Rab GDP dissociation inhibitor: putting rab GTPases in the right place

PFEFFER, S R, DIRAC-SVEJSTRUP, A B, SOLDATI, Thierry


PMID : 7615494

Available at:
http://archive-ouverte.unige.ch/unige:18934

Disclaimer: layout of this document may differ from the published version.
**Minireview**

**Rab GDP Dissociation Inhibitor: Putting Rab GTPases in the Right Place***

Suzanne R. Pfeffer, A. Barbara Dirac-Scejstrup, and Thierry Soldati

*From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305-5307*

Rab GDP dissociation inhibitors (GDIs)\(^1\) are relatively abundant cytosolic proteins that bind prenylated Rab-GTPases with high specificity. GDIs have the capacity to deliver Rab proteins to their specific membrane-bound compartments and to retrieve Rabs from their fusion targets after they have completed a catalytic cycle. This Minireview will summarize the current available information regarding this interesting class of protein escorts.

Rab proteins represent a family of at least 30 different Ras-like GTPases that function in the processes by which membrane vesicles identify and/or fuse with their targets (see Refs. 1–4 for review). Almost every membrane-bound organelle in the secretory and endocytic pathways bears a distinct set of Rab proteins on its surface. Rabs are doubly geranylgeranylated at or near their carboxyl termini, which makes them to be recognized by distinct sets of proteins on organelle surfaces (9, 10).

Like Ras, Rab proteins interconvert between two conformations. Active Rab has GTP bound; GTP hydrolysis converts the protein into its inactive, GDP-bound conformation. Thus, transport vesicles bear Rab proteins with bound GTP, concomitant with or after membrane fusion. Rabs are converted into their GDP-bound states. In this manner, target membranes acquire vesicle-derived Rab proteins in their GDP-bound conformations.

In pulse-chase experiments, Novick and co-workers (11) showed that Rab proteins cycle from the plasma membrane back to secretory granules via a cytosolic intermediate. Indeed, at steady state, the bulk of a given Rab is membrane-associated; however, between 10 and 50% can be detected in the cytosol. Given that Rabs are stably prenylated, it was not initially clear how membrane dissociation might be accomplished. In 1990, Takai and co-workers (12) reported the discovery and purification of a protein from bovine brain cytosol that blocked the dissociation of \(^{3H}\)GDP from Rab3A. The protein was termed GDP dissociation inhibitor (GDI), and the corresponding cDNA (13) predicted a \(M_r\) of 50,565, consistent with the electrophoretic mobility of the purified protein (\(~54,000\) kDa).

Araki et al. (14) discovered that bovine brain GDP could displace Rab3A from membranes in its GDP- but not GTP-bound conformation. Membrane displacement resulted in the formation of soluble, equimolar complexes of Rab3A bound to GDI. As first predicted by Matsui et al. (15), it is now clear that bovine brain GDI can interact with a variety of Rab proteins including yeast Sec4 and can also release essentially all Rab proteins from membranes, in the presence of GDP but not GTP (15–22). The ability of GDI to distinguish GTP- and GDP-bound conformations of Rab proteins provides GDI with the capacity to retrieve "spent" Rab proteins from target membranes into the cytosol, allowing their return to the correct organelle for subsequent rounds of intracellular transport.

