A Novel Form of the G Protein β Subunit Gβ5 Is Specifically Expressed in the Vertebrate Retina*

(Received for publication, June 7, 1996, and in revised form, August 16, 1996)

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The G protein β subunit, Gβ5, is predominantly expressed in the central nervous system. In rodent brain, Gβ5 is expressed as a protein with an apparent molecular mass of 39,000 daltons (39 kDa). We have identified an additional Gβ5 immunoreactive protein of apparent size 44 kDa in the vertebrate retina. Molecular cloning and sequencing of polymerase chain reaction products revealed that the cDNA encoding the larger species of Gβ5 (Gβ5L) was identical to the shorter form with the addition of 126 base pairs of 5' DNA sequence potentially encoding an in-frame 42-amino acid extension. Sequencing of nucleotides Gβ5 genomic clones demonstrated that the 126-base pair of retinal-specific coding material is derived from a hitherto undetected 5' exon. During sucrose density gradient fractionation of bovine retinas, the 44-kDa Gβ5L protein co-purified with rod outer segment membranes. Incubation of rod outer segment membranes with the nonhydrolyzable guanine nucleotide, GTPγS (guanosine 5'-3-O-(thio)triphosphate), which released the Gβ subunit of transducin (Gβ1), failed to remove Gβ3L. The 39-kDa Gβ5 protein displayed differential association with retinal and brain membranes. In the retina, Gβ5 was present as a soluble protein and was undetectable in the membrane fraction, whereas in the brain approximately 70% of Gβ5 was associated with cellular membranes. In transient COS-7 cell expression experiments, Gβ3L, formed functional Gβγ dimers and Gaβ heterotrimers, and activated phosphoinositide-specific phospholipase Cβ in a manner indistinguishable from the 39-kDa Gβ3 protein. The cloning of the retinal-specific Gβ3L cDNA suggests the existence of potentially novel G protein-mediated signaling cascades in photoreception.

In eukaryotic cells, a family of signal-transducing guanine-nucleotide binding proteins (G proteins) orchestrates many physiological processes by coupling activated cell surface receptors to intracellular second messenger systems. G protein-coupled receptors (GPCRs), which possess a stereotypical seven-transmembrane-spanning domain architecture, bind and mediate the signaling of a variety of molecules, including hormones, neurotransmitters, odors, and light. To date, several hundred GPCRs have been cloned or characterized (1). In contrast, the number of heterotrimeric G proteins, as well as the number of G protein-regulated effectors, is much more limited.

G proteins are heterotrimeric, composed of α, β, and γ subunits (Ga, Gβ, Gγ). Activation of a GPCR by ligand binding stimulates the exchange of bound GDP for GTP on the Ga subunit and results in the dissociation of the Ga subunit from a tightly complexed Gβγ dimer. The released Ga and Gβγ subunits in turn regulate the activity of effector proteins, the better characterized of which include cGMP phosphodiesterase, adenylyl cyclases, phosphoinositide-specific phospholipase C β enzymes, and ion channels. In addition, the dimeric Gβγ subunits are involved in GPCR desensitization by recruitment of receptor kinases to the plasma membrane, and in signal transfer from seven transmembrane GPCRs to mitogen-activated protein kinase cascades (reviewed in Refs. 2 and 3).

The enormous diversity of the GPCR superfamily combined with the relatively limited set of G proteins and intracellular effectors poses problems for the generation of specific cellular responses to signaling molecules. It is believed that signal-sorting G proteins participate in the generation of highly specific and appropriate intracellular signals in several ways. Signaling of a particular receptor can be routed through specific G protein heterotrimers by somatic or temporal compartmentalization of the receptor and downstream signaling molecules with particular G protein subunits. For example, specificity in visual signal transduction by the light receptor, rhodopsin, is mediated in part by tissue- and cell type-specific expression of rod and cone cell Ga and Gγ subunits (4). Somatic compartmentalization of other G protein α and γ subunits has been described (5, 6). To date, Gβ5, which is readily detected only in the central nervous system, is the only G protein β subunit known to be expressed in a tissue-restricted fashion (7). Alternatively, GPCR signaling specificity may be achieved at the level of G protein-receptor coupling. There is a body of evidence to suggest that specific receptors display marked preferences for particular heterotrimeric combinations of G protein α, β, and γ subunits (8–11). While it is known that not all Gβγ dimeric combinations occur in vivo, there still remain a large number of combinatorial possibilities for G protein heterotrimer assembly. Thus, mechanisms to generate G protein subunit diversity have implications for signal transduction specificity.

