Interaction of Farnesylated PRL-2, a Protein-tyrosine Phosphatase, with the β-Subunit of Geranylgeranyltransferase II*

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Protein of regenerating liver (PRL)-1, -2, and -3 comprise a subgroup of closely related protein-tyrosine phosphatases featuring a C-terminal prenylation motif conforming to either the consensus sequence for farnesylation, CAAX, or geranylgeranylation, CCXX. Yeast two-hybrid screening for PRL-2-interacting proteins identified the β-subunit of Rab geranylgeranyltransferase II (βGGT II). The specific interaction of βGGT II with PRL-2 but not with PRL-1 or -3 occurred in yeast and HeLa cells. Chimeric PRL-1/-2 molecules were tested for their interaction with βGGT II, and revealed that the C-terminal region of PRL-2 is required for interaction, possibly the PRL variable region immediately preceeding the CAAX box. Additionally, PRL-2 prenylation is prerequisite for βGGT II binding. As prenylated PRL-2 is localized to the early endosome, we propose that this is where the interaction occurs. PRL-2 is not a substrate for βGGT II, as isoprenoid analysis showed that PRL-2 was solely farnesylated in vitro. Co-expression of the α-subunit (α) of GGT II, βGGT II, and PRL-2 resulted in α/βGGT II heterodimer formation and prevented PRL-2 binding. Expression of PRL-2 alone inhibited the endogenous α/βGGT II activity in HeLa cells. Together, these results indicate that the binding of αGGT II and PRL-2 to βGGT II is mutually exclusive, and suggest that PRL-2 may function as a regulator of GGT II activity.

Protein prenylation is a post-translational modification with an important role in targeting proteins to membranes and in protein-protein interactions (reviewed in Refs. 1 and 2). Prominent among a variety of prenylated proteins are numerous small GTP-binding proteins, including Ras, RhoB, and the Rab proteins, and prenylation is essential for their cellular functions in signal transduction and vesicle trafficking (2). Isoprenoid modification can be catalyzed by one of three different transferases, depending on the isoprenoid and the prenylation motif of the target protein. Farnesyltransferase (FT)1 and geranylgeranyltransferase I (GGT I) are α/β heterodimers which share a common α-subunit and utilize the C15 farnesyl or the C20 geranylgeranyl, respectively (3–5). Both recognize the prenylation motif CAAX, where C is cysteine, A is aliphatic, and X is preferred as Met, Ser or Gln by FT, and as Leu for GGT I. Ras and Rho proteins can be farnesylated by FT or geranylgeranylated by GGT I (6–9). The Rab proteins are the only known substrates of GGT II, a distinct α/β dimer that prenylates XXCC, XCCX, or CCXX C-terminal sequences when the Rab proteins are bound to a carrier called REP (Rab escort protein) (10, 11).

Ras oncogenes are associated with many human cancers, and as Ras transforming function requires farnesylation (12, 13), FT inhibitors (FTI) have been developed and tested as anti-cancer therapeutics. While Ras-mediated oncosis is in many cases compromised by such inhibitors (14–17), in other cases it appears that other non-Ras farnesylated proteins are the effective targets of the inhibitor (18). One candidate is RhoB, which is farnesylated and geranylgeranylated in cells, and a gain in the level of geranylgeranylated RhoB is observed upon FTI treatment (19, 20). This prenylation switch has been proposed to mediate a gain of the tumor growth inhibitory function of RhoB (21), however, recent studies with human cancer cells suggest that both prenylated forms of RhoB are potent anti-transforming molecules and that RhoB thus may not be the unidentified FTI target in cancer cells (22). The nature and role of other farnesylated proteins that could account for the anti-tumorigenic effects of FTI need to be investigated.

