PDZ Domain Protein GIPC Interacts with the Cytoplasmic Tail of Melanosomal Membrane Protein gp75 (Tyrosinase-related Protein-1)*

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Tyrosinase and tyrosinase-related proteins (TRPs) are a family of melanosomal membrane proteins involved in mammalian pigmentation. Whereas the melanogenic functions of TRPs are localized in their amino-terminal domains that reside within the lumen of melanosomes, the sorting and targeting of these proteins to melanosomes is mediated by signals in their cytoplasmic domains. To identify proteins that interact with the cytoplasmic tail of gp75 (TRP-1), the most abundant melanosomal membrane protein, we performed yeast two-hybrid screening of a melanocyte cDNA library. Here, we show that the cytoplasmic domain of gp75 interacts with a PDZ domain-containing protein. The gp75-interacting protein is identical to GIPC, an RGS (regulator of G protein signaling)/GAIP-interacting protein, and to SEMCAP-1, a transmembrane semaphorin-binding protein. Carboxyl-terminal amino acid residues, Ser-Val-Val, of gp75 are necessary and sufficient for interaction of gp75 with the single PDZ domain in GIPC. Although endogenous and transfected GIPCs bind efficiently to transiently expressed gp75, only a small amount of GIPC is found associated with gp75 at steady state. Using a strategy to selectively synchronize the biosynthesis of endogenous gp75, we demonstrate that only newly synthesized gp75 associates with GIPC, primarily in the juxtanuclear Golgi region. Our data suggest that GIPC/SEMCAP-1 plays a role in biosynthetic sorting of proteins, specifically gp75, to melanosomes.

In mammalian melanocytes, melanin pigment is synthesized in specialized organelles known as melanosomes. Tyrosinase is the critical enzyme required for melanin synthesis, and tyrosinase-related protein-1 (TRP-1) and TRP-2 (dopachrome tautomerase) influence the nature of the pigment produced (1, 2). Photodynamic inactivation of melanosomes is mediated by signals in their cytoplasmic domains, suggesting the presence of several signals for intracellular transport in the cytoplasmic tail (20). Thus, through their interactions with cytosolic proteins, cytoplasmically exposed amino acid sequences seem to influence the biological functions of mammalian pigmentation-related proteins. In this study, by using the cytoplasmic tail of gp75 as bait for yeast two-hybrid screening we identified a PDZ domain-containing protein, GIPC/SEMCAP-1 (referred to as GIPC hereafter), as a gp75-interacting protein. GIPC, which was identified earlier based on its interaction with GAIP, a regulator of G protein signaling (21), and as neural semaphorin-interacting protein SEMCAP-1 (22), binds specifically to the carboxyl terminus of newly synthesized gp75 but not tyrosinase. Binding of GIPC with the melanosomal gp75 during biosynthesis suggests a possible role for this interaction in intracellular sorting and targeting of melanosomal membrane proteins.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Analysis—For bait plasmid construction a DNA fragment encoding the cytoplasmic tail of gp75 was amplified by polymerase chain reaction using the plasmid pSVK3hgp75 containing the full-length gp75 cDNA (14) as template and primers with 5′-EcoRI and 3′-XhoI restriction sites and cloned into EcoRI-XhoI-digested pHybLex/
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Zeo plasmid (Invitrogen, Carlsbad, CA) to produce an in-frame fusion cDNA with the LexA DNA-binding domain protein. The transformation of Saccharomyces cerevisiae strain EGY48 and selection of Zeocin-resistant colonies were performed according to manufacturer instructions. Zeocin-resistant colonies were transformed with the reporter plasmids pJG-GIPC, pSH18/H11003. The plasmids pJG-GIPC and pSH18/H11003 were digested with the enzymes Zeo/"Ura" and then plated on Zeo/"ura"/leu plates containing 2% galactose, 1% raffinose, and 80 mg/ml X-gal to test background activation of the reporter genes by the bait fusion protein alone.

The human melanocyte cDNA library cloned in pJG vector (Invitrogen) as B42 activation domain fusion constructs was a gift from Dr. Tony Zervos (Cutaneous Biology Research Center, Cambridge, MA). A freshly isolated colony of EGY48 transformant selected for bait and β-galactosidase (β-gal) plasmids was transformed with 150 μg of melanocyte cDNA library plasmid. Aliquots (200 μg) of the transformation mixture containing a total of 13.6 × 10⁶ transformants were plated on 40 Zeo/"Ura"/Trp plates containing 2% dextrose and incubated at 30 °C for 2 days. An amplified library was titered and stored at −80 °C. The transformants were screened for the interacting proteins by plating the following strategy. Briefly, rabbits were inoculated with 100 μg of purified protein mixed with complete Freund’s adjuvant. After three booster injections of 50 μg of antigen in incomplete Freund’s adjuvant, sera were obtained and tested for anti-GIPC reactivity by Western blotting. Two weeks after the third boost, responding rabbits were boosted twice at 1-week intervals, and serum was collected 1 week after the last boost by bleeding into EDC-treated BD tubes.

For the isolation of anti-GIPC IgG, sera were first preclutered with GST-Sepharose, and IgG from GST-preclutered preimmune and immune sera was purified using protein A-Sepharose affinity chromatography (Amersham Pharmacia Biotech). GIPC-specific IgG was isolated by GST-GIPC-Sepharose affinity chromatography (23). GST-preclutered sera were used for immunoblotting analyses, and GST-preclutered IgG fraction or GST-GIPC affinity-purified IgG3 were used for immunofluorescence staining.

Cell Culture—The isolation and culture of human neonatal foreskin melanocytes was described earlier (24). Human pigmented metastatic melanoma cell lines SK-MEL-19 and SK-MEL-23. cl.22 (clone 22) and the nonpigmented gp75-negative SK-MEL-33. cl.22a (clone 22a) cell cultures were grown in 10% fetal bovine serum (FBS) supplemented with 150 μg/ml Zeocin (Invitrogen, Carlsbad, CA). Primary melanocytes (WM75, WM35, and WM98-1) and metastatic (WM451/451Lu and WM1205) melanoma cell lines were cultured as described (24).

