In comparison with the α subunit of G proteins, the role of the β subunit in signaling is less well understood. During the regulation of effectors by the βγ complex, it is known that the β subunit contacts effectors directly, whereas the role of the β subunit is undefined in receptor-G protein interaction. Among the five G protein β subunits known, the β1 subunit type is the least studied. We compared the ability of βγ complexes containing β4 and the well characterized β1 to stimulate three different effectors: phospholipase C-β2, phospholipase C-β3, and adenylyl cyclase type II. β1γ2 and β1γ2 activated all three of these effectors with equal efficacy. However, nucleotide exchange in a G protein constituting α3β1γ2 was stimulated significantly more by the M2 muscarinic receptor compared with αα3β4γ3. Because α3 forms heterotrimers with β1γ2 and β1γ2 equally well, these results show that the β subunit type plays a direct role in the receptor activation of a G protein.

The G protein βγ complex regulates the activity of a diverse set of effectors, including phospholipases, adenylyl cyclases, and ion channels (1). There is evidence that the β subunit in the complex interacts directly with effectors (2–5). There are five β subtypes (β1–β5) as well as an alternatively spliced version of β2 (known as β2-long) (6–11). β1–β5 share over 80% identity with one another, whereas β2 shares only ~50% identity with the other β subunits (12). The divergence between β2 and the other β subunits is consistent with the functional differences between β2 and β5 observed in effector regulation in a variety of systems (4, 13, 14). The high sequence similarity of β1–β4 suggests that their functions are conserved. Although some experiments indicated little difference in effector modulating capability among these β subunit types, other experiments suggest otherwise. The G protein–coupled receptor kinase GRK3 binds βγ complexes consisting of β1, β2, and β3, but only β1 and β2 bind to the related kinase GRK2 (15). Other results indicate the selective mediation of cross-talk between G proteins and protein kinase C modulation of N-type channels by the β1 subunit type (16).

Experiments focusing on the specific role of individual G protein subunit types have provided evidence for a certain level of selectivity in the interaction of α subunit types with receptors (17). Evidence for similar selectivity of interaction between γ subunit types and receptors also exists (18–20). In contrast there is limited evidence for β subunit type selectivity in receptor interaction. Whole-cell experiments using antisense oligonucleotides directed against specific β subunit cDNAs selectively disrupted signaling from particular receptors (21). Although the selective interaction of β subunit types with receptors could give rise to this result, such selectivity has not been shown so far.

Among the five β subunits, β1 is the least studied. Its role in effector regulation and receptor interaction remains unclear. To examine its effect on the βγ regulation of effectors and G protein interaction with a receptor, we expressed purified recombinant β1γ2 and β1γ2 complexes and compared their abilities to regulate the activity of three different G protein effectors: PLC-β2, PLC-β3, and adenylyl cyclase type II (AC-II). Next, we examined the abilities of heterotrimers made up of α3β1γ2 and αα3β4γ3 to couple to the M2 muscarinic receptor in a reconstituted system containing purified M2 and G protein subunits. The results indicate that in comparison to β1, the β4 subunit does not differentially modulate effector function but does differentially affect the receptor activation of a G protein. These results indicate that the particular β subunit type present in a heterotrimer influences the effectiveness of the receptor activation of that G protein. Because these experiments were performed with purified receptor and G protein subunits, these results also indicate that the β subunit plays a direct role in the receptor activation of a G protein.

EXPERIMENTAL PROCEDURES

Materials—PIP2 and phosphatidyethanolamine were obtained from Avanti Polar Lipids. [3H]PIP2 was from PerkinElmer Life Sciences. Where a source is not stated, reagent was obtained from Sigma.