The PRL subgroup (PRL-1, -2, -3) of protein-tyrosine phosphatases (PTPs) are closely related intracellular enzymes (23–27) with the highest sequence homology to two dual specificity PTPs, Cdc14p and PTEN. The PRLs are unique among PTP superfamily members in possessing a C-terminal prenylation motif and being prenylated in vivo. Prenylated PRLs are found in the early endosome and at the plasma membrane (28). Inhibition of prenylation, by treatment of cells with a farnesyltransferase inhibitor, results in PRL translocation to the nucleus (28). The prenylation motifs of the PRLs (CICC, CCVQ, CCVM) conform to either the CAAX motif preferentially recognized by FT or to the CCXX motif recognized by GGT II, although their relocalization in response to FT inhibition indicates that they are likely farnesylated proteins in vivo. The prenylation-dependent subcellular localization of the PRLs suggests that regulated prenylation is a mechanism which controls the access of these PTPs to early endosomal or nuclear substrates. Cellular substrates of the PRL phosphatases have not yet been identified, although PRL-1 interacts with a basic leucine zipper protein, ATF-7, and can dephosphorylate it in vitro (29).

Although the specific substrates of the PRLs are unknown, PRL expression is associated with two distinct cell processes. PRL-1 was first identified as an immediate early gene expressed in regenerating liver and in serum-treated fibroblasts,
and overexpression of PRL-1 in NIH 3T3 cells results in cell transformation, suggesting that the gene product plays a role in proliferation (23, 30). Another role for PRL-1 has been proposed in the development and maintenance of differentiating epithelial tissues, as it is expressed in several developing tissues in fetal rat, and in the adult rat is found in terminally differentiated cells such as renal tubular epithelium, bronchiolar epithelium of the lung, and in villus but not crypt enterocytes of the intestine (31, 32). Similarly, PRL-3 is specifically expressed in the differentiated epithelial cells of the villus but not in the proliferating crypt cells of the mouse small intestine (28).

Yeast-two hybrid screening was carried out to identify PRL-2 interacting proteins, the nature of which could give insight into the cellular role of this PTP. The β-subunit of a prenyltransferase, GGT II, was found to specifically interact with PRL-2. This was intriguing, as this enzyme is only known to prenylate Rab proteins. The association of βGTT II with PRL-2 in mammalian cells was confirmed, and found to depend on the prenylation status of PRL-2, even though PRL-2 was farnesylated in cells. Association also required a unique region of PRL-2 that is not present in PRL-1 or -3. We present evidence that the binding of PRL-2 and αGTT II to βGTT II is mutually exclusive, and propose that through displacement of αGTT II, PRL-2 may function as a regulator of Rab GGT II activity.

**EXPERIMENTAL PROCEDURES**

Expression Plasmids—The pAS2-1 vector (CLONTECH) was used to generate the yeast expression PRL-PTP plasmids. In general, the cDNAs encoding the full-length PRL-1, -2, and -3 were amplified by polymerase chain reaction, and each subcloned in-frame into BamHI and PstI cut pAS2-1 vector. The pXJ40-myc vector (a gift from V. Yu) was used to generate the PRL-PTP expression plasmids for in vitro transfections. A BamHI-XhoI fragment encoding each full-length PRL-PTP or a mutant PRL-2 lacking the four C-terminal amino acids (PRL-2cd) was excised from pGEX-KG-PRL-1, -2, -3 or pGEX-KG-PRL-2cd (28), respectively, and subcloned in-frame into pXJ40-myc. The plasmid pXJ40-myc-PRL-2 (CSVQ) was generated by in-frame insertion into BamHI/XhoI-cut pXJ40-myc of a polymerase chain reaction fragment from amplification of PRL-2 using an appropriate forward primer incorporating a BamHI site, and a reverse primer with a nucleotide substitution giving the desired C165S mutation and an engineered XhoI site. To generate the PRL-1/2 chimera expression plasmids, a PRL-1 or PRL-2 DNA fragment was excised from pXJ40-myc-PRL-2 or pXJ40-myc-PRL-1 at EcoRI and/or BglII sites, and was replaced by the corresponding fragment from the other PRL-PTP. The βGTT II cDNA (lacking the nucleotides encoding the four N-terminal amino acids) was amplified from the pGADGH-GTT II-B plasmid and subcloned into an NcoI/XhoI-cut pGEX-KG vector. The βGTT II cDNA was then excised using NcoI and SacI flanking sites, and subcloned into an NcoISacI-cut pBKS-flag vector (a gift from S. Lin). The DNA encoding flag-βGTT II was released by digestion with EcoRI and SacI, and subcloned in-frame into pXJ40 to create pXJ40-flag-βGTT II. The rat αGTT II cDNA was a gift from M.-C. Shabr and was subcloned in-frame into a BamHI/KpnI-cut pXJ41-HA vector. All of the plasmids were sequenced prior to use.