Cell Lysis and Fractionation—Cells were harvested, washed in phosphate-buffered saline (PBS), and lysed in 50 mM phosphate buffer, pH 7.0, containing 150 mM NaCl, 1% Triton X-100, and a mixture of protease inhibitors (Roche Diagnostics, Indianapolis, IN). Detergent lyses were cleared by centrifuging at 15,000 × g for 20 min. For preparation of cytosolic and membrane-bound proteins, cells were resuspended in PBS containing protein inhibitor mixture and lysed by freeze/thaw cycles in liquid nitrogen. After centrifugation for 1 h at 100,000 × g at 4 °C, the cytosol fraction was collected and adjusted to 1% Triton X-100. The membrane pellet was solubilized in lysis buffer and cleared as described above. In experiments to determine the nature of membrane-associated GIPC, the membrane fraction was washed with buffer containing 0.5 mM NaCl for 1 h and centrifuged at 100,000 × g (25). The supernatant was collected, and the membrane pellet was lysed and cleared as described above. Nuclei were isolated, and nuclear extracts were prepared as described (26).

Sucrose Density Gradient Fractionation—SK-MEL-19 melanoma cells at 70% confluence were washed with PBS, harvested, suspended in 20 mM HEPES (pH 7.2, 5 mM KCl, 5 mM NaCl, 0.1 mM EDTA, and protease inhibitor mixture), homogenized in Dounce homogenizer, and centrifuged at 700 × g for 10 min in gradient buffer containing 0.25 mM sucrose. The postnuclear supernatant was fractionated by discontinuous sucrose density gradient as described (27). Fractions (1 ml) were collected from the top and stored at −20 °C until used for immunoblot analysis. A microcentrifuge pellet obtained at the bottom of the tube, presumably consisting cellular debris, contained all proteins analyzed and is not shown.

Transfection and Co-immunoprecipitation—COS-7 and clone 22a melanoma cells were co-transfected with 8 μg of pCMV5a-gp75 or pCMV5a-gp75/tyr (chimeric gp75 with tyrosinase cytoplasmic tail) or 18 μg of pSVK3-gp75A14 (gp75 truncated its last 14 amino acids of the cytoplasmic tail) and 2 μg of pCMV5a-full-length GIPC-FLAG or pCMV5a-NH2-GIPC-FLAG plasmids using LipofectAMINE Plus reagent (Life Technologies, Inc.) according to manufacturer instructions. pCMV2-FLAG-control (an unrelated 82-kDa soluble protein, 2 μg) was used for control transfections. After 36 h of transfection, the cells were washed with cold PBS and lysed in 1 ml of lysis buffer as described above. Clear centrifuged lysates were incubated with mAb T99 (1–6 μg/ml) on ice for 2 h followed by protein A-Sepharose for 1 h. Immunoprecipitates were washed three times with lysis buffer, twice with lysis buffer containing 0.5 mM NaCl, and analyzed by SDS-PAGE and immunoblotting with anti-FLAG mAb M2 (Sigma).

Co-precipitation of Endogenous gp75 and GIPC—Melanoma cells were cultured in the presence or absence of 5 mM hexamethylene bisacetamide (HMBA) for 5 days and harvested immediately thereafter allowing to recover from HMBA treatment for 8–48 h. gp75 in detergent lysates was precipitated with mAb T99, immunoprecipitates were washed twice with lysis buffer, and GIPC in the immunoprecipitates was detected by immunoblot analysis with anti-GIPC serum.

Immunoblot Analysis—Proteins from cell lysates, cell fractions, sucrose fractions, and immunoprecipitates were separated by SDS-PAGE.
RESULTS

Identification of gp75 Cytoplasmic Domain-interacting Protein GIPC—We employed the yeast two-hybrid method to screen a human melanocyte cDNA expression library with a LexA DNA-binding domain-gp75 cytoplasmic tail fusion protein as bait. We obtained 27 positive clones from $13 \times 10^6$ primary transformants. DNA sequence analysis showed that these 16 positive clones contained two overlapping cDNAs of 1,437 and 1,348 base pairs differing at their 5′ ends. Fig. 1A shows two representative yeast clones streaked on leucine-deficient (leu−) plates. In the absence of galactose (gal), which is required for the expression of the prey protein, yeast clones failed to grow on leu−/dextrose-containing plates (Fig. 1A, leu−/dext−). Thus, the growth of these clones on leu− plates (Fig. 1A, leu−/gal−) and activation of the β-gal reporter gene (Fig. 1A, leu−/gal−/X-gal) depend on the expression and interaction of the protein product encoded by melanocyte-derived cDNA with the LexA-gp75 tail fusion protein.

A BLAST search of GenBank™ data base using the 243-amino acid sequence predicted from the 1.4-kb partial melanocyte cDNA showed sequence identity with three PDZ domain-containing mammalian protein entries. These are 1) a protein fragment encoded by melanocyte-derived cDNA with the protein product encoded by melanocyte-derived cDNA with the protein fragment encoded by TIP-2 (GenBank™ accession no. AF02884), a cDNA isolated by yeast two-hybrid screening using human T cell leukemia virus transactivator Tax as a bait (28), 2) a human homologue of GIPC (GenBank™ accession no. AF164219), a protein identified by binding of its carboxy-terminal cytoplasmic domain GIPC-FLAG fusion protein or control-FLAG were immunoprecipitated (IP) with anti-gp75 mAb TA99, and the immunoprecipitates were analyzed by immunoblotting (IB) using anti-FLAG mAb M2 (top panel). The expression levels of gp75 and the FLAG fusion proteins in total cell lysates were probed with rabbit anti-gp75 antisera (left bottom panel) or mAb TA99 (right bottom panel) and mAb M2 (middle panel). Immunoglobulin heavy and light chains of mAb TA99 are indicated on the left. The positions of prestained molecular mass markers (kDa) marked with ink on the blot are shown.