Construction of Recombinant Baculoviruses—β3-Expressing baculovirus was constructed using the Bac-to-Bac baculovirus expression system from Life Technologies, Inc. Because sequencing revealed a very long cytosine-rich region at the 5’-end of the original β3 cDNA (kind gift from Dr. M. I. Simon, California Institute of Technology), a synthetic oligonucleotide cassette made up of DX19103 (5’-GCCCGCATGACGGAGCTGGAGCAG) and DX19104 (5’-CTGCTCCAGTCGGCCTATGC) was constructed to replace this region. The cassette encodes the first six amino acids of β3. It was introduced into the β3pFastBac construct using NotI/PvuII sites. The methods used for the construction of baculoviruses expressing His-PLC-β2, His-PLC-β3, adenylyl cyclase type II, the G protein β1 subunit, and the G protein His-γ1 subunit have been published (22–26).

Expression and Purification of βγ Complexes from SF9 Insect Cells—SF9 cells were maintained in suspension in IPL-41 medium (Life Tech-

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The abbreviations used are: PLC, phospholipase C; SF9 cells, Spodoptera frugiperda cells; AC-II, adenylyl cyclase type II; PIP2, phosphatidylinositol 4,5-bisphosphate; CHAPS, 3-[3-cholamidopropyl]dimethylammonium]-1-propanesulfonic acid; GTPγS, guanosine 5’-O-(3-thiotriphosphate); RGS, regulator of G protein signaling.
Procedures. 10 ng of purified PLC-β2 (A) or 5 ng of purified PLC-β3 (B) were assayed for stimulation with the indicated concentrations of β1γ2 or β2γ2 in a 1:1 mixture containing [3H]PIP2, as described under Experimental Procedures. Basal activity is defined as activity in the absence of the βγ complex. The fold stimulation over basal activity is shown. [3H]phosphatidylinositol 1,4,5-trisphosphate production was measured by scintillation counting. Data in A are the means of three independent experiments performed in duplicate (+ S.E.). When examined with the unpaired t test, differences in the activities between β1γ2 and β2γ2 at all concentrations were not statistically significant. B is representative of two independent experiments; each experiment was done in duplicate.

**Fig. 1. Expression and purification of βγ complexes.** A, immunoblot analysis of βγ protein expression in Sf9 cells with the βγ-specific antibody (B4-2). Cells were infected with the βγ baculovirus for varying amounts of time and then lysed as described under “Experimental Procedures.” 10 μg of proteins from each sample were loaded. Lane 1, uninfected cells; lane 2, cells infected for 40 h; lane 3, 48 h; lane 4, 64 h; and lane 5, 72 h. B, Coomassie Blue staining of purified G protein recombinant β1γ2 and β2γ2 subunits separated by SDS-polyacrylamide gel electrophoresis on a 12% gel. 

**Fig. 2. β1γ2 and β2γ2 stimulation of PLC-β2 and PLC-β3 activities.** 1 ng of purified PLC-β2 (A) or 5 ng of purified PLC-β3 (B) were assayed for stimulation with the indicated concentrations of β1γ2 or β2γ2 in a 1:1 mixture containing [3H]PIP2, as described under Experimental Procedures. Basal activity is defined as activity in the absence of the βγ complex. The fold stimulation over basal activity is shown. [3H]phosphatidylinositol 1,4,5-trisphosphate production was measured by scintillation counting. Data in A are the means of three independent experiments performed in duplicate (+ S.E.). When examined with the unpaired t test, differences in the activities between β1γ2 and β2γ2 at all concentrations were not statistically significant. B is representative of two independent experiments; each experiment was done in duplicate.