**Yeast Two-hybrid System**—The interaction screen was performed essentially as recommended in the CLONTECH user manual. A HeLa cDNA library (CLONTECH) was transformed into the yeast strain Y190, which had been pretransformed with pAS2-1-PRL-2. The transformants were plated on His, Leu, and Trp—medium containing 25 mM 3-aminol-1,2,4-triazole (Sigma). Plasmids were isolated from positive colonies that fulfilled all criteria, and retested. One plasmid that was consistently positive for interaction with PRL-2 was sequenced and compared against the Entrez data base using a Blast search, and was identified as the cDNA encoding the β-subunit of human GGT II.

**Cell Culture and Transient Transfections**—HeLa cells were maintained in Dulbecco’s modified Eagle’s medium and transiently transfected using LipofectAMINE reagent (Life Technologies, Inc.). The empty expression vector pXJ40-myc was used to normalize the amount of DNA in each transfection. After 24 h of culture, the cells were harvested in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 2 mM phenylmethylsulfonyl fluoride). The cytosol fraction was prepared by passing the cells 6–8 times through a 26-gauge needle, followed by clarification of the lysate by centrifugation at 12,000 rpm for 30 min at 4 °C. The supernatant (cytosol) was used for immunoprecipitation and Western blotting. For farnesyltransferase inhibition, 10 μM FTI-277 (a gift from S. M. Sebti) (33) in Dulbecco’s modified Eagle’s medium, 10% fetal calf serum was added to the cells 5 h after transfection.

**Western Blots, Immunoprecipitation, and Immunofluorescence**—Anti-Myc (9E10, Santa Cruz) and anti-FLAG (M2, Sigma) antibodies were used for immunoprecipitation and Western blotting. Typically the lysates were incubated with the specific antibody overnight at 4 °C. Immunofluorescence was carried out using fluorescein isothiocyanate-conjugated Myc antibody and described (28).

**In Vitro GGT II Assay**—HeLa cells were transiently transfected with pXJ40-myc vector (mock) or pXJ40-myc-PRL-2 (PRL-2) for 18 h prior to harvest. Cell lysate was prepared by scraping the cells into lysis buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 10 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride and then passing the cells 6–8 times through a 26-gauge needle. The lysate was clarified by centrifugation at 12,000 rpm for 30 min at 4 °C, and the supernatant was used as the source of GGT II. The in vitro enzymatic reaction was initiated by adding 80 μg of cell lysate to buffer containing 10 mM MgCl₂, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 10 mM dithiothreitol, containing 2 μM [3H]GGPP (22 Ci/mmol, PerkinElmer Life Sciences) and with or without 2 μM Rabα protein (Calbiochem). The 50-μl reaction was incubated at 30 °C and stopped after 5 h by adding SDS sample buffer, and then resolved by SDS-PAGE. The gel was dried and exposed to hyperfilm at 80 °C for 2 weeks and the labeled Rabα protein was quantified by densitometry.

**Metabolic Labeling and HPLC**—[3H]Mevalonolactone (40 Ci/mmol, [3H]MVA) was purchased from PerkinElmer Life Sciences. HeLa cells were maintained and transfected with pXJ40-myc-PRL-2, and treated with or without FTI-277, as described above. For the final 18 h, cells were treated with [3H]MVA at 100 μCi/ml and 30 μM lovastatin (Calbiochem). Cell lysate was prepared by scraping the cells into 50 mM Tris, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride and then passing the cells 6–8 times through a 26-gauge needle. The lysate was clarified by centrifugation at 12,000 rpm for 30 min at 4 °C, and the supernatant was used to isolate myc-PRL-2 by immunoprecipitation with anti-Myc antibody (9E10, Santa Cruz). The immunoprecipitate was washed twice with acetone and CHCl₃/MeOH, 1:2 (v/v) at −20 °C, dried by vacuum centrifugation, and processed for isoprenoid analysis as described by Casey et al. (6).