**Multiple Rab-GDI Isoforms**

Northern blot analyses indicated that GDI was expressed in a variety of rat tissues (13). Two RNAs were detected in rat brain (3.1 and 2.3 kilobases); the 2.3-kilobase RNA was also detected in lung, thymus, heart, liver, spleen, kidney, and small intestine. The existence of two Rab-GDI mRNAs indicated that there may be more than one GDI isozyme. Nishimura et al. (23) have now shown that rat brain expresses two GDI isoform RNAs (GDI-α and GDI-β, respectively), whereas other rat tissues produce predominantly the GDI-β mRNA. The two rat proteins are \(~86\%\) identical in amino acid sequence; the rat α-form is \(~99\%\) identical to bovine brain GDI, which is now termed GDI-α. Shisheva et al. (24) reported the primary structures of two mouse GDI isozymes, which they termed GDI-1 and GDI-2. These workers reported that GDI-1 RNA was the predominant form in mouse kidney whereas GDI-2 was the major form in lung. Brain, skeletal muscle, and pancreas contained both. Although a few years ago Ueda et al. (16) presented data suggesting that a GDI isolated from rat liver cytosol appeared not to interact with Rab11, Rab 11 has now been shown to interact with both GDI-α and GDI-β (23).

Whereas mouse GDI-1 and GDI-2 are 90 and 72% identical in deduced amino acid sequence to bovine brain GDI-α, respectively (24), rat GDI-α and GDI-β are 99 and 86% identical to bovine GDI-α, respectively (23). The nature of the differences in sequence among the bovine, mouse, and rat isozymes would be consistent with the existence of more than two GDI isoforms in mammalian cells. Perhaps the mouse GDI-2 gene encodes a third γ form, and the true mouse α gene, which might be predicted to be closer in sequence to the bovine α gene, has yet to be cloned. Indeed, sequences of additional GDI isoforms will soon be available.\(^2\)

Despite the existence of multiple isoforms of GDI, functional differences have not yet been detected. GDI-α and GDI-β show comparable capacities to slow GDP dissociation from Rab3A and Rab11 (23), and both release a variety of Rabs from cellular membranes (22, 24). The proteins differ in their relative abundance in different cell types (22, 25), and GDI-β shows a more particulate distribution than GDI-α (25). The significance of these differences is not yet clear and will require further study. The facts that yeast contain a single GDI gene that is essential for viability (26) suggests that GDI isozymes may overlap significantly in terms of their functional capabilities.

\(^*\) This minireview will be reprinted in the 1995 Minireview Compendium, which will be available in December, 1995.

\(^\dagger\) To whom correspondence should be addressed. Tel.: 415-723-6169; Fax: 415-723-6783.

\(^1\) The abbreviations used are: GDI, GDP dissociation inhibitor; CHO, Chinese hamster ovary; REP, Rab escort protein.

\(^2\) B. Goud and A. Shisheva, personal communication.
Structural Requirements for Rab Protein-GDI Interaction

Using partial proteolysis, Araki et al. (27) generated carboxyl-terminally truncated Rab3A protein and showed that the carboxyl terminus was essential for Rab3A interaction with GDI and with membranes. Carboxyl-terminal prenylation was critical for GDI interaction, because GDI did not alter the rate of GDP dissociation from bacterially expressed Rab3A nor did these proteins form a complex (27). However, carboxyl methylation of the prenylated protein was not required (28, 29). An intact carboxyl terminus and geranylgeranylation was also required for Rab9-GDI interaction (18).

Monogeranylated Rab proteins also complex with GDI (18, 19, 29), although perhaps to a lesser extent than diprenylated Rab proteins (cf. Ref. 29). Palmitoylation of one cysteine, followed by farnesylation or geranylgeranylation of the second cysteine, led to a similar decrease in the ability of Rab6 to interact with GDI (29).

In addition to prenylation, GDI interaction seems to involve structural determinants of Rab proteins (cf. Ref. 29). GDI interacts only with Rab proteins and not with other doubly geranylgeranylated constituents (17). GDI also binds preferentially to RabA in their GDP-bound conformations. This suggests that GDI may recognize structural determinants thought to undergo a nucleotide-dependent conformational change, such as the effector domain. The importance of Rab structural determinants is underscored by the unexpected observation that Sec4p, the yeast secretory vesicle Rab, interacts with yeast GDI even in the absence of prenylation (20).