**G Protein β Subunit Types Interact with Muscarinic Receptor**

GTP Hydrolysis—Heterotrimeric G protein subunits were formed by incubating 10 μM concentrations of α and βγ subunits on ice for 30 min in a buffer containing 25 mM Hepes, 100 mM NaCl, 2 mM MgCl2, 0.1 mM EDTA, 10 μM GDP, and 1 mM dithiothreitol. The reconstituted M2 was incubated with heterotrimeric Gα on ice for 30 min with or without RGS4 protein (kind gift from Dr. M. Linder, Washington University). The receptor-G protein complex was then mixed with carbocich or water before γ-[32P]GTP was added to initiate the reaction at 25 °C. The reaction mixture contained different concentrations of G protein, 1 μM receptor, 0.2 μM GTP, 5 μM GDP, and 1 mM carbocich or 200 μM GTP in a buffer of 20 mM Hepes (pH 8.0), 100 mM NaCl, 2 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.5 μM bovine serum albumin. RGS4 concentration was 0.1 μM. Excess 200 μM GTP (1000× more than γ-[32P]GTP) instead of carbocich serves as a nonspecific control of GTP hydrolysis. Aliquots taken at the indicated time points were diluted with 0.5 ml of ice-cold buffer containing 5% activated charcoal and 50
Fig. 3. \( \beta_G4 \gamma_2 \) and \( \beta_G1 \gamma_2 \) stimulation of adenyl cyclase type II. An adenyl cyclase assay was performed as described under "Experimental Procedures." Various concentrations of \( \beta \gamma \) complex were used as indicated. Activities are expressed as fold stimulation over control, which was measured in the presence of 100 nM GTP-S-bound \( \alpha_s \) without the \( \beta \gamma \) complex. Data shown are the means of two independent experiments performed in duplicate (± S.E.). Differences in the activities elicited by \( \beta_G1 \gamma_2 \) and \( \beta_G4 \gamma_2 \) were not statistically significant. The plot shown is representative of at least three other independent experiments performed in duplicate.

mm K\(_2\)PO\(_4\). Samples were centrifuged, and radioactivity in supernatants was quantified by scintillation counting.

Measurement of G Protein Heterotrimer Formation—To measure the formation of heterotrimer, a fixed concentration of \( \beta \gamma \) complex was mixed with various concentrations of \( \alpha_s \) subunit. Initially, 360 nM \( \beta \gamma \) complex was incubated with increasing concentrations of the \( \alpha_s \) subunit (11.25, 22.5, 45, 90, 180, and 360 nM) in ice for 30 min in a 10-μl buffer containing 20 mM Hepes (pH 8.0), 100 mM NaCl, 2 mM MgCl\(_2\), 0.1 mM EDTA, 1 mM dithiothreitol, and 0.5 mg/ml bovine serum albumin. Five μl of this mixture were then diluted 10 times to a total of 50 μl of buffer containing 50 mM Na-Hepes (pH 7.2), 3 mM EGTA, 1 mM EDTA, 5 mM MgCl\(_2\), 100 mM NaCl, and 1 mM dithiothreitol. 10 μl of this diluted sample containing \( \beta \gamma \) and various concentrations of the \( \alpha_s \) subunit were then added to a total of 60 μl of PLC reaction buffer containing [\(^{3}\)H]PIP2 substrate and enzyme for determining the PLC-\( \beta \gamma \) activity as described above (6 nM final concentration of \( \beta \gamma \) complex).

RESULTS

\( \beta_G4 \gamma_2 \) and \( \beta_G1 \gamma_2 \) Protein Expression and Purification—To study the function and properties of the G protein \( \beta \) subunit, we constructed a baculovirus expressing \( \beta \)4. To confirm the viral expression of \( \beta \)4, SF9 cells were infected with this virus for varying lengths of time; cells were then harvested, lysed, and checked for protein expression by immunoblotting with the B4-2 antibody against the \( \beta \)4 protein (Fig. 1A). To produce \( \beta_G1 \gamma_2 \) dimers, we simultaneously co-infected SF9 cells with the \( \beta \)4 virus and a virus expressing a His-tagged \( \gamma_2 \) subunit. \( \beta_G2 \gamma_2 \) was purified by nickel-nitrilotriacetic acid chromatography. \( \beta_G1 \gamma_2 \) was expressed and purified using a similar approach. \( \beta \)4 consistently runs with slightly faster mobility than does \( \beta \)1 (Fig. 1B). This is consistent with a report from Asano et al. (35), who studied the native \( \beta \)4 protein expressed in bovine tissues. We confirmed that the purified \( \beta_G2 \gamma_2 \) and \( \beta_G1 \gamma_2 \) proteins contain the same concentration of detergents by using thin layer chromatography with the appropriate detergent standards (data not shown).