Mammalian cDNA clones coding for at least 20 distinct Ga subunit proteins have been described (12, 13). Tandem duplication

polymerase chain reaction; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; GTPγS, guanosine 5'-3-O-(thiotriphosphate); GPCR, G protein-coupled receptor.
followed by evolutionary divergence of Ga genes has been proposed as a fundamental genetic mechanism to increase the number and diversity of Ga subunits (14). Alternative splicing has been shown to occur for certain Ga gene transcripts and is an additional mechanism to increase G protein subunit diversity (15, 16). In addition to the known Ga subunits, cDNAs encoding 5 Gβ (7) and at least 12 Gγ (17) subunits have been described. The Gβ5 protein products are relatively similar, displaying amino acid identities ranging from 53 to 90%. The Gγ proteins, on the other hand, are much less well conserved.

In this paper, we report that the Gβ5 gene is expressed as two distinct proteins in a tissue-specific manner. While Gβ5 expression in brain is confined to a single 39,000-dalton (39-kDa) species, an additional Gβ5 protein, with an apparent molecular mass of 44 kDa, is present in the vertebrate retina. We have cloned a retinal-specific Gβ5 cDNA and show that this cDNA is identical to our previously described brain cDNA Gβ5 clones with the exception of an additional 126 base pairs (bp) of sequence, encoding an additional 43 amino acids, at the 5′ end. Sequence analysis of bacteriophage clones comprising part of the murine Gβ5 genomic locus has revealed that the additional coding material derives from an upstream exon located approximately 2,900 bp (2.9 kilobase pairs) away from the initiator methionine of the 39-kDa brain Gβ5 isoform. Fractionation of bovine retinas reveals that the long 44-kDa form of Gβ5 (hereafter referred to as Gβ5m) is localized to the rod outer segment (ROS) membranes of the photoreceptor cell layer. While the function(s) of the retinally expressed Gβ5 proteins is unclear, this unexpected versatility of the Gβ5 gene has implications for G protein subunit diversity and the generation of complex, finely tuned signal transduction circuits.

EXPERIMENTAL PROCEDURES

Cloning, Sequencing, and Northern Analysis of Retinal Gβ5—Partial DNA sequence of a human retinal cDNA clone encoding a potentially longer form of Gβ5 was initially identified during a search of The Institute for Genetic Research (TIGR) Human cDNA (HCD) data base using the full-length amino acid sequence of mouse Gβ5 as the query. Using the DNA sequence of the TIGR clone as a guide, we cloned the coding region of the corresponding mouse cDNA by reverse transcription-polymerase chain reaction amplification (RT-PCR). Amplification of first-strand cDNA prepared from mouse retina (3) was carried out with Pfu DNA polymerase (Stratagene) using the following primers: 5′-ggaattcATGTNGATCAGACCTTTCT-3′ and 5′-gctctagaTTATGCCCAAACTC-3′. As a control, 400 ng of commercially obtained mouse brain total RNA (Clontech) was carried out with Pfu DNA polymerase (Stratagene) was screened using the Gβ5 as the query.