GDI Is Required for Rab Protein Recycling in Living Cells

If GDI serves to recycle Rab proteins after each round of intracellular transport, mutations in GDI would be expected to interfere with a variety of intracellular transport events. Garrett et al. (26) have now shown that depletion of yeast GDI using a regulated promoter led to multiple defects in protein transport and that the GDI1 gene is in fact allelic with an already identified pleiotropic secretory mutant gene, sec19. Moreover, depletion of GDI led to the concomitant depletion of the cytoplasmic pool of Sec4p. These data strongly support a Rab retrieval role for GDI in living cells. When Rab proteins accumulate at their fusion targets in their GDP conformations, subsequent transport events cannot take place.

GDI Presents Functional Rab Proteins to the Transport Machinery in Vitro

The ability of GDI to retrieve Rab proteins from membranes and the existence of a cytosolic pool of Rab proteins bound to GDI suggested that GDI functions to present them to the transport machinery located on distinct organelles. This hypothesis was confirmed by the observation that purified GDI-α does indeed have the capacity to deliver cytosolic Rab proteins to the appropriate organelle, using permeabilized cells (10) or enriched membrane fractions (9). Prenylated Rab5 and Rab9 proteins were reconstituted with purified GDI-α, and membrane transfer was then monitored. Membrane targeting was accompanied by the displacement of GDI, followed by the exchange of bound GDP for GTP. Since membrane association preceded the nucleotide exchange process (9, 10), it was proposed that a GDI displacement factor catalyzes the recruitment of Rab proteins onto specific organelle membranes.

Rab proteins that are competent for membrane recruitment in vitro are functional in these systems in that they have been shown to stimulate endosome fusion (10) or receptor transport (9). Direct proof that GDI presents functional Rab proteins to specific organelle membranes comes from immunodepletion experiments in which removal of GDI and Rab proteins bound to GDI led to complete loss of the ability of cytosol to stimulate intracellular transport of proteins between late endosomes and the trans-Golgi network in vitro (30). Rab1GDI complexes were also required for the transport of proteins from the endoplasmic reticulum and through the Golgi stack (31).

GDI increases the efficiency with which Rab proteins can be utilized by the transport machinery by increasing the selectivity with which they are delivered to their correct membrane targets (30). This was shown in experiments designed to test whether GDI served only a solubilizing function for prenylated Rab proteins. Delipidated serum albumin could solubilize prenylated Rab9 and deliver it to membranes in a functional form. However, unlike Rab9 delivered by GDI, Rab9 delivered by serum albumin complexes associated nonspecifically with membrane fractions. By binding very tightly to Rab9 (K_{D} < 23 nM (32)), GDI appears to suppress the promiscuous interaction of Rab proteins with inappropriate target membranes. In this manner, GDI increases the efficiency of Rab utilization.

A Model for GDI Function

Fig. 1 summarizes our current view of GDI function. Unoccupied GDI retrieves GDP-bearing Rab proteins from target membranes after a vesicular transport event (Step 1). Prenylated Rab-GDI complexes are then recognized by target membrane-specific proteins. Recent experiments have shown that Rab proteins are recruited with high selectivity (9, 10) by a process that displays an apparent K_{m} of <50 nM. While it appears that the membrane interaction site for Rab-GDI complexes is Rab-specific, it is not yet clear whether the protein (or proteins) that comprise this site interact directly with GDI, in addition to that portion of the Rab protein that would be presented by GDI (Fig. 1, Step 2). In the last step, GDI is displaced (Step 3) and returned to the cytosol; the Rab associates with membranes first in its GDP-bound conformation prior to what is presumed to be a catalyzed nucleotide exchange event. Zerial and coworkers (39) have very recently described an activity in preparations of clathrin-coated vesicles that

![Fig. 1. A model for GDI function.](image-url)
may represent a GDF for Rab5.