Co-immunoprecipitation of GIPC with gp75—That the interaction of the protein fragment encoded by GIPC cDNA with the cytoplasmic tail of melanocyte-specific gp75 may also be mediated by the PDZ domain is suggested by the following observations. First, class I PDZ domain proteins, including GIPC, are known to specifically bind the carboxy-terminal amino acid motif Xaa-Ser/Thr-Xaa-Val (where Xaa is any amino acid). The carboxy-terminal sequence of gp75 is Gln-Ser-Val-Val (14). Second, the protein fragment encoded by all the 16 partial cDNAs contained no protein-protein interaction domain other than the PDZ domain. To test whether the full-length, intracellular transmembrane gp75 also interacts with GIPC, we cloned 0.7- and 1.0-kb GIPC cDNAs encoding partial and com-
complete open reading frames in the pFLAG-CMV plasmid to generate FLAG-epitope fusion proteins. COS-7 cells were transiently transfected with the gp75 cDNA expression plasmid together with partial and full-length GIPC-FLAG fusion proteins. Detergent lysates of transfected cells were immunoprecipitated with gp75-specific mAb TA99. Western blot analysis of immunoprecipitated proteins with anti-FLAG mAb M2 (Fig. 1B) showed that both the partial 28-kDa ΔNH2,GIPC and full-length 38-kDa GIPC co-precipitated with gp75 (lanes 1 and 3 in the upper panel). No M2 antibody-reactive proteins were precipitated from detergent extracts of cells cotransfected with either empty vector pFLAG (Fig. 1B, lane 2 in the upper panel) or the unrelated control protein control-FLAG fusion plasmid pControl-FLAG (Fig. 1B, lane 4 in the upper panel). These data show that the PDZ domain of GIPC efficiently binds intracellular membrane-bound gp75.

Carboxyl-terminal Amino Acids of gp75 Are Necessary and Sufficient for Interaction with GIPC in Melanocytic Cells—In melanocytic cells, the cytoplasmic tail of gp75 is involved in the sorting and targeting of this protein to melanosomes. Therefore, we tested whether in melanocytic cells the cytoplasmic tail of gp75 is accessible for interaction with GIPC. We used a nonpigmented gp75-negative melanoma cell line clone 22a to coexpress FLAG-GIPC with either full-length gp75 or a deletion mutant lacking carboxyl-terminal 14 amino acid (ΔC14). In earlier studies we showed that 1) the deletion of carboxyl-terminal amino acids of gp75 does not affect its recognition by anti-gp75 mAb TA99 and 2) in transiently transfected melanoma cells, ΔC14 mutant, which lacks the PDZ-binding motif but contains the endosomes/lysosomes sorting signal, displays an intracellular distribution similar but not identical to the wild-type protein (14). Detergent extracts of transfected cells were immunoprecipitated with anti-gp75 mAb TA99 and immunoblotted with anti-FLAG mAb M2 (Fig. 2, upper panel). In cells coexpressing wild-type gp75 and GIPC-FLAG fusion protein, the mAb M2-reactive 38-kDa band corresponding to the co-precipitated GIPC-FLAG fusion protein could be detected (lane 1). This interaction was specific as shown by the failure of co-precipitation of control-FLAG with gp75 (lane 2). The coexpression of GIPC with gp75ΔC14 did not result in its co-precipitation with gp75 (lane 2). Immunoblotting of total cell lysates showed approximately equal expression of GIPC-FLAG fusion protein (Fig. 2, middle panel) and full-length and truncated gp75 (bottom panel) in the transfectants. These data demonstrate that the PDZ domain protein GIPC binds efficiently to the carboxyl-terminal amino acids of gp75 in melanocytic cells. This is consistent with the canonical mode of PDZ domain interaction with the carboxyl-terminal amino acids of target membrane proteins (32).

Specific Interaction of GIPC with the Unique Carboxyl Domain of gp75—gp75 is a member of the tyrosinase family of melanosomal membrane proteins, and its cytoplasmic tail, which contains the conserved dileucine sorting motif, shares limited sequence homology with other members of the tyrosinase family (14). Examination of amino acid sequences of the cytoplasmic domains of gp75, tyrosinase, TRP-2, and other known melanosomal proteins including gp100, Melan A/MART-1, and P (pink-eyed dilution) protein showed that the PDZ-binding motif, however, is found only in the gp75 tail (Fig. 3A). It therefore seems that the unique carboxyl-terminal amino acid sequence of gp75 is sufficient for its interaction with GIPC. We hypothesized that other tyrosinase family proteins, which lack the extended carboxyl-terminal PDZ-binding motif, do not interact with GIPC. We tested this by yeast two-hybrid analysis and co-precipitation experiments using wild-type and carboxyl-terminal sequence mutant proteins. In Fig. 3B, β-gal activity in two representative clones of double transformants of S. cerevisiae expressing GIPC and wild-type or mutant cytoplasmic tails of gp75 and tyrosinase is shown. As predicted, wtgp75 tail (Fig. 3B, wtgp75) showed a strong interaction with GIPC as seen by the activation of β-gal, and the gp75 tail lacking terminal residues Gln-Ser-Val-Val did not interact with GIPC (Fig. 3B, gp75ΔC14). The cytoplasmic tail of tyrosinase did not show interaction with GIPC (Fig. 3B, Tyr). Substitution of the tyrosinase carboxyl-terminal amino acid residues His-Leu with valine, which results in a carboxyl-terminal sequence Tyr-QSVV, allowed efficient interaction of the tyrosinase tail with GIPC (Fig. 3B, Tyr-QSVV). Although interaction of GIPC with the mutant tyrosinase-QSVV tail was qualitatively comparable with its interaction with the gp75 tail (as shown by the in-plate β-gal assay in Fig. 3B), a quantitative difference between these interactions could be noted (Fig. 3C). However, β-gal activity of both the wild-type gp75 tail and tyr-QSVV transformants was significantly higher than the activity seen for the positive control (Fig. 3C, jun⁺). Consistent with the in-plate assay, β-gal activity was undetectable in gp75ΔQSVV and tyrosinase transformants. These data suggest that although the terminal three amino acids Ser-Val-Val of gp75...
are necessary for GIPC binding, other residues in the gp75 tail may also influence this binding. A lack of interaction between the tyrosinase tail and GIPC in vivo was confirmed by coexpression of GIPC with a chimeric gp75 containing the lumenal and transmembrane domains of gp75 and the cytoplasmic tail of tyrosinase in 22a cells (Fig. 3, D and E). As shown in Fig. 3E, although the immunoprecipitation of gp75 from wtgp75 transfectants co-precipitated GIPC (lane 1, upper panel), no co-precipitated GIPC band could be seen from chimeric gp75/tyr transfectants (lane 2, upper panel). Utilization of anti-gp75 mAb TA99 for immunoprecipitation of both wild-type gp75 and gp75/tyr chimera transfectants allowed us to conclude that the lumenal domain of gp75 does not contribute to its interaction with GIPC.

