subtracted from the titration experiment data. The titration experiments were carried out at 25 °C. All experiments were performed at least three times. The data obtained were fitted using the MicroCal-ITC implementation of the Origin7 software package and summarized in Table 1.

**Crystalization, X-ray Data Collection, and Structure Determination**—Yeast Rab GDI was crystallized using the hanging drop vapor diffusion method at 20 °C by 2 μl of 20 mg/ml protein solution mixed with 2 μl of reservoir solution (50 mM MES, pH 6.5, 20% polyethylene glycol 3350). Prior to data collection, crystals were cryoprotected in a buffer containing 17% ethylene glycol, 50 mM MES pH 6.5, 12% polyethylene glycol 3350 and flash-frozen in liquid nitrogen.

**GDI-Sec4 complex crystals** were obtained by the same method at 20 °C. 2 μl of 25 mg/ml protein complex solution were mixed with 2 μl of reservoir solution (50 mM sodium citrate, pH 5.5, 12% MME-polyethylene glycol 2000). The cryoprotectant solution contained 15% ethylene glycol, 50 mM sodium citrate, pH 5.5, 12% MME-polyethylene glycol 2000. GDI-Ypt31 complex crystallization is reported elsewhere (12).

X-ray diffraction data were collected at the SLS synchrotron radiation source (PSI, Villigen), beamline X10SA, and processed using the XDS program suite (13). The data collection statistics are reported in Table 2.

The GDI-Ypt31 crystal structure was determined by the molecular replacement method with Molrep (14) using the GDI structure from the GDI-Ypt1-geranylgeranyl complex (Protein Data Bank code 1UKV) as a search model. The GDI-Ypt31 crystal contained one complex per asymmetric unit, and the molecular replacement solution unambiguously indicated the position of the GDI molecule in the asymmetric unit. The resulting difference electron density maps clearly reflected the position and secondary structure elements of the Ypt31 molecule bound to the GDI that allowed the GTPase model building using the program O (15). Several iterative cycles of crystallographic refinement with Refmac5 (16) and manual model rebuilding were performed to obtain the final GDI-Ypt31 complex structure, which has been deposited to the Protein Data Bank (3CP).

The GDI-Sec4 complex structure was determined by the molecular replacement method with the GDI structure as a search model. The Matthews coefficient calculated for the GDI-Sec4 crystal indicated that the asymmetric unit probably contains two GDI-Sec4 complexes, whereas only one contrast solution was found by the molecular replacement search with

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**TABLE 1**

Dissociation constants determined for the interaction of Mrs6 and GDI with yeast Rab proteins and their mutants

| Rab                  | Rab-GDI $K_d$ (μM) | Rab-MRS6 $K_d$ (μM) |
|----------------------|--------------------|--------------------|
| Ypt1Δ2               | 12.20 ± 1.7        | NM                 |
| Ypt6                 | 25.00 ± 3.1        | NM                 |
| Sec4Δ2               | 0.33 ± 0.02        | 0.36 ± 0.01        |
| Ypt7Δ2               | 2.3 ± 0.2          | NM                 |
| Ypt3Δ2               | 5.8 ± 0.5          | 0.5 ± 0.09         |
| Ypt31                | 18.9 ± 2           | 1 ± 0.03           |
| Ypt32                | 1.5 ± 0.2          | 0.44 ± 0.04        |
| Ypt51                | 5.9 ± 0.3          | 1 ± 0.4            |
| C-terminal truncated Rabs |                    |                    |
| Ypt1Δ14              | ND                 | 33 ± 5.1           |
| Sec4Δ29              | 4.2 ± 0.3          | 4 ± 0.1            |
| Ypt7Δ26              | ND                 | 40 ± 9             |
| Ypt7Δ10              | 6.6 ± 1.1          | 4 ± 0.4            |
| A/Δ box mutants of Ypt1-CAAX |            |                    |
| Ypt1-CAAX            | 30 ± 2.2           | 0.46 ± 0.1         |
| Ypt1-CAAX-farnesyl   | 0.1 ± 0.02         | 0.89 ± 0.02        |
| Ypt1-V191A-CAAX-farnesyl | 0.7 ± 0.1       | 9.4 ± 0.6          |
| Ypt1-L193A-CAAX-farnesyl | 10 ± 0.9         | 4.4 ± 0.3          |
| Ypt1-V191H-CAAX-farnesyl | 20 ± 2            | 6 ± 0.2            |
| Ypt1-L193H-CAAX-farnesyl | 18 ± 1.7         | 3.2 ± 0.1          |