The cellular concentration of GDI-α in CHO cells has been estimated to be ~170 nm (32). In addition, CHO cell lines contain more GDI-β than GDI-α (22, 25). Furthermore, the level of GDI-α per mg of cytosol protein is much higher in bovine brain (12) and Madin-Darby canine kidney cells (33) than in CHO cells. The presence of individual Rab proteins at nanomolar concentrations in the cytosol and the high affinity of GDI for prenylated Rabs suggest that there is adequate unoccupied GDI to direct Rab recycling.

**Rab Escort Protein-1 Shares Properties with GDI**

Rab proteins are prenylated by Rab geranylgeranyltransferase (34). A required accessory factor for this enzyme, termed Rab escort protein (REP), is similar in sequence to GDI-α (35). Current data support a model in which REP presents Rabs to prenyltransferase and then could transfer them to another target molecule (35). An obvious candidate recipient molecule would be GDI. However, it was recently shown that prenylated Rab-REP complexes were able to deliver prenylated Rabs to endosome membranes in permeabilized cells (36). The characteristics of REP-mediated Rab delivery paralleled those previously described for GDI-mediated Rab delivery in the same cell type (10). Moreover, REP-1 depressed the rate of GDP release from Rab5 and could retrieve Rab5 from membranes with potency comparable with that of GDI (36). These experiments suggest that newly prenylated Rabs might be delivered directly to membranes by REP, rather than GDI.

Despite the fact that REP shares many properties with GDI, yeast REP cannot substitute for GDI in yeast cells in which the GDI gene has been disrupted. Thus, for some subtle and yet to be discovered reason, REP and GDI must play distinct roles in *vivo*. In this regard, it is important to note that GDI's are more abundant in the cytosol than REP (19, 32, 36). In addition, Rab-REP complexes seem to be of lower affinity than Rab-GDI complexes. The weaker interaction of REP with prenylated Rabs may lead to some mistargeting of Rab proteins in the same manner as Rab-bovine serum albumin complexes lead to mistargeting (30), which might subsequently be corrected by the availability of excess GDI.

The role of REP is to bind unprenylated Rabs and ensure their prenylation. If REP bound very tightly to prenylated Rabs, release of these proteins from REP would be rate-limiting for prenylation. Perhaps by differing in their affinities for prenylated Rabs, REP is designed to facilitate prenylation while GDI can facilitate accurate Rab targeting and recycling.

**GDI-α Inhibits Intracellular Transport in Vitro**

Another characteristic of GDI-α is that it inhibits the transport of proteins between membrane-bound compartments when added to *in vitro* reactions that reconstitute such events (21, 30, 31). GDI-α inhibits the transport of proteins from the endoplasmic reticulum to the Golgi (31), between Golgi cisternae (21, 31), and from late endosomes to the trans-Golgi network (30). Indeed, this property was used to reveal a requirement for a Rab protein in endoplasmic reticulum-to-Golgi transport and in intra-Golgi transport (21, 30). In the case of the transport of proteins between endosomes and the trans-Golgi network, cytosolic Rab9-GDI complexes represent a significant fraction of the total activity of the cytosol in terms of the ability of cytosol to stimulate that transport process (30, 37). In order for Rab proteins to function, they must first be delivered to the correct membrane-bound compartment. GDI is a potent inhibitor of Rab protein membrane delivery (9, 10). Perhaps by blocking the delivery of Rabs that are essential, cytosol-derived transport factors, *in vitro* transport reactions are suppressed. Another model would be that GDI inhibits transport by scavenging Rabs from membranes. However, according to this scenario, it is not clear why such complexes could not be reutilized (and therefore be active, rather than inhibitory) during an *in vitro* transport reaction.

In summary, GDI is an elegantly designed recycling and presentation factor for prenylated Rab proteins. The emerging diversity of GDI's and the significance of their (apparent) post-translational modification (33, 38) are not yet understood. Another major remaining challenge will now be to identify proteins with which GDI interacts.

**REFERENCES**


---

3 P. Novick, personal communication.
4 M. Seabra, personal communication.