Expression of GIPC in Melanocytic Cells—Northern analysis of primary melanocytes and a panel of pigmented and nonpigmented melanoma cells using the 1.4-kb cDNA obtained from the yeast two-hybrid screen revealed a single band of 1.8-kb RNA in all cell lines tested (data not shown). To characterize the human GIPC protein, we generated polyclonal antibodies against the GST-GIPC fusion protein. Fig. 4A shows Western blot analysis of melanoma cell extracts with preimmune and anti-GST-GIPC immune serum. Immune serum from GST-GIPC-immunized rabbits detected three major bands including the prominent band at 38 kDa, the expected molecular mass of GIPC. A one-step GST-Sepharose affinity chromatography produced a serum that specifically detected the 38-kDa GIPC band. We used GST-precleared serum to study the expression of GIPC in human melanocytic cells. The steady-state level of GIPC expression in neonatal foreskin melanocytes (NMC), primary (WM75, WM35, and WM98-1) and metastatic (WM451, WM1205, clone 22, clone 22a, and SK-MEL-19) melanoma cell lines, pigmented (WM75, clone 22, and SK-MEL-19) and nonpigmented melanoma cell lines (WM98-1, WM451, WM1205, and clone 22a), and gp75+ (NMC, WM75, clone 22, and SK-MEL-19) and gp75– (WM35, WM98-1, WM451, WM1205, and clone 22a) cell lines is shown in Fig. 4B. Nearly equal amounts of GIPC are expressed in normal and malignant melanocytes, and GIPC seems to be regulated independently of gp75, pigmentation, or melanoma tumor progression.

Intracellular Distribution of GIPC—To study the intracellular distribution of GIPC and investigate whether it colocalizes with gp75 in human melanoma cells, we performed double immunofluorescence staining. Paraformaldehyde-fixed and detergent-permeabilized SK-MEL-19 melanoma cells were incubated with either preimmune serum or anti-GIPC antibody followed by TRITC-conjugated anti-rabbit IgG and then FITC-conjugated anti-gp75 mAb TA99. The anti-GIPC antibody showed patchy staining in the juxtanuclear region, punctate staining in the cytoplasm, and a variable nuclear staining (Fig. 5A-d; also see Fig. 8). No specific staining could be seen with the preimmune serum (Fig. 5A-a). gp75 showed similar juxtanuclear and punctate staining, consistent with its localization in the Golgi, endosomes, and melanosomes (Fig. 5, A-b and e). Staining for the two proteins showed only limited overlap localized primarily to a few vesicles near the intense patchy areas of GIPC staining (Fig. 5A-f). Accordingly, immunoprecipitation of endogenous gp75 with mAb TA99 showed co-precipitation of only a weak band corresponding to GIPC (data not shown). Because coexpression studies in COS and melanoma cells showed that GIPC interacts with the membrane-bound gp75, we investigated whether GIPC in melanoma cells is associated with membranes. The distribution of GIPC in postnuclear supernatant, crude membrane, and cytosolic fractions is shown in Fig. 5B. A significant amount (~50%) of GIPC was found to be associated with the membrane fraction. After incubation of the crude membrane fraction with 0.5 M NaCl for 1 h, the bulk of GIPC remained in the insoluble membrane fraction. Almost all the cellular gp75, on the other hand, is present in the membranes and could not be dissociated by high salt. Thus, although a significant amount of GIPC is found associated with membranes in melanoma cells, only a small fraction of this protein appeared to interact with the cytoplasmic domain of gp75.
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4 Characterization of anti-GIPC antiserum and expression of GIPC in melanocytic cells. A, equal volumes of human melanoma SK-MEL-19 cell lysate were electrophoresed in multiple lanes by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and probed with preimmune and immune sera and GST-precleared immune serum (1:800) followed by horseradish peroxidase-conjugated donkey anti-rabbit antibody (1:10,000). A specific band of endogenous GIPC detected by immune serum and GST-precleared antiserum but not by preimmune serum is shown by the arrow on the right. B, GIPC expression in melanocyte and melanoma cells. Total cell lysate (50 μg of protein) from neonatal foreskin melanocytes and primary and metastatic melanoma cells were electrophoresed by SDS-PAGE and immunoblotted with GST precleared anti-GIPC serum (1:1,000). Western blotting for γ-tubulin is shown as control for protein loading. Prestained molecular mass markers are shown on the right.

The affinity-purified anti-GIPC antibody showed variable nuclear staining (Fig. 5, A–d and f). We therefore investigated whether GIPC could be detected biochemically in melanoma nuclei. Western blotting showed a weak band corresponding to the molecular mass of GIPC in melanoma nuclear extracts (Fig. 5B, right panel) confirming the immunofluorescence observation (see “Discussion”).