The resulting amplification product was directionally cloned into the pCDNAⅢ+ expression vector (Invitrogen) and the entire sequence of several clones determined on both strands. A 1295V mouse genomic DNA library in the cloning vector ϕX174 (Stratagene) was screened using the Gβ5 cDNA as a probe according to standard methods (18). Isolated phage clones were rescreened with an oligonucleotide covering the first 18 bases of the Gβ5 coding region to identify clones containing the 5′ end of the gene. Positive clones were subcloned into pBlueScript SKⅡ (Stratagene), analyzed by restriction enzyme digestion, sequenced using dye-labeled primers on an Applied Biosystems model 373 automated sequencer. One clone was identified that contained approximately 8 kb of 5′ untranslated DNA. This 8-kb region was cloned into pBlueScript SKⅡ and partially sequenced by primer walking.

For Northern analysis, 40 μg of total RNA, prepared from mouse tissues using the guanidine thiocyanate–phenol method (Trizol reagent, Life Technologies, Inc.), was fractionated on formaldehyde–agarose gels, transferred to Magna NT nylon membranes (MSI) and probed with an end of the gene. Positive clones were sequenced using dye-labeled primers on an Applied Biosystems model 373 automated sequencer. One clone was identified that contained approximately 8 kb of 5′ untranslated DNA. This 8-kb region was cloned into pBlueScript SKⅡ and partially sequenced by primer walking.

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RESULTS

Identification and cDNA Cloning of a Retinal-specific Form of Gβ5—We have reported previously that the G protein β subunit, Gβ5, is expressed predominantly in the central nervous system. When immunoblotting of crude membrane preparations from whole mouse brain, are probed with antisera CT215, which recognizes a 16-amino acid peptide corresponding to the amino terminus of Gβ5, a protein of the appropriate molecular mass (39 kDa) can be readily detected. In contrast, Gβ5 immunoactive material is not detected on Western blots of membrane protein isolated from mouse heart, spleen, kidney liver, or skeletal muscle (7). However, we had noticed that when crude membrane preparations from murine retinas were immunoblotted and probed with antisera CT215, another immunoactive protein, with an apparent molecular mass of 44 kDa was present, in addition to the 39-kDa Gβ5 protein (Fig. 1). The presence of a larger protein immunologically related to
Gβ5 in retina can be explained in several ways. The larger protein could be transcribed from a gene closely related to Gβ5, or, finally, it could be a splice variant of the Gβ5 gene. Interestingly, the 44-kDa immunoreactive species was not observed in retinal protein samples obtained from rd mutant mice (Fig. 1, lane 3). Mouse strains carrying the rd mutation display retinal degeneration and a specific loss of rod photoreceptors (reviewed in Ref. 24). This result suggests that the 44-kDa protein, but not the 39-kDa Gβ5 protein, is predominantly if not exclusively localized to rod cells in the retinal photoreceptor cell layer.

Concurrent with our identification of a larger protein immunologically related to Gβ5 in retina, we had performed homology searches, using the amino acid sequence of mouse Gβ5 to query the Institute for Genetic Research (TIGR) human cDNA expressed sequence tag (HCD EST) data base (25), as part of our on-going efforts to identify additional members of the G protein β subunit family. This search returned 43 significant matches, 5 of which were determined to be partial sequences of the human Gβ5 homologue. The DNA sequence of one of these (EST19480), which had been isolated from a human retinal cDNA library, was found to potentially encode a longer form of Gβ5. The DNA sequence of this partial cDNA clone overlapped bases 31–108 of Gβ5, which encode the first 36 amino acids of the protein. Significantly, EST19480 also had 193 bp of unrelated sequence at its 5′ end, 126 bases of which had the potential to code for an additional in-frame 42 amino acids at the amino terminus of Gβ5.