The final dried sample was dissolved in 60 μl of 50% CH₃CN, 25 mM H₃PO₄ (solvent A), and a portion was injected onto a C18 reverse-phase HPLC column. The column was eluted with a 4-ml linear gradient of solvent A to 100% CH₃CN, 25 mM H₃PO₄ (solvent B), followed by 1 ml of solvent B at a flow rate of 100 μl/min. Fractions of 100 μl were collected and their radioactivity determined by scintillation counting. Trans,trans-farnesol (FOH, Sigma) and all-trans-geranylgeraniol (GGOH, American Radiolabeled Chemicals, Inc.) were used as elution standards.

**RESULTS**

**Physical Association of PRL-2 with GGT II β-Subunit in Vivo**—To identify PRL-2 interacting proteins, we performed a yeast two-hybrid screen with full-length PRL-2 fused to the GAL4 DNA-binding domain as bait. The expression of the fusion protein in Y190 yeast cells was verified by immunoblotting (data not shown). A human HeLa cell cDNA library fused to the GAL4 activating domain under the control of the constitutive alcohol dehydrogenase 1 promoter was transfected into Y190 cells that expressed DNA-binding domain-PRL-2. Ten million colonies were selected on medium lacking Trp, Leu, and His and supplemented with 25 mM 3-aminol-1,2,4-triazole. Among others, one colony was identified that showed growth on medium lacking Trp, Leu, and His and supplemented with 25 mM 3-aminol-1,2,4-triazole. The plated plasmid was isolated and sequenced. The insert was a frame with the cDNA that coded for the human GGT II β-subunit.

PRL-2 is a member of a group of three closely related proteins which also includes PRL-1 and PRL-3 (23–27). In order to test the specificity of the interaction of PRL-2 with the β-subunit of GGT II, we used PRL-1 and PRL-3 as bait and co-
transfected the yeast with a plasmid expressing GAL4(DNA-binding domain)-βGGT II. The expression of the fusion proteins in Y190 yeast cells was verified by immunoblotting (data not shown). Despite the high homology between different PRL-PTP family members, only PRL-2 interacted with the GGT II β subunit in the yeast two-hybrid system (Fig. 1). As GGT II is a prenyltransferase, we also tested the dependence of the interaction on the presence of the C-terminal prenylation sequence, the CAAX box, of PRL-2. A prenylation-deficient mutant of PRL-2 lacking the CAAX box, PRL-2(cd), did not interact with βGGT II (Fig. 1).

To validate the yeast two-hybrid results, we examined the in vivo interaction of PRL-2, -1, or -3 and βGGT II in mammalian cells. HeLa cells were transiently co-transfected with Myc-tagged PRLs and FLAG-tagged βGGT II. Since βGGT II is a soluble protein mainly localized in the cytosol (10), non-detergent-extracted soluble fractions from the HeLa cells were used to carry out anti-Myc and anti-FLAG immunoprecipitations. Virtually all the expressed βGGT II was indeed present in this soluble cytosolic fraction (Fig. 2A, upper panel, compare lanes 1–4 with lanes 5–8). More PRL-2 was present in the cytosol than in the pellet (Fig. 2A, lower panel, compare lanes 3 and 7), while about one-third of the PRL-1 partitioned into the cytosol (Fig. 2A, lower panel, compare lanes 2 and 6). However, comparatively less of the total PRL-3 was found in the soluble extracts (Fig. 2A, lower panel, compare lanes 4 and 8). PRL-2 and βGGT II co-immunoprecipitated with one another, as evidenced by the presence of FLAG-βGGT II in Myc-PRL-2 immunoprecipitates (Fig. 2B, lane 6), and by the presence of Myc-PRL-2 in anti-FLAG immunoprecipitates (Fig. 2C, lane 6). No βGGT II was detected in myc-PRL-1 immunoprecipitates and Myc-PRL-1 was not present in anti-FLAG immunoprecipitates (Fig. 2, B and C, lane 5). Likewise, no association was detected between PRL-3 and βGGT II (Fig. 2, B and C, lane 7). These results confirm the in vivo interaction of PRL-2 and βGGT II and demonstrate that this involves specific features or properties of PRL-2.