* RabΔ2—Rab with two C-terminally truncated amino acid residues, considered here as full-length due to the presence of the extended flexible C terminus.

The experimental conditions of the ITC measurements are described in detail under “Experimental Procedures.” NM, not measured; ND, not detectable.
Molrep (14). The solution was used for subsequent phasing. The calculated difference electron density maps clearly indicated the structural features of Sec4 bound to the GDI molecule and some additional density for another protein molecule. The core secondary structure elements of Sec4 molecule were built, and the GDI molecule was rebuilt manually using the program O (15), the initial complex structure was refined with Refmac5 (16), and the resulting electron density maps more clearly outlined the secondary structure elements of the additional molecule. Its Ca trace was built, and the protein molecule was recognized as a free GDI. Finally, the entire asymmetric unit containing the GDI-Sec4 complex and the free GDI was iteratively rebuilt, and refined resulting in the model were deposited to the Protein Data Bank (3CPH).

The yeast GDI crystal structure was determined by the molecular replacement method (14) with the GDI from the GDI-Ypt1 complex structure as a search model. Two contrast molecular replacement solutions corresponding to the two molecules in the asymmetric unit were found. The calculated electron density maps and the protein model correlated very well in the GDI domain I region and poorly in domain II. The second domain was removed from the protein model, the model was refined as a rigid body, and the resulting electron density maps were used for manual building of the domain II. After several rounds of manual rebuilding and crystallographic refinement, the yeast GDI crystal structure was deposited to the Protein Data Bank (3CPI). The protein model refinement statistics are summarized in Table 2. Model superimposition and r.m.s. deviation calculations were performed using the Swiss-Pdb Viewer (17).

RESULTS AND DISCUSSION

During the course of this research, the crystal structures of the yeast GDI apo-form and structures of two unprenylated Rabs (Ypt31 and Sec4) in complex with GDI (Fig. 1A) were solved (Table 2). These protein complex structures represent models of the transient Rab-GDI complex when GDI is bound to a membrane-anchored Rab protein poised to extract Rab to the cytosol, whereas the previously determined prenylated Ypt1-GDI crystal structures (5, 10) represent the state after Rab retrieval from the membrane. The structural comparison reveals significant conformational changes in the GDI molecule occurring upon Rab binding. ITC titration experiments in combination with mutational analyses reveal varying affinities of the different unprenylated or farnesylated yeast Rabs to GDI and MRS6 (Table 1). The thermodynamic properties of GDI-Rab interactions together with structural analysis of GDI-Rab in different conformational states provide mechanistic insights into the GDI competence in prenylated Rab membrane extraction.

Structural Features of GDI-Rab Complexes—The structures of prenylated Ypt1-GDI, Ypt31-GDI, and Sec4-GDI demonstrate that GDI binds the Rab molecule via three interaction sites. These are 1) the GDI-Rab binding platform (RBP), located in domain I, which interacts extensively with the globular part of the Rab molecule; 2) the GDI C terminus coordinating region (CCR), located in the cleft between domain I and domain II, which coordinates the flexible extended C terminus of Rab; 3) domain II of GDI, consisting solely of α helices, which form a prenyl-lipid binding pocket, exhibiting an open conformation and accommodating the prenyl moiety of a modified Rab if present (5, 10) (Fig. 1A).