Stimulation of Effectors by \( \beta_G4 \gamma_2 \) and \( \beta_G1 \gamma_2 \)—To search for potential differences between \( \beta \)4 and \( \beta \)1 in effector regulation, we focused on three major effectors regulated by G protein \( \beta \gamma \) complexes: PLC-\( \beta \), PLC-\( \beta \gamma \), and AC-II. The effect of \( \beta \gamma \) complex on these enzymes was examined. Fig. 2A shows the activation of purified PLC-\( \beta \) by \( \beta_G1 \gamma_2 \) and \( \beta_G4 \gamma_2 \) complexes. Consistent with previous reports where brain \( \beta \gamma \) was tested (36), both \( \beta \gamma \) complexes stimulate PLC-\( \beta \) more than 4-fold above basal activity with similar effectiveness. The PLC-\( \beta \)2 stimulatory properties of both \( \beta \gamma \) complexes are thus essentially identical. Although PLC-\( \beta \)2 and PLC-\( \beta \)3 are isozymes that are both stimulated by the G protein \( \beta \gamma \) complex, there is evidence that the residues in the \( \beta \) subunit that contact these two enzymes are distinct (37). This raised the possibility that although the two \( \beta \gamma \) complexes showed little difference in the activation of PLC-\( \beta \), they might interact differentially with PLC-\( \beta \). We therefore examined the stimulation of PLC-\( \beta \) by \( \beta_G1 \gamma_2 \) and \( \beta_G4 \gamma_2 \). Both \( \beta \gamma \) complexes activate PLC-\( \beta \) 20-fold over basal activity (Fig. 2B). The higher stimulation of PLC-\( \beta \) compared with PLC-\( \beta \) is consistent with previous reports (36). As in the case of PLC-\( \beta \), the effectiveness with which \( \beta_G1 \gamma_2 \) and \( \beta_G4 \gamma_2 \) activate PLC-\( \beta \) is similar. Because detergents in the \( \beta \gamma \) preparations may themselves activate PLC-\( \beta \) (38), we also tested boiled preparations of \( \beta_G1 \gamma_2 \) and \( \beta_G4 \gamma_2 \). Stimulation from boiled samples was minimal. Moreover, boiled samples of \( \beta_G2 \gamma_2 \) also showed essentially the same profile of activity, indicating that detergent concentrations and any other nonprotein stimulators of PLC activity are present at equivalent levels (data not shown).

AC-II is stimulated by the G protein \( \beta \gamma \) complex in the presence of the activated \( \alpha_s \) subunit. To test whether \( \beta_G1 \gamma_2 \) and \( \beta_G4 \gamma_2 \) stimulate this enzyme differentially, we examined AC-II stimulation in the presence of GTP-S-bound \( \alpha_s \). SF9 cell membranes expressing AC-II were used as a source of the enzyme (29). In the presence of GTP-S-bound purified \( \alpha_s \), \( \beta_G1 \gamma_2 \) and \( \beta_G4 \gamma_2 \) activate AC-II significantly over the basal level. Fig. 3 shows that, as in the case of PLC-\( \beta \), the extent of maximal stimulation (8-fold) by \( \beta_G2 \gamma_2 \) or \( \beta_G1 \gamma_2 \) and the effectiveness of both \( \beta \gamma \) complexes in stimulating AC-II are similar.