To further explore whether endogenous GIPC and gp75 can co-sediment in same-cellular fractions, we performed sucrose density gradient fractionation. The postnuclear supernatant isolated from SK-MEL-19 cells was fractionated on discontinuous sucrose density gradient (0.25–1.5 M), and the distribution of various proteins was studied by Western blot analysis (Fig. 6). Based on the enrichment of specific proteins, fractions collected from the top to bottom were designated as soluble and low density (fractions 1–3), intermediate density (fractions 4–8), and high density (fractions 9–11). GIPC showed a tri-partite distribution. Although a significant proportion of the protein was distributed among low and high density fractions, a small amount of GIPC could also be detected in the intermediate fractions (low dense > intermediate). Low density fractions contained the bulk of cellular clathrin, and high density fractions were enriched for the lysosomal membrane protein LAMP-1, whereas intermediate fractions were enriched for adaptor protein AP-1. gp75 and tyrosinase were enriched in intermediate and high density fractions, which also contained varying amounts of melanin pigment presumably associated with melanosomes at various stages of maturation. A small amount of mature gp75 and tyrosinase could also be seen in clathrin-enriched low density fractions.

Fig. 4. Characterization of anti-GIPC antiserum and expression of GIPC in melanocytic cells. A, equal volumes of human melanoma SK-MEL-19 cell lysate were electrophoresed in multiple lanes by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and probed with preimmune and immune sera and GST-precleared immune serum (1:800) followed by horseradish peroxidase-conjugated donkey anti-rabbit antibody (1:10,000). A specific band of endogenous GIPC detected by immune serum and GST-precleared antiserum but not by preimmune serum is shown by the arrow on the right. B, GIPC expression in melanocyte and melanoma cells. Total cell lysate (50 μg of protein) from neonatal foreskin melanocytes and primary and metastatic melanoma cells were electrophoresed by SDS-PAGE and immunoblotted with GST precleared anti-GIPC serum (1:1,000). Western blotting for γ-tubulin is shown as control for protein loading. Prestained molecular mass markers are shown on the right.

Fig. 5. Intracellular distribution of endogenous GIPC in melanoma cells. A, confocal immunofluorescence analysis of SK-MEL-19 melanoma cells. Paraformaldehyde-fixed Triton X-100-permeabilized cells were stained with preimmune rabbit IgG (a–c) or affinity-purified anti-GIPC IgG (d–f) followed by TRITC-conjugated anti-rabbit IgG and FITC-conjugated anti-gp75 mAb TA99 as described under “Experimental Procedures.” A limited overlap of staining for GIPC and gp75 in vesicles is shown by arrows (f). Scale bar, 10 μm. B, Western blot analysis of GIPC distribution in melanoma cell fractions. Total cell lysate, cytosol, and membrane fractions were prepared from SK-MEL-19 cells as described under “Experimental Procedures.” The membranes were washed with buffer containing 0.5 M NaCl, and a high speed supernatant (SN) and pellet were obtained. An equal amount (50 μg) of each protein fraction was separated by SDS-PAGE and immunoblotted with anti-GIPC and anti-γ tubulin antibodies (left panel). Total cell lysates and nuclear extracts were immunoblotted (IB) with GST-precleared preimmune (PI) and immune (I) sera (right panel). FNS, postnuclear supernatant.

Fig. 6. Intracellular distribution of membrane-associated GIPC in melanoma cells. SK-MEL-19 melanoma cells (1 × 10⁷) are homogenized in gradient buffer, and the postnuclear supernatant was loaded on a discontinuous 20–50% sucrose density gradient and centrifuged at 100,000 × g for 2 h. 1-ml fractions were collected from the top (fractions numbered 1–11 from top to bottom), analyzed by SDS-PAGE, and immunoblotted with antibodies against GIPC, gp75, tyrosinase, clathrin heavy chain, AP-1, LAMP-1, and γ-tubulin. Note the co-fractionation of GIPC in intermediate density fractions 6 and 7 with gp75, tyrosinase, clathrin, and AP-1.
and reactivation of the gene. We showed earlier that endogenous gp75 is biosynthesized by selective repression and reactivation of the gp75 gene. We tested this interaction by Western blotting with anti-GIPC serum and anti-gp75 mAb TA99, respectively. The arrows (top) show that GIPC co-precipitated with newly synthesized gp75. Molecular mass markers are shown on the right.

FIG. 7. Endogenous GIPC interacts with newly synthesized endogenous gp75. SK-MEL-19 cells were left untreated (C) or treated with 5 mM HMBA (H) for 5 days. Cell lysates were prepared immediately or after 15-, 36-, and 48-h recoveries from HMBA treatment. Lysates were immunoprecipitated (IP) with anti-gp75 mAb TA99, separated by SDS-PAGE, and immunoblotted (IB) with anti-GIPC antibody (top) as described under "Experimental Procedures." The expression of endogenous GIPC (middle) and gp75 (bottom) in total cell lysates was detected by Western blotting with anti-GIPC serum and anti-gp75 mAb TA99, respectively. The arrows (top) show that GIPC co-precipitated with newly synthesized gp75. Molecular mass markers are shown on the right.

GIPC Interacts with Newly Synthesized gp75—We consistently found that only a small amount of endogenous GIPC associates with gp75 at steady state. However, because we also found that endogenous GIPC in melanoma cells readily co-precipitated with transiently expressed gp75 (data not shown), we considered the possibility that interaction of GIPC with gp75 may be transient. The presence of a small amount of GIPC and gp75 in intermediate density clathrin-containing fractions (fractions 6 and 7), in which AP-1 is also enriched (Fig. 6A), suggested that such an interaction might be restricted to a transport compartment.

To examine this possibility, we used a strategy to synchronize biosynthesis of endogenous gp75 by selective repression and reactivation of the gp75 gene. We showed earlier that treatment of pigmented melanoma cells with a polar planar compound HMBA selectively and completely extinguishes gp75 (33). This inhibition of gp75 expression occurs at the level of transcription and is reversible (34). We tested the interaction of GIPC with newly synthesized gp75 in cells recovering from inhibition by HMBA (Fig. 7). The treatment of pigmented SK-MEL-19 melanoma cells with HMBA for 5 days extinguishes expression of gp75, and removal of this drug allows gp75 re-expression (Fig. 7, bottom). The expression of GIPC was not affected by treatment with HMBA or during recovery from HMBA (Fig. 7, middle). Immunoprecipitation with anti-gp75 mAb TA99 followed by Western blotting with the rabbit anti-GIPC antibody showed a weak co-precipitated band of 38 kDa in control untreated cells (arrow in lane C, upper panel). As expected, after the extinction of gp75 expression by HMBA the co-precipitated 38-kDa band was undetectable (lane H, upper panel). Immunoprecipitation of newly accumulating gp75 from lysates of cells recovering from HMBA treatment clearly showed a 38-kDa GIPC band. The intensity of this band increased with prolonged recovery up to 36 h (lanes 15 h and 36 h), but decreased after 36 h to a level nearly comparable with that of untreated cells (arrow in lane 48 h, upper panel). Similar data were obtained using clone 22, another pigmented melanoma cell line (data not shown). These results show that GIPC only binds newly accumulating gp75.