Using the DNA sequence of EST19480 as a guide, we designed oligonucleotide primers to amplify the coding region of the corresponding mouse retinal transcript. Reverse transcription and amplification of total murine retinal RNA resulted in the appearance of the predicted 1,188-bp product, which was cloned and sequenced in its entirety. The sequence of this clone, which we have designated Gβ5Long (Gβ5L), was identical to our previously reported mouse Gβ5 cDNA sequence except for the addition of a 126-base pair extension at its 5′ end. As determined for the EST19480 cDNA, the additional 126 bp of coding material in the Gβ5L cDNA has the potential to direct the synthesis of a 42-amino acid peptide, which is in-frame with the rest of the Gβ5 sequence. The complete sequence of the Gβ5L coding region is shown in Fig. 2. The Gβ5L 1,185-bp open reading frame potentially encodes a protein of 395 amino acids with a predicted molecular mass of 43.6 kDa, in excellent agreement with the size of the retinal protein we had observed following SDS-PAGE. Homology searches of DNA and protein sequence data bases failed to identify any significant match to the retinal-specific coding material.

The retinal-specific 126-bp extension has no homology to the 5′-untranslated portion of Gβ5 cDNAs isolated from mouse brain (7), suggesting that it arises by retinal-specific usage of a 5′ exon. We therefore isolated mouse genomic bacteriophage λ clones covering most of the Gβ5 genomic locus and sequenced the first 3,923 bp upstream from the initiator ATG codon found in brain Gβ5 transcripts. The beginning of the retinal-specific

**Fig. 1.** Presence of 39- and 44-kDa Gβ5-immunoreactive proteins in mouse retina. Crude membrane protein (10 μg) from mouse brain (B), retina (R), and retina from an rd mutant mouse (rd R) were fractionated on a 16% Tricine polyacrylamide gel and blotted onto a nitrocellulose membrane. Immobilized proteins were probed with the Gβ5-specific antiserum, CT215, and visualized by the ECL method. Positions of protein size standards are indicated.

**Fig. 2.** Nucleotide sequence and predicted amino acid sequence of Gβ5L. Nucleotide and predicted amino acid sequences of murine Gβ5L, cloned by RT-PCR from retinal RNA, are shown. Nucleotide positions are given on the right side of the sequence. The initiator methionine utilized in the 39-kDa Gβ5 protein is circled.
sequences present in brain G protein. In addition, genomic sequencing revealed that the 5’ splice donor and acceptor dinucleotide sequence upstream of the Gβ5l coding region, no amplification of the 1,188-bp product was observed, even though this product was readily amplified from retinal RNA (data not shown). This result indicates that Gβ5l is not expressed in brain and, coupled with our earlier results regarding the expression pattern of Gβ5, defines Gβ5l as a retinal-specific gene product.

### Retinal Gβ5l, but Not Gβ5, Is Present in Rod Outer Segment Membranes

The observation that the 44-kDa Gβ5 protein is specifically lost from the retinas of mice that have undergone retinal degeneration suggests that Gβ5l is located in the photoreceptor cell layer. To characterize the retinal expression pattern of Gβ5l further, we exploited the observation that ROS can easily be separated from the inner segments and soluble retinal proteins by means of centrifugation through sucrose density gradients (19). We used the polyclonal antiserum CT215 to follow the distribution of the two Gβ5 proteins in these fractions and to compare it with the distribution of the β subunit of transducin, Gβ1, which is known to be localized to the outer segment membrane. The results of these experiments are shown in Fig. 5. Upon low speed (5,000 × g) centrifugation of gently homogenized bovine retina through 45% sucrose, the ROS membranes and soluble proteins remain in supernatant, while the inner segments and unbroken cells are found in the pellet. Both the short and long forms of Gβ5, as well as Gβ1, were found in this first supernatant fraction (S1). The ROS membranes can be separated from retinal soluble protein by centrifugation at 15,000 × g. At this point, the outer segment membranes are found in the pellet fraction and soluble proteins remain in the supernatant. When we further purified bovine ROS in this way, the short form of Gβ5, which is known to be localized to the outer segment membrane, was readily amplified from retinal RNA (data not shown). This result indicates that Gβ5l is not expressed in brain and, coupled with our earlier results regarding the expression pattern of Gβ5, defines Gβ5l as a retinal-specific gene product.