Conserved GDI-Rab Binding Platform—Ypt1, Sec4 and Ypt31 exhibit 60% primary structure homology. Comparison of the prenylated Ypt1-GDI, Sec4-GDI, and Ypt31-GDI complex structures showed that the three GTPases bound to GDI can be superimposed with a Ca r.m.s. deviation of ~1 Å, excluding Switch I and the C-terminal variable regions (Fig. 1A). The
three complex structures demonstrate the conserved GDI-Rab interface with which the Rab molecule makes direct contacts to the Rab binding platform of GDI, primarily by using the conserved residues from the Switch I and Switch II regions. These regions, along with the C terminus, are known as the most structurally variable and flexible parts in Rab GTPases. Interaction with GDI generates similar Switch II conformations in the different Rabs, making extensive contacts with GDI, whereas the major part of Switch I remains unaffected by the interactions, and its structural organization in the three GTPases is quite different. The Rab GDI binding epitope is formed by a number of conserved amino acid residues (Ile41, Gly42, Asp/Glu44, and Phe45) from Switch I; Trp62, Asp63, Ala65, Gln67, Phe/Tyr70, Thr/Ala72, Thr74, Ser/Thr75, Ser/Ala76, and Arg99 from Switch II (Fig. 2A).

**Contribution of the C Terminal Coordinating Region to Rab Binding**—The second GDI-Rab binding site (*i.e.* the C terminus coordinating region) (Fig. 1A) is formed by residues 93–112 from domain I and 226–235 from domain II and represents a hydrophobic cavity on the surface of the protein located between the GDI domains. In the prenylated Ypt1-GDI structures, the CCR is occupied by side chains of hydrophobic amino acid residues Val190 and Val192 penetrating the hydrophobic cavity on the GDI surface. The observed structural similarity in the GDI CCR organization in the GDI Sec4 complex indicates that the Rab C-terminal A box serves as a lid for the hydrophobic CCR cavity on the GDI surface.

**ITC experiments** confirmed the thermodynamic preference of shielding the GDI CCR hydrophobic cavity upon complex formation. C-terminally modified yeast Rabs lacking the AXA motif showed weaker GDI/MRS6 binding properties compared with the full-length Rabs (Table 1). Truncation of the entire C-terminal part, including the AXA region (Ypt1Δ14 and Ypt7Δ26), diminishes GDI binding affinities to values undetectable in ITC experiments, whereas the MRS6 binding affinities of the truncated proteins remain detectable but very low. Ypt7 truncation, removing the second aliphatic residue from the AXA characteristic sequence, makes Ypt7Δ10 binding to GDI 3 times weaker compared with full-length protein, and the Ypt7Δ10 mutant binds MRS6 with comparable affinity. However, Ypt7 has an alternative AXA box slightly upstream in the sequence, rendering this result difficult to interpret. The C-terminal truncated Sec4Δ29 lacking the entire flexible C-tail binds GDI 10 times and MRS6 100 times weaker than the full-length protein. Thus, the Rab-GDI/MRS6 AXA box mediated interactions contribute significantly to the complex affinities.
The mobile effector loop, a region that is highly conserved among REP/GDIs, (residues 225–228), is a part of GDI CCR and was shown to direct GDI to the membrane and regulate the ability of GDI to retrieve Rab to the cytosol (18), indicating functional importance of GDI CCR.

Regulation of the GDI Lipid Binding Pocket Conformation—In the prenylated Ypt1-GDI complex, the lipid binding pocket is open and accommodates the prenyl moiety (5) (Fig. 1A), whereas the apo-form of bovine α-GDI shows a closed lipid binding pocket (7). Since have we studied the yeast Rab membrane extraction/delivery system, we have now solved the crystal structure of yeast apo-GDI, which exhibits ~50% amino acid sequence identity to the bovine equivalent. The yeast apo-GDI structure shows a protein conformation similar to that of bovine GDI (7) (Ca r.m.s. deviation ~0.9 Å), with a closed lipid binding pocket. Thus, the GDI lipid binding pocket can adopt two conformations, one being the open form when lipid is bound and the other being closed when neither lipid nor Rab is bound. The question that arises at this point is whether the opening of the lipid site is due to the presence of lipid solely or whether the binding of Rab triggers the opening of the lipid binding site, which can then accommodate a prenyl moiety if present.