FIG. 8. Colocalization of endogenous GIPC and newly synthesized gp75 in melanoma cells. A, SK-MEL-19 cells grown in 8-well chamber slides were left untreated (a–f), treated with HMBA (g–i), or allowed to recover from treatment with HMBA (j–o) as described in the legend for Fig. 7. After fixation and permeabilization, the cells were stained with preimmune IgG (a–c) or anti-GIPC IgG (d–o) followed by TRITC-conjugated anti-rabbit antibody and FITC-conjugated anti-gp75 mAb TA99. The distribution of GIPC and gp75 were examined by confocal microscopy. The arrows (f, l, and o) indicate colocalization of the two proteins in the juxtanuclear region. Scale Bar, 20 μm. B, double immunofluorescence staining and confocal microscopy of clone 22 cells recovering from HMBA at 36 h. Higher magnification of boxes in the merged panel are shown in C. Note the colocalization of GIPC and gp75 in patchy areas (solid line box) and distinct vesicles (broken line box). Scale Bar, 20 μm.

At steady state, the transient interaction of GIPC with newly synthesized gp75 molecules, which constitute a relatively small proportion of intracellular gp75 compared with a large pool of preexisting gp75 protein, accounts for co-precipitation of a small amount of GIPC. Synchronization of gp75 synthesis either by transient transfection or selective repression and reactivation increased the pool of newly synthesized protein, allowing for visualization of this interaction.

Double immunofluorescence microscopy of HMBA-treated and untreated SK-MEL-19 cells and cells recovering from HMBA confirmed these biochemical observations. Fig. 8A, a–e, shows the staining of untreated cells. a shows background staining with the preimmune rabbit IgG. The staining of en-
The yeast two-hybrid system was used to identify proteins that interact with the cytoplasmic domain of melanosomal membrane protein gp75 and found GIPC/SEMCAP-1 as a gp75-interacting protein. In melanocytic cells, GIPC co-precipitated and colocalized with newly accumulated gp75 in the Golgi region, suggesting a role for GIPC in intracellular transport of gp75 during biosynthesis.

Specific Interaction of gp75 with PDZ Domain of GIPC—GIPC is a member of a large family of PDZ domain proteins. PDZ domains that recognize the sequence motif Ser/Thr-Xaa-Val are classified as class I PDZ domains (35). The requirement of gp75 carboxyl-terminal Ser-Val-Val residues for its interaction with the single PDZ domain present in GIPC is consistent with a canonical mode of class I PDZ domain interaction (32, 35). It is interesting to note that although GIPC/SEMCAP-1, which was isolated independently as a GAIP-binding protein from a rat pituitary library and as neurally enriched semaphorin-binding protein, is expressed widely in mammalian tissues. Thus, the specificity of GIPC interactions may be determined by tissue distribution of the interacting target proteins. For example, because GIPC/SEMCAP-1 and M-SemF, but not GAIP, are expressed in the brain, it has been proposed that M-SemF (but not GAIP) is the biologically relevant target for GIPC in this tissue (22). The expression of GIPC targets GAIP and M-SemF in melanocytes has not been investigated. Although the carboxyl-terminal sequence motif of gp75 is a potential target for other class I PDZ domain proteins, co-immunoprecipitation of endogenous GIPC with gp75 and immunofluorescence data presented here support its specific interaction with GIPC in melanocytic cells. Additionally, among 27 positive clones isolated by yeast two-hybrid screening, we found only GIPC (16 of 27 positives) but no other PDZ domain proteins.

Expression of GIPC in Melanocytic Cells—Although gp75 expression in melanoma cells is widely variable and readily modulated (33, 36), nearly identical amounts of GIPC are present in all mouse and human melanocytic cells and nonmelanocytic cells (e.g., COS cells, mouse fibroblasts, and rat PC12 cells; data not shown). This is reminiscent of a tightly regulated expression pattern of proteins with essential or housekeeping functions (e.g., GAPDH and tubulin) and suggests that PDZ interactions mediated by GIPC play important roles in the biology of melanocytic cells. Because we isolated multiple clones of GIPC by yeast two-hybrid analysis and found that GIPC efficiently associates with gp75 when coexpressed by transient transfection in both melanoma and nonmelanoma cells, we were intrigued by the fact that only a small amount of GIPC associated with endogenous gp75. However, nearly 50% of cellular GIPC is associated with membranes in melanoma cells, and a significant amount of GIPC co-sedimented with gp75 and melanin-containing dense vesicles. Thus, a steady state the bulk of membrane-bound GIPC is not associated with gp75, suggesting that in melanocytes GIPC interacts with multiple target proteins.

Interestingly, a small amount of GIPC was also found to co-fractionate with AP-1 and clathrin-containing vesicles. In this context, it is of interest to note that the GIPC-interacting protein RGS-GAIP seems to be localized to clathrin-coated vesicles, and in HeLa cells immunogold electron microscopic studies showed that GIPC is associated with small vesicles located near the cell membrane (21). Interaction of GIPC/SEMCAP-1 with SemF, on the other hand, seems to create complex protein aggregates at specialized domains on the plasma membrane (22).

Nuclear Localization of GIPC—Using the antisera raised against melanoma GIPC, we found variable staining of nuclei and a small amount of protein in the nuclear extracts. It seems that the intracellular distribution of GIPC is also variable, suggesting a possible redistribution of the protein in response to different cellular or environmental conditions.