### Northern analysis of Gβ5l expression

Total RNA (40 μg) isolated from normal mouse retina (lane 1) and brain (lanes 2 and 3; two independent preparations) were hybridized with an oligonucleotide probe (corresponding to bases −1 to −43 of the mouse genomic DNA sequence shown in Fig. 3). Positions of the RNA size standards, in kb, are shown on the left.
membranes with the nonhydrolyzable guanine nucleotide, GTPγS, the 44-kDa Gβ3 protein continued to remain completely associated with the membrane. As expected, incubation of the ROS membranes with GTPγS effected the release of approximately 60% of Gβ1.

The 39-kDa Gβ3 Brain Protein Is Membrane-associated—Our observation that the retinal 39-kDa Gβ3 protein was essentially soluble and not associated with cell membranes was unexpected. We therefore examined the membrane association of the Gβ3 protein in mouse brain, and compared its distribution to that of the Gβ1 protein (Fig. 7). In contrast to our observations in retina, in brain both of the G protein β subunits were associated with cell membranes. At least 70% of the detectable Gβ3 protein in mouse brain was found in the membrane pellet (P), and the Gβ3 protein present in this fraction could not be extracted in the absence of detergents (data not shown). A solution of 1% cholate, which solubilized about 50% of the total membrane proteins, was able to solubilize nearly all of Gβ3 (compare supernatant and pellet fractions of the 1% cholate lane S1, crude ROS membranes; lane P1, crude ROS membranes; lane S2, supernatant 2 (soluble proteins); lane P2, crude ROS membranes; lane ROS, ROS membranes purified by sucrose density gradient centrifugation. The amount of the loaded protein was normalized to the volume of the total homogenate (5 μl or about 20 μg of total protein). Immunoblots were probed with antisera directed against Gβ3 (upper panel) or Gβ1 (lower panel).

Transient Expression of Gβ Proteins in COS-7 Cells—The Gβ3 proteins are members of a much larger multiprotein family termed WD repeat proteins. The β subunits of G proteins are able to form stable dimers with G protein γ subunits, and these Gβγ dimers are capable of regulating the activity of several effector enzymes, including the β2 and β3 isotypes of phosphoinositide-specific phospholipase C. In contrast, non-Gβ WD repeat proteins do not dimerize with Gγ subunits nor do they activate Gβγ effectors (26). To determine whether the Gβ3L protein was capable of dimerizing with Gγ subunits, we transiently co-transfected COS-7 cells with cDNA expression plasmids encoding Gβ3L and various Gγ subunits. The ability to form functional dimers was assessed by the ability of the co-expressed Gβ3L and Gγ subunits to activate a co-transfected Gβγ effector enzyme, PI-PLCβ2. Our initial experiments were complicated by the fact that Western blot analysis of transfected cell extracts showed that COS cells expressed both short and long forms of Gβ3 (Fig. 8A). This was apparently due to inappropriate initiation of translation at the second methionine codon (i.e., the initiator codon utilized in brain) in the transfected cells. To circumvent this problem, we altered the second methionine codon to encode alanine by site-directed mutagenesis. Western analysis of extracts prepared from COS-7 cells transfected with the mutant Gβ3L ([Ala43]Gβ3L) showed the presence of a single immunoreactive band that co-migrated with the 44-kDa retinal protein (Fig. 8B). We then asked whether [Ala43]Gβ3L could dimerize with various Gγ subunits and activate PI-PLCβ2. The results of one such co-transfection experiment are shown in Fig. 8B. Co-transfection of either Gβ3 or [Ala43]Gβ3L in combination with Gγ2 resulted in a 4–5-fold activation of PI-PLCβ2. Co-transfection of other Gγ subunits with either of the Gβ3L species resulted in less activation of the enzyme. In each case, the stimulation of PI-PLCβ2-catalyzed inositol phosphate release was dependent on the presence of functional Gβγ dimers. Replacement of the Gγ2 protein with the [Leu71]Gγ2 mutant (Fig. 8B, Gγ2*) in which the site of isopenylation (Cys71) was removed by site-directed mutagenesis reduced enzyme stimulation by approximately 60% (Fig. 8B). These results for activation of PI-PLCβ2 by the 39-kDa Gβ3L are in agreement with our previous observations (7) and further demonstrate that the 44-kDa retinal Gβ3L also possesses the ability to dimerize with Gγ proteins and activate a Gβγ effector enzyme. An additional immunoreactive protein, with an apparent molecular mass of 32–35 kDa, was observed in extracts of COS-7 cells transfected with the Gβ3L expression vectors (Fig. 8A). This may represent premature translational termination or carboxyl-terminal degradation of the Gβ3L protein. However, since removal of COOH-terminal sequence from
J. Carbrera and V. Z. Slepak, unpublished observations.