Yeast apo-GDI domain II has a well ordered hydrophobic core stabilizing the α helices D, E, F, G, and H in a tightly packed state, defining the closed conformation of the lipid binding pocket (Fig. 3A). All three Rab-GDI complex structures demonstrate a distinctly different domain II structural arrangement (Fig. 3A). The binding of Rab rearranges the packing of the GDI domain II helices, exposing a part of the hydrophobic core residues and forming a hydrophobic cleft on the surface of GDI. In the prenylated Ypt1-GDI complex, the cleft is occupied by the lipid moiety, whereas in the Ypt31-GDI and Sec4-GDI, the pocket is open and empty (Fig. 1A). This observation indicates that pocket opening is not initiated by the lipid group but is allosterically regulated by Rab binding to GDI in response to the formation of Rab interactions with the RBP and the CCR.

Superimposition of apo-GDI on the Rab-bound GDI (Ca r.m.s. deviation 1.6 Å) showed that the β sheets in the GDI molecule build a relatively rigid protein carcass, whereas the α helical regions showed significant shifts in Ca coordinates, demonstrating their involvement in the structural rearrangement on Rab binding (Fig. 3B). In the GDI domain-I, the four helices A, C, I, and N form a bundle. Helix I and the loop adjacent to the helix C belong to RBP and make direct contact with the GTPase upon its binding. Therefore, Rab binding may promote the rearrangement of the GDI helices by pushing helix I toward the GDI core, whereas the loop following helix C is pushed away from the core, resulting in displacement of helices C and N (Fig. 3, A and B). The displaced helices C and N make direct contact with domain II of GDI and appear to induce a conformational change resulting in structural reorganization of domain II.

The majority of GDI domain II (helices E, H, G, and F) retains its structure (Ca r.m.s. deviation is 0.36 Å) (Fig. 3C) upon Rab binding. However, there is a change in its orientation relative to domain I, and helix D is not tightly packed within domain II anymore. The side chain of Phe192 located on helix G flips and pushes the loop following helix D away, stabilizing the pocket in the open conformation (Fig. 3C).

The observed solvent exposure of the hydrophobic pocket is thermodynamically unfavorable. This, presumably, is the reason for the moderate affinity of GDI to unprenylated Rabs (Table 1).
Role of C-terminal Rab Prenylation in GDI/MRS6 Binding—

The direct measurement of the affinity of prenylated Rabs to GDI/MRS6 is difficult to perform due to the insolubility of geranylgeranylated Rabs in water. Since it is known that farnesylated proteins are normally soluble, we decided to use this modification to emulate the geranylgeranyl group. In order to do this, Ypt1 was genetically modified by substitution of the C-terminal geranylgeranylation motif with a so-called CAA box sequence recognizable by farnesyl transferase, an enzyme that performs enzymatic cysteine farnesylation.

The enzymatically prenylated Ypt1-CAA box-farnesyl protein was purified as a homogeneous soluble monomer and was used directly for ITC titrations. GDI-Ypt1 and Ypt1-CAAAX binding affinities differ only by a factor of 2.5 (Table 1). The affinities of GDI for prenylated (Ypt1-CAAAX-farnesyl) and unprenylated (Ypt1-CAAAX) Rabs differ by more than 2 orders of magnitude, fitting the established model of a marked preference of GDI for the prenylated forms of Rab proteins (19). The measured affinities of MRS6 for prenylated and unprenylated Ypt1-CAAAX are comparable, and the dissociation constants differ by just a factor of 2. Thus, the presence of the farnesyl moiety at the C terminus of Rab dramatically increases its affinity to GDI, whereas the affinity to MRS6 does not change significantly.