PRL-2 and GGT II β-Subunit Are Associated in the Early Endosome—PRL-2 is localized in the early endosome compartment in a prenylation-dependent manner (28). In transfected CHO cells and NIH 3T3 cells, PRL-2 showed strong perinuclear staining and weak plasma membrane staining visualized by immunofluorescence. A prenylation-deficient PRL-2 mutant was redirected to the nucleus and a farnesyltransferase inhibitor, FTI-277, caused the same nuclear relocalization (28). We found essentially the same results in transiently transfected

HeLa cells, but with enhanced diffuse cytoplasmic staining of expressed wild-type PRL-2 following treatment with FTI-277, and of expressed PRL-2(cd) (Fig. 3). The readily apparent cytoplasmic staining may reflect a higher level of PRL-2 expression in transiently transfected HeLa cells compared to that in the stably transfected CHO and NIH 3T3 cells. Together these results indicate that prenylation plays an important role in the subcellular localization of PRL-2.

To determine whether the linked properties of PRL-2 prenylation status and subcellular localization affect its interaction with GGT II, co-immunoprecipitation experiments were carried out from lysates of HeLa cells expressing FLAG-tagged βGGT II and Myc-tagged PRL-2 or PRL-2(cd) mutant. As observed in previous experiments, the prenylated and early endosome-localized wild-type PRL-2 and βGGT II co-immunoprecipitated with one another (Fig. 4A, lanes 4 and 7). However, the non-prenylated PRL-2(cd) mutant failed to interact with βGGT II (Fig. 4A, lanes 5 and 8). The lack of interaction cannot
merely be due to physically separate pools of cytoplasmic βGGT II and nuclear PRL-2(cd), as some PRL-2(cd) is present in the cell cytoplasm (Fig. 3) and likely represents the soluble PRL-2(cd) protein that was extracted together with soluble βGGT II (Fig. 4A, lane 2). Likewise, in the yeast two-hybrid system we observed that the prenylation deficient mutant PRL-2(cd) did not interact with βGGT II, despite these expressed proteins being directed to the yeast nucleus (Fig. 1). Nevertheless, wild-type PRL-2 did interact with βGGT II in the yeast nucleus (Fig. 1). These results suggested that co-localization of PRL-2 and βGGT II is not sufficient for interaction, and that the early endosomal localization of PRL-2 per se is not required for the physical association of PRL-2 and βGGT II in vivo. However, prenylation or the C-terminal prenylation sequence of PRL-2 is necessary for interaction.

To distinguish between these latter possibilities, we investigated the association between βGGT II and prenylated PRL-2 or unprenylated PRL-2 possessing the CAAX box. HeLa cells were transiently transfected with PRL-2 and βGGT II in the presence or absence of the farnesyltransferase inhibitor FTI-277 for 16 h prior to harvest. Whole cell lysates were prepared and co-immunoprecipitations performed. Once again, wild-type PRL-2 from FTI-277 untreated cells was observed to interact with the GGT II β-subunit as visualized by immunoblotting (Fig. 4B, lanes 4 and 7). Upon treatment with FTI-277, the expression level of PRL-2 was virtually unchanged (Fig. 4B, lanes 1 and 2) but the association with the GGT II β-subunit was lost (Fig. 4B, lanes 5 and 8). This was consistent with the study of PRL-2(cd) described above, and indicated that not only the CAAX box, but modification of PRL-2 by prenylation was required for interaction. As prenylated PRL-2 is in the early endosome and not in the cytoplasm or in the nucleus, this is the subcellular pool of PRL-2 that can interact with βGGT II.