The activity of PI-PLC is part of the G protein signaling system. These experiments showed that the stimulation of PI-PLC by Gβγ subunits or [Ala43]Gβ5Lγ1 was decreased 71% and 59%, respectively, in the presence of Gαq due to apparent suppression of signaling by the Gβγ dimer as a result of heterotrimer formation (Fig. 8C).

**DISCUSSION**

The Gβγ dimers of heterotrimeric G proteins have emerged in recent years as important regulators of ion channels, adenylyl cyclases, phosphoinositide-specific phospholipases, G protein-coupled receptor kinases, and mitogen-activated protein kinase cascades (2, 3). Therefore, diversity within the Gβ subunit family has important implications for our understanding of cellular control of second messenger systems. With this in mind, we were interested to observe the presence of an additional protein immunologically related to the G protein β subunit, Gβ5, in crude membrane preparations of mouse retina. To understand the molecular basis for this additional Gβ-related protein, we have cloned, by RT-PCR, the cognate cDNA. Analysis of cDNA and genomic clones comprising part of the murine Gβ locus revealed that the longer Gβ5 isof orm, Gβ5L, results from retinal-specific utilization of a 5’ exon located approximately 3 kb away from the initiator codon employed in brain Gβ5 expression. During the sequenc ing of Gβ5 cDNA and genomic clones, we detected two differences, at positions 516 and 534, from our previously published Gβ5 expression. During the sequencing of Gβ5L with the second methionine codon, as we would expect that retinal Gβ5L would be approximately 49 kDa, instead of the observed 44 kDa.

Several unanswered questions remain concerning the basis of expression of the multiple Gβ5 isof orms. For example, the available data suggest, but we have not confirmed, that transcription of both Gβ5L and Gβ5 is directed by elements contained within the first intron, but that the two isof orms utilize different transcriptional start sites. It remains to be determined whether the expression of the 39-kDa Gβ5 protein in retina is due to inappropriate initiation at the second methionine codon, as we observed in our COS-7 cell transfection experiments. Additionally, we still do not understand the basis for the two mRNA species seen on Northern analysis of retinal and brain RNA. Continued investigation into these phenomena may shed some light on mechanisms of retinal-specific gene expression.

In the retina, the 44-kDa Gβ5 protein, but not the 39-kDa Gβ5L protein, is associated with photoreceptor cell membranes. Because Gβ5L is expressed only in retina and co-fractionates with rhodopsin-containing membranes, it is tempting to speculate that it is likely to be somehow involved in photoreception. However, the expression levels of Gβ5 and Gβ5L are much lower than that of the transducin Gβ subunit (0.02–0.04% of total retinal and brain protein). The fact that Gβ5L cannot be eluted from the rod outer segment membrane by incubation with GTPγS suggests that it may have a role there distinct from Gβ1. In this regard, it is interesting to recall that a fraction of

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3 A. Katz and M. I. Simon, unpublished observations.

4 J. Carbrera and V. Z. Slepak, unpublished observations.
transducin α subunit, which binds GTPγS but does not dissociate from the rod membrane, has been described (27). Alternatively, it is possible that Gβ5L is associated primarily with other Gβγ-binding proteins present in photoreceptors, such as phosducin, or is involved in regulation of downstream effectors, such as retinal PI-PLC (28).