The observed increase in affinity of GDI to Rab upon prenyl group binding might be due to the Rab lipid moiety filling the open hydrophobic lipid binding pocket in GDI, diminishing the solvent-exposed hydrophobic surfaces of the GDI-Rab complex. The large increase in affinity of GDI to Rab upon prenyl group binding appears to be the driving force for the membrane extraction process, as suggested previously (20). This property is exclusive to GDI (in contrast to MRS6) and explains the efficiency of GDI in Rab retrieval from membranes. This is in agreement with the recently published data on geranylgeranylated Rab molecules (21).

Importance of the Rab AXA Box for GDI/MRS6 Binding to Prenylated Rabs—To investigate the importance of the hydrophobic component in the AXA box coordination for prenylated Rab GDI and MRS6 binding, the two aliphatic amino acid residues (Val191 and Leu193) were individually mutated to alanines in Ypt1-CAAAX. The mutant proteins were enzymatically farnesylated, and binding affinities to GDI and MRS6 were measured (Table 1). The mutation of the first aliphatic residue valine to alanine (V191A) decreased the affinity of Rab to GDI by a factor of 7 and the second (L193A) by a factor of 100. The substitution of the aliphatic residues with the more bulky and polar histidines (V191H and L193H) led to a 200-fold decrease in binding affinities (i.e., levels that are comparable with that of unprenylated YPT1-CAAAX to GDI) (Table 1). These data suggest that the Ypt1 AXA box and prenyl moiety binding contribute cooperatively to increase their binding affinities to GDI upon complex formation. It can be further explained by GDI CCR not only binding the AXA box but also directing the prenylated C terminus to the GDI lipid binding pocket.
All four farnesylated mutants demonstrated similar MRS6 binding properties, displaying a 5–7-fold increase in the dissociation constants (Table 1) compared with Ypt1-CAA-farnesyl, indicating the less critical contribution of the prenylated C terminus to the complex formation.

Structural-Functional Differences in GDI/Rab and REP1/Rab Complexes—Yeast Rab GTPases bind GDI and MRS6 with variable affinities: from 0.33 to 25 μM for GDI and from 36 nM to 1 μM for MRS6 (Table 1). The pronounced heterogeneity of affinities of Rab/MRS6/GDI interactions nevertheless conforms to a certain rule; the GDI-Rab affinities are always lower than those of the corresponding MRS6-Rab affinities. This demonstrates that MRS6 is a stronger binder of unprenylated Rabs compared with GDI. Comparison of the REP1 structures from the Rab7-REP1 and geranylgeranyl transferase-REP1 complex structures (Protein Data Bank code 1VGO and 1LTX, respectively) revealed that the REP1 Rab binding platform is not altered upon Rab binding (Fig. 3D) (REP1 domain-I Cα r.m.s. deviation 0.54 Å). Thus, the REP1 RBP is preformed for Rab acceptance. By contrast, the GDI requires RBP adjustment to bind the GTPase (Fig. 3B). Moreover, the structural changes in domain I of GDI appear to be coupled to the opening of the lipid binding site, which must in itself cost energy and therefore contribute negatively to the overall affinity. The higher affinity of REP for freshly synthesized Rabs, compared with GDI, will lead to preferential REP-Rab binding, which is biologically reasonable, since REP-supported prenylation is essential for the Rab biological activity.

Summarizing the presented structural and biophysical data, we propose a putative mechanism of prenylated Rab membrane extraction driven by GDI (Fig. 4). Prenylated Rabs are localized on the membrane and anchored to the lipid bilayer by prenyl moieties. GDI uses its RBP for initial inter molecular recognition of Rab to form a low affinity complex. The Rab C-terminal AXA box binds to the GDI CCR, increasing the complex affinity and causing the coupled structural adjustment that leads to opening of the lipid binding pocket. By virtue of the latter interaction, the opened GDI lipid binding pocket will be located in the vicinity of the Rab lipid anchor, poised to interact with the membrane-inserted lipid groups. Release of the lipid moieties from the membrane and binding into the open hydrophobic pocket of GDI results in the high affinity GDI-Rab complex formation and release from the membrane.
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