**DISCUSSION**

We used the yeast two-hybrid system to identify proteins that interact with the cytoplasmic domain of melanosomal membrane protein gp75 and found GIPC/SEMCAP-1 as a gp75-specific interacting protein. In melanocytic cells, GIPC co-precipitated and colocalized with newly accumulated gp75 in the Golgi region, suggesting a role for GIPC in intracellular transport of gp75 during biosynthesis.

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to increased synthesis of interacting protein gp75 (compare GIPC distribution in steady-state cells in Fig. 5D and the top left panel in Fig. 9 versus cells recovering from HMBA in Figs. 8j and the top right panel in Fig. 9). Because, GIPC lacks recognizable nuclear localization signals it is possible that GIPC is translocated to the nucleus in association with other cytosolic proteins(s). This possibility is highlighted by the following observations. 1) GIPC, indeed, was first isolated as a partial protein fragment named TIP based on its interaction with the carboxyl terminus of human T cell leukemia virus-1-encoded protein Tax, a transcriptional activator (28). However, endogenous cellular transcription factors to which GIPC can bind have not yet been identified. 2) The PDZ domain protein CASK has been shown to be translocated to the nucleus in association with the Tbr-1 transcription factor after the binding of CASK to the cytoplasmic tail of the transmembrane adhesion molecule syndecan (37).

**Transient Interaction of Newly Synthesized gp75 with GIPC**—We found that endogenous and transfected GIPC efficiently associate with coexpressed gp75 in both melanoma or non-melanoma cells. Moreover, GIPC co-sedimented with gp75-containing vesicles. However, immunoprecipitation of gp75 from total cellular lysates of melanoma cells or from gp75-enriched fractions showed only a weak co-precipitated band of GIPC. We considered several possible explanations for the discrepancy in interaction of GIPC with transfected and endogenous gp75. It is possible that GIPC binds only a small distinct subpopulation of mature gp75 molecules, or GIPC may bind all gp75 molecules only transiently either during the biosynthesis and/or degradation of gp75. Alternatively, the inefficient co-precipitation of GIPC could be caused by the rapid degradation of GIPC upon its association with membranes and/or gp75. To examine whether membrane-bound GIPC is degraded rapidly, we estimated the half-life of total, cytosolic, and membrane-associated GIPC in gp75- and gp75- melanoma cells by pulse-chase metabolic labeling and immunoprecipitation. Newly synthesized cytosolic and membrane-bound GIPC was degraded with a similar half-life (18 h) in both gp75- and gp75- cell lines (data not shown), suggesting that our inability to efficiently co-precipitate GIPC with gp75 was not caused by a preferential degradation of membrane or gp75-associated GIPC.

Our strategy to selectively synchronize synthesis of gp75 by inhibiting gp75 transcription and then releasing the inhibition allowed us to demonstrate that endogenous GIPC in melanocytic cells interacts only with newly synthesized gp75 molecules. We examined the possibility that this could be demonstrated by pulse-chase metabolic labeling followed by immunoprecipitation with anti-gp75 mAb TA99. However, only a weak co-precipitating radioactive band corresponding to GIPC could be detected at the 8-h chase point (data not shown). This is consistent with the data from the co-immunoprecipitation of steady-state proteins. This highlights the importance of the unique strategy we employed to demonstrate this transient interaction by synchronization of endogenous gp75 biosynthesis.

**Role of GIPC in Intracellular Sorting of gp75**—We showed that the interaction of GIPC with the gp75 cytoplasmic tail is dynamic and limited to newly synthesized gp75 protein localized in and near the Golgi. A transient interaction during biosynthesis of gp75 suggests that the carboxyl PDZ-binding motif plays a role in intracellular sorting. A sequence motif in the cytoplasmic tail of gp75 (and other melanosomal membrane proteins) that is known to interact with cytosolic proteins is the dileucine sorting sequence (14). The binding of adaptor complexes, specifically AP-3, to this sequence is known to mediate the sorting of melanosomal proteins along the lysosome/melanosome pathway (15). Mutations in AP-3 produce the mocha phenotype in mouse and Hermansky-Pudlak-like syndrome in man (reviewed in Ref. 16). The role GIPC plays in intracellular protein sorting remains to be investigated. In transiently transfected mouse fibroblasts and COS cells, the intracellular distribution of gp75ΔC14 protein lacking the PDZ-binding motif appears grossly similar to but distinct from that of wild-type protein (14). Preliminary pulse-chase experiments indicated that carboxyl-terminal residues determine the stability of newly synthesized gp75 expressed in nonpigmented melanoma cells (data not shown). It is tempting to speculate that GIPC participates in the sorting of gp75 to a vesicular compartment, presumably premelanosomes. The observation that GIPC binds GAIP, a $G_{	ext{on}}$-interacting protein localized to vesicles, implicates GIPC in G protein-mediated control of vesicular trafficking (21). Similarly, the interaction of GIPC/SEMCAP-1 with the plasma membrane protein SemF appears to link SemF to G protein-signaling pathways (22). Recently, it has been reported that mutations in the novel intracellular G protein-coupled receptor OA1 are associated with an X-linked ocular albinism type 1 (OA1) (38). A defect in melanosome biogenesis in skin melanocytes and retinal pigment epithelial cells seems responsible for the hypopigmentary phenotype in OA1 (39). It is not clear whether GIPC or its interaction with gp75 plays a role in OA1-mediated G protein signaling and pigment regulation.