PRL-2 Is Not a Substrate for GGT II—It is well documented that GGT II is a prenyltransferase that recognizes the prenylation motifs of XXC, XXC, or CCX, and whose exclusive substrates are Rab GTPases (1). The C-terminal prenylation motif of PRL-2, CCVQ, fits the CCXX sequence recognized by geranylgeranyltransferase II or the CAAX motif recognized by farnesytransferase or GGT I. Indeed, PRL-2 can be geranylgeranylated or farnesylated in vitro (25, 28). To examine the possibility that prenylation of PRL-2 by GGT II was involved in the interaction of these proteins, we examined the lipid modification of PRL-2 in vivo. HeLa cells transiently expressing Myc-tagged PRL-2 were labeled with the isoprenoid precursor [3H]mevalonolactone, and isoprenoid analysis of the immunoprecipitated PRL-2 was carried out. SDS-PAGE and autoradiography of the PRL-2 immunoprecipitate revealed that PRL-2 was labeled, and that it contained the majority of the label in the immunoprecipitate (Fig. 5A, lane 2). A duplicate Myc-PRL-2 immunoprecipitate was processed for isoprenoid analysis, and the sample resolved by HPLC reverse phase chromatography. The [3H] in the collected fractions was quantitated, and the major labeled elution peak was found to co-migrate with an authentic trans,trans-farnesol standard (Fig. 5B). No obvious labeled elution peak co-migrated with an authentic all-trans-geranylgeraniol standard. Thus PRL-2 is normally farnesylated, and not geranylgeranylated, in vivo. Treatment of PRL-2 expressing cells with the farnesyltransferase inhibitor FTI-277 results in relocalization of PRL-2 from the early endosome to the nucleus (28). Inhibition of farnesylation sometimes results in a gain in geranylgeranylation (8, 9, 20), and we examined if this occurred with PRL-2. Using the HeLa cell expression and labeling system as above, cells were treated with or without FTI-277 and the isoprenoid content of the immunoprecipitated PRL-2 was analyzed. As expected, PRL-2 was labeled in the absence of FTI-277 and the major elution peak co-migrated with authentic trans,trans-farnesol standard. FTI-277 treatment resulted in the absence of labeled PRL-2 in the PRL-2 immunoprecipitate PRL-2 (Fig. 5A, lane 4) and in the disappearance of the elution peak co-migrating with trans,trans-farnesol (Fig. 5C). No obvious elution peak co-migrated with the authentic all-trans-geranylgeraniol standard. Moreover, co-expression of ectopically tagged βGGT II did not alter the prenylation status of PRL-2 (data not shown). Thus the HPLC analysis showed that PRL-2 was indeed modified by a farnesyl isoprenoid and that the modification could be inhibited by a farnesyltransferase inhibitor, consistent with our previous study (28). Furthermore, PRL-2 is not geranylgeranylated in vivo.

Another approach was also employed to test whether PRL-2 geranylgeranylation by GGT II or recognition of a Rab-like prenylation motif in PRL-2 (CCXX) was involved in the interaction with βGGT II. The CCVQ prenylation motif of PRL-2 was altered by site-directed mutagenesis to CSVQ. This PRL-2 mutant has the same prenylation motif as Drosophila PRL-1 (dPRL-1) (28, 34) and can only be the substrate of a CAAX
prenyltransferase. Whole cell lysates from HeLa cells transiently expressing Myc-tagged wild-type PRL-2 or CSVQ mutant PRL-2 together with FLAG-tagged \( \text{G}^{\beta} \text{GGT II} \) were probed for the expression of both proteins (Fig. 6, lane 1–3) and immunoprecipitated with anti-Myc antibody (IP myc) (lanes 2 and 4) and analyzed by SDS-PAGE and fluorography. In B, the immunoprecipitate described in A, and in C, immunoprecipitated PRL-2 from FTI-277-treated HeLa cells, were determined by C\({}_2\)s reverse-phase HPLC following methyl iodide cleavage. Elution times of the trans,trans-farnesol (FOH) and all-trans-geranylgeraniol (GGOH) standards are indicated by arrows.