The Gβ5L protein is substantially larger than mammalian Gβ subunits identified previously. Indeed, the presence of an extended NH2-terminal domain provides a superficial resemblance to the yeast S. cerevisiae Gβ subunit, ste4 (29), and to certain members of the WD repeat multiprotein family (26), although this region of Gβ5L has no homology to these proteins. Nevertheless, the results of our COS-7 cell co-transfection experiments clearly show that Gβ5L is a bona fide G protein β subunit and interacts with other members of the heterotrimeric G protein signal transduction cascade in a manner indistinguishable from Gβ3. This observed similarity in behavior is not unexpected since Gβ3L and Gβ5L share all residues involved in Gγ and Go contact (30, 31). The fact that Gβ3L and Gβ5L display a marked preference for Gγ1 in the PI-PLC activation assay is perhaps somewhat surprising in view of the recent report that Gβ3L dimerizes rather poorly with Gγ2 in a yeast two-hybrid assay (32). However, it should be remembered that our assay is a functional one and does not quantitate dimer formation.

An interesting question concerns the biochemical differences we observed for the 39-kDa Gβ1 protein. Even though the 39-kDa Gβ1 protein is expressed in both brain and retina, these proteins can be distinguished on the basis of their co-fractionation from the rod membrane, has been described (27). Nevertheless, it seems reasonable to conclude that the 42-kDa Gβ5L protein is associated with membrane localization of the Gβ5L protein, resulting in membrane localization of the Gβ5L dimer (reviewed in Ref. 33). It is thought that dimerization with a Gγ protein is essential for Gβ subunit stability and function. Some of the observed solubility of retinal Gβ3 may be due to differences in the prenyl moiety associated with the gamma subunit since brain Gγ proteins are geranylgeranylated, while retinal Gγ1 is farnesylated (33). However, this cannot completely account for the lack of membrane association of the 39-kDa Gβ5L, since at least 50% of retinal Gβ3 is found in the membrane pellet (Fig. 5). Perhaps retinal Gβ3 is complexed with a non-isoprenylated Gγ protein.

A retinal endoproteolytic activity has been identified that is capable of producing a non-isoprenylated form of Gγ1 (34). The difference in localization of Gβ3 and Gβ3L within the retina cannot be totally ascribed to differences in dimerization with Gγ proteins, however, since on the basis of the COS-7 cell co-transfection assay, both forms dimerize with Gγ proteins. Nevertheless, it seems reasonable to conclude that the 42-amino acid NH2-terminal extension present in Gβ3L is sufficient to allow efficient targeting of Gβ3L to the outer segment membrane. This protein sequence may therefore prove to be a useful tool to study protein compartmentalization in the retina. Purification of native Gβ3 and Gβ3L proteins from retina should provide insight into the protein-protein interactions in which these subunits participate in vivo.

Gγ proteins reported to be present in bovine retina include Gγ1 and Gγ3, which were found in rod and cone cell outer segments, respectively, and Gγ5, which was detected in the plexiform layers (4). In addition, a novel cone-specific Gγ subunit (Gγb) has been recently identified (35). Preliminary immunohistochemical analysis of mouse retinal sections with anti-serum CT215 indicates that Gγb immunoreactivity is present in the plexiform layers, similar to the reported distribution for bovine Gγb. The absence of the Gβ5L protein from the inner segment fraction of bovine retina (Fig. 5) suggests that this immunoreactivity may be due to the 39-kDa Gβ5L protein, although other explanations, including species differences, are certainly possible. More detailed immunohistochemical and in situ hybridization experiments currently in progress should allow definitive sublocalization of the 39- and 44-kDa Gβ5L proteins in retina and brain.

Acknowledgments—We thank our colleagues in the Simon laboratory for materials and N. Gautam, D. Oprian, and P. Casey for helpful discussions. Automated DNA sequencing was performed by S. Marsh, R. Colaoco, and B. Perry.

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