**Possible Role for GIPC-gp75 Interaction in Pigmentation**—Among tyrosinase-related proteins, gp75 exhibits several unique features. 1) In melanocytes and pigmented melanomas, gp75 is the most abundant melanosomal protein (13). 2) Human gp75, unlike its murine counterpart, does not seem to function as a DHICA (5,6-dihydroxyindole-2-carboxylic acid) oxidase, raising the possibility that gp75 may have nonenzymatic functions in melanin pigmentation (12). 3) The 36-amino acid residue-long tail of gp75 is the longest among TRPs and contains some unique features as well as some shared with other TRPs (14). The relative abundance of GIPC in melanosytic cells and its interaction with the unique carboxyl terminus of gp75 suggests a possible role for this interaction in the biological functions of the enigmatic brown locus protein gp75. In this context it is worth noting that we have mapped the human GIPC gene to chromosome 19p13.1, where BRHC (OMIM locus 113750), the human brown hair color 1 locus, is also mapped (40, 41).^2

**Alternative Functions of GIPC**—The best known function of PDZ domains is to organize the assembly of protein complexes, especially in submembranous areas, by binding to the cytoplasmic tails of membrane proteins (42). Generally, multiple PDZ domains present in these proteins allow binding and assembly of protein complexes. Other functional domains such as the Src homology (SH)-3 domain, tyrosine phosphatase, guanylate kinase, and calcium/calmodulin protein kinase domains on PDZ proteins or the interacting target proteins are thought to mediate the signaling functions of these complexes. For example, clustering of NMDA (N-methyl-D-aspartate) receptors in neuronal cells by PSD-95, a protein with multiple PDZ domains, has been shown to couple the receptor activation to specific excitotoxic signaling (30). GIPC, however, has only one PDZ domain and no other recognizable sequence motif involved in signaling (21, 22). This raises the possibility that gp75-bound GIPC interacts with other proteins through novel non-PDZ interactions. Alternatively, the functions of GIPC may involve the acyl carrier protein (ACP) domain present at its C terminus. De Vries et al. (21) suggested that GIPC could act as a carrier for palmitoyl moieties for palmitoylation of target proteins including GAIP and the α

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^2 McKusick, V. A. (1997) Online Mendelian Inheritance in Man, www.ncbi.nlm.nih.gov/entrez/dispomim.cigizid=113750.
subunits of G protein. Thus, palmitoylation of the cytoplasmic tails of TRPs and/or other melanosomal membrane proteins by GIPC also merits investigation.

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REFERENCES

1. Pawlek, J. M., and Chakraborty, A. K. (1998) in The Pigmentary System: Physiology and Pathophysiology (Nordlund, J. J., Boissy, R. E., Hearing, V. J., King, R. A., and Ortonne J.-P., eds) Oxford University Press, New York
2. Oetting, W. S (2000)
3. 20. Xu, Y., Vijayasaradhi, S., and Houghton, A. N. (1997) J. Invest. Dermatol. 110, 324–331
4. De Vries, L., Lou, X., Zhao, G., Zheng, B., and Fraquhar, M. G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12340–12345
5. Wang, L.-H., Kalb, R. G., and Strittmatter, S. M. (1999) J. Biol. Chem. 274, 14137–14146
6. Voussoufian, H. (1997) BioTechniques 24, 198–202
7. Fang, D., and Setaluri, V. (2000) Biochem. Biophys. Res. Commun. 279, 53–61
8. Christgau, S., Schierbeck, H., Aanstoot, H. J., Augard, L., Begley, K., Kefsd, H., Hejnaes, R., and Baekkeskov, S. (1991) J. Biol. Chem. 266, 21257–21264
9. Kingston, R. E. (1999) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) John Wiley & Sons, New York
10. Radke, K., Carter, C., Moss, P., Dehazya, P., Schliwa, M., and Martin, G. S. (1991) J. Cell Biol. 113, 130–141
11. Lozano, J. A., and Garcia-Borron, J. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 16470–16475
12. Jenkins, N. A., and Hearing, V. J. (1992) EMBO J. 11, 537–543
13. Jiang, I. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2801–2805
14. Hearing, V. J., and Figueroa, K. (1998) J. Biol. Chem. 273, 6778–6783
15. Hearing, V. J., and Tsukamoto, K. (1991) Int. J. Cancer 47, 288–292
16. Halaban, R., Lozano, J. A., and Garcia-Borron, J. C. (1994) J. Biol. Chem. 269, 17993–18001
17. Awad, H. Y., Peretz, J. M., Laursen, R., Har, M., and Gilchrest, B. (1999) J. Biol. Chem. 274, 16470–16478
18. Xu, Y., Vijayasaradhi, S., and Houghton, A. N. (1997) J. Invest. Dermatol. 110, 324–331
19. Hsueh, Y.-P., Wang, T.-F., Yang, F.-C., and Sheng, M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 15889–15894
20. Schiaffino, M. V., d’Addio, M., Alloni, A., Bassirrotto, C., Valeri, A., Cossete, K., Puri, C., Bassi, M. T., Colla, C., De Luca, M., Taccetti C., and Ballabio A. (1999) Nat. Genet. 23, 108–112
21. d’Addio, M., Pizzigoni, A., Bassi, M. T., Bassirrotto, C., Valeri, A., Incerti, B., Clemetti, M., De Luca, M., Ballabio, A., and Schiaffino, V. (2000) Hum. Mol. Genet. 9, 3011–3018
22. Von Kap-Herr, C., Kandala, G., Mann, S. S., Hart, T. C., Pettenati, M. J., and Setaluri, V. (2000) Cytogenet. Cell Genet. 89, 234–235
23. Eiberg, H., and Mohr, J. (1987) Clin. Genet. 31, 186–191
24. Ponting, C. P., Phillips, C., Davies, K. E., and Blake, D. J. (1997) BioEssays 19, 469–479
25. Porrello, L., Cotese, K., Bassi, M. T., Colla, C., De Luca, M., Taccetti, C., and Ballabio, A. (1999) J. Biol. Chem. 274, 14137–14146
26. Fang, D., and Setaluri, V. (1999) Biochem. Biophys. Res. Commun. 266, 21257–21264
27. Radke, K., Carter, C., Moss, P., Dehazya, P., Schliwa, M., and Martin, G. S. (1991) J. Cell Biol. 113, 130–141
28. Fang, D., and Setaluri, V. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6210–6215
29. Halaban, R., Svedeen, S., Cheng, E., Smirou, Y., Aron, R., and Hebert, D. N. (2000) Nucl. Acids Res. 74, 807–820