The C-terminal Region of PRL-2 Is Required for Association with \( \text{G}^{\beta} \text{GGT II} \)—PRL-1 and PRL-3 are prenylated and present in the early endosome (28), yet no interaction of these PRLs with \( \text{G}^{\beta} \text{GGT II} \) was detected, suggesting an additional unique feature of PRL-2 is required. To identify the region of PRL-2 involved in interaction with the \( \text{G}^{\beta} \text{GGT II} \) subunit, we generated a series of swap mutants (Fig. 7A) by replacing segments of PRL-2 with the corresponding regions from its closest homologue, PRL-1. HeLa cells were transiently transfected with wild-type Myc-tagged PRL-2 or PRL-1/-2 swap mutants (swap-1 to -5) together with FLAG-tagged \( \text{G}^{\beta} \text{GGT II} \). Whole cell lysates were prepared and probed for Myc-tagged protein (Fig. 7B, bottom panel) and FLAG-tagged protein (Fig. 7B, top panel), demonstrating equivalent levels of expression from the various transfections. Anti-Myc immunoprecipitates were prepared from the lysates and probed for the presence of PRL-2 or \( \text{G}^{\beta} \text{GGT II} \). Besides wild-type PRL-2 (Fig. 7B, lane 2), swap-1 (Fig. 7B, lane 3), swap-3 (Fig. 7B, lane 5), and swap-5 (Fig. 7B, lane 7) associated with the \( \text{G}^{\beta} \text{GGT II} \) subunit. All of these proteins have in common the feature of possessing at least the C-terminal half of PRL-2 (amino acids 93–167). The PRL-1/-2 chimeric proteins possessing the C-terminal half of PRL-2 failed to interact with \( \text{G}^{\beta} \text{GGT II} \) (Fig. 7B, lanes 4 and 6). Amino acid alignment of the C-terminal halves of PRL-2 and PRL-1 revealed virtually identical sequences, with the exception of a 3-amino acid insertion immediately preceding the CAXX box sequence of PRL-1 (27). Apart from this, there are three conservative substitutions near the C terminus (Lys\(^{161}\), Ser\(^{163}\), and Ile\(^{172}\) in PRL-1 for Arg\(^{158}\), Thr\(^{160}\), and Val\(^{166}\) in PRL-2, respectively), one near the N terminus of this region (Ile\(^{109}\) in PRL-1 with Val\(^{97}\) of PRL-2), and a non-conservative substitution of Gly\(^{122}\) in PRL-1 with Cys\(^{119}\) in PRL-2. As
PRL-2 also possesses the same or similar amino acid differences with PRL-1, two or more of these could account for the differential interaction of PRL-2 with βGGT II.

The GGT II α-Subunit Disrupts the Interaction of PRL-2 with βGGT II—GGT II functions as an αβ heterodimer to prenylate Rab proteins when they are bound to an escort protein termed REP (11, 35). To determine whether PRL-2 could bind to βGGT II when the latter was complexed with the GGT II α-subunit (αGGT II), we co-expressed α and βGGT II in the presence or absence of PRL-2 and examined protein associations. The HA-tagged αGGT II was expressed as a ~68-kDa band (Fig. 8, lanes 2 and 3, upper panel), and was only detectable when co-expressed with βGGT II (data not shown) (36). In the absence of PRL-2, αGGT II complexed with βGGT II as shown by reciprocal immunoprecipitations and immunoblotting (Fig. 8, lanes 5 and 6, upper and middle panels). When PRL-2 was expressed together with α and βGGT II, no PRL-2 was detectable in the αGGT II or the βGGT II immunoprecipitates, although in both cases the other GGT II subunit was present in the complex (Fig. 8, lanes 8 and 9). Immunoblotting of these immunoprecipitates detected faint reactive bands in the vicin-
activity (Fig. 9B). Considering that the efficiency of PRL-2 transfection is about 40% (as assessed by indirect immunofluorescent visualization of Myc-PRL-2 with anti-Myc antibody and an fluorescein isothiocyanate-labeled secondary antibody), this supports the idea that PRL-2 can act as an inhibitor of GGT II, and that the mechanism of inhibition involves displacement of the αGGT II subunit.

**DISCUSSION**

The phosphatase PRL-2 is physically associated with βGGT II *in vivo* (Figs. 1 and 2). The association of PRL-2 with βGGT II depends on PRL-2 prenylation, as a potent farnesyltransferase inhibitor, PTI-277 (33), can abolish the association (Fig. 4B). Moreover, the C-terminal prenylation motif deletion mutant PRL-2(cd) fails to interact with βGGT II *in vivo* (Figs. 1 and 4A). The association is not substrate-like, because HPLC analysis demonstrates that PRL-2 is modified by a farnesyl isoprenoid and this modification is inhibited by a FT inhibitor (Fig. 5). Additionally, a farnesylation-only mutant of PRL-2, PRL-2CSVQ, is not impaired in its ability to interact with βGGT II, clearly showing that geranylgeranylation of PRL-2 is not required. In a previous study we showed that all the PRLs can be prenylated *in vitro*, and that *in vivo* prenylation is key to their plasma membrane and early endosome localization (28). Taken together with the present results, it appears likely that PRL-2 and βGGT II are preferentially associated in the early endosome.

The crystal structure of GGT II shows that the β subunit contains most of the residues in the active site (37). However, βGGT II is active only when complexed to αGGT II (36). We have demonstrated that PRL-2 associates with βGGT II, but that the presence of αGGT II and formation of the αβ heterodimer excludes PRL-2 binding. This is the situation when all three proteins are heterologously expressed and present in the cell in high amounts. If only PRL-2 is transiently expressed, an inhibition of cellular GGT II activity is observed, suggesting that the higher amount of PRL-2 relative to endogenous αGGT II can inhibit αGGT II binding to βGGT II. We propose that PRL-2 and αGGT II bind to βGGT II in a mutually exclusive manner, and that regulation of the relative amounts of PRL-2 and αGGT II can modulate GGT II activity. This could be accomplished by the regulation of PRL-2 or αGGT II gene expression, or by translocation of PRL-2 to the nucleus where, as we have shown, βGGT II is inaccessible for PRL-2 binding. The key factor in determining PRL-2 subcellular localization is its farnesylation (28), and this can potentially be enhanced by environmental conditions which increase isoprenoid synthesis such as stress (heat shock, UV radiation, and arsenite) or heat and light (38–40). In this way, farnesylation of PRL-2 would be stimulated and act to inhibit GGT II activity and Rab geranylgeranylation, suggesting a cellular mechanism by which the activities of the different prenyltransferases may be reciprocally balanced.

In light of the requirement for the αβ GGT II heterodimer for GGT II activity, it is intriguing that while βGGT II is widely expressed, αGGT II expression appears to be negligible in certain tissues such as lung, kidney, and in particular, muscle (36). PRL-2 is highly expressed in these αGGT II-deficient tissues, particularly in muscle (27), and in such situations farnesylated PRL-2 may be the predominant βGGT II binding partner. Treatment with FT inhibitors would result in nuclear localization of PRL-2, and might thus be especially effective in increasing Rab geranylgeranylation in these tissues.

Among the family of PRLs, only PRL-2 can interact with βGGT II (Figs. 1 and 2), suggesting that some element other than prenylation may play an important role in association. In the swap test for βGGT II binding, only the chimeric PRL proteins possessing an intact PRL-2 C-terminal sequence can interact with βGGT II (Fig. 7). Apart from a few mainly conservative amino acid substituitions, a 3-residue insertion just before the CAAX boxes of PRL-1 and -3 is the most divergent feature between these PRLs and PRL-2. Possibly this region of PRL-2 is directly involved in the interaction with GGT II, as with some GGT II Rab substrates where the least conserved C-terminal 25 amino acids, or hypervariable region, influences their direct interaction with βGGT II (41). Alternatively, this region may indirectly provide specificity by directing the various PRLs to different subcellular locations or microlocations such that only PRL-2 is appropriately positioned to interact with βGGT II. The 3-residue pre-CAAX box insert is distinct even between PRL-3 and PRL-1, and thus could account for different subcellular localizations/functions among all the PRLs. It has been proposed that divergent C-terminal sequences of small GTPases dictate their specific association with membrane structures. Studies of Ras proteins have revealed that the trafficking of Ras to the plasma membrane is largely dependent on a second signal (besides farnesylation) within the hypervariable region (42, 43). Different isoforms of Ras may be targeted to specific subregions of the plasma membrane on the basis of this second membrane localization signal (44, 45). In stably transfected Chinese hamster ovary and NIH 3T3 cells, we have observed that PRL-2 is enriched in perinuclear regions and its staining at the plasma membrane is much less obvious compared with that of PRL-1 and -3. Transferrin receptor/PRL co–internalization experiments suggest that PRL-2 may reside in the later recycling subcompartment of the early endosome, while large amounts of PRL-1 and -3 reside in the plasma membrane and early subcompartment. Whether the C-terminal amino acid variation between the PRLs differentially determines their precise membrane destination is unknown, but could explain their localization and perhaps the βGGT II binding differences.

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