M2 Receptor Activation of Go, Containing the \( \beta \)1 and \( \beta \)4 Subunits—The receptor stimulation of a G protein can be measured as GTP-S binding to the \( \alpha \) subunit or as GTPase activity of the \( \alpha \) subunit in the presence of an agonist. We have recently determined that in a reconstituted system containing the purified M2 muscarinic receptor and G protein subunits, the GTPase assay is much more sensitive and allows us to measure G protein activation with a relatively low concentration of receptor (1 nm) at ratios of receptor:G protein that are close to 1:1 (34). These conditions are potentially closer to the dissociation constant for M2 interaction with \( \alpha_s \) (which has not been determined) than to the ratio of receptor to G protein used in the less sensitive GTP-S assay. Subtle differences in the receptor interaction of G protein heterotrimers are more likely to be revealed under the conditions used in the GTPase assay. This notion is borne out in the analysis of the G protein \( \gamma \) subunit interaction with M2 where differences in coupling were detected using these conditions (34).

The M2 stimulation of \( \alpha_s \) \( \beta_G1 \gamma_2 \) and \( \alpha_s \) \( \beta_G4 \gamma_2 \) was examined by assaying GTPase activity in the presence of RGS4. The RGS4 protein, a GTPase-activating protein for the Go family, in-
creases the pool of G protein heterotrimers available to the receptor and considerably increases the sensitivity of the reaction (by ≈10-fold). As expected, the addition of carbachol increases the GTP hydrolysis rate significantly (Fig. 4). More importantly, the M2-activated GTP hydrolysis of αβγ2 was ≈200% higher than that of αβ1γ2. This difference was consistently observed at all G protein concentrations tested (Table I).

Statistical analysis with the unpaired t test indicated that these differences are significant (p < 0.05). To further verify these data, two independent preparations of each purified βγ complex containing β1 or β4 were examined again. These preparations provided similar results. As mentioned before, the concentration of detergent (CHAPS) in the purified β1γ2 and β4γ2 stocks was determined using thin layer chromatography (data not shown). This analysis indicated that the detergent concentrations in the samples were similar and were not the cause for the differential receptor activation. Furthermore, when the ability of the β1γ2 and β4γ2 complexes were examined in PLC-β3 activation assays, the results indicated that the functional proportions in the stocks of both subunit complexes were the same. Finally, the possibility that these differences arose from the differential interaction of the β subunit types with the RGS4 protein was tested by assaying the M2-stimulated GTPase activity in the absence of the RGS protein. Again αβ1γ2 was consistently 2–3-fold more active than αβ4γ2 (Table I), indicating that the differential stimulation of GTPase activity resulted from receptor rather than RGS protein interaction.

Efficiency of Heterotrimerization of β1γ2 and β4γ2 Subunits with αo—Because receptors interact effectively only with the heterotrimer and not with the individual subunits, the difference observed in the M2 receptor-stimulated activity between αβ1γ2 and αβ4γ2 could be attributable to the differential heterotrimer formation between αo and these two βγ complexes. The residues in β1 and β4 that contact the α subunit are conserved, indicating that the β1 and β4 affinity for αo is likely to be the same (39). However, it was possible that heterotrimerization was differentially affected by divergent residues in β1 and β4 that were located at a distance from residues that contacted the α subunit. To examine this possibility, we used a recently developed assay for measuring G protein heterotrimer formation (34). This assay is based on evidence that the βγ complex has overlapping sites for binding the α subunit and the PLC-β3 enzyme (40). Thus heterotrimerization prevents βγ complex interaction with PLC-β3, leading to the inhibition of the βγ complex-stimulated PLC-β3 activity. Because the assay is sensitive, it can be used to examine the αo-βγ interaction at the same subunit concentrations (1–10 nM) used in the M2-stimulated GTPase assays above. In contrast, the ADP-ribosylation assay that has been used extensively in the past is less sensitive (requiring more than 1 μM subunits), and in addition, it is complicated by the lack of knowledge regarding the mechanistic basis of the βγ enhancement of the α subunit.

| Composition | 1 nMα | 2 nMα | 4 nMα | 6 nMα |
|-------------|-------|-------|-------|-------|
| αβ1γ2 + RGS4 | 0.51 ± 0.04 | 0.98 ± 0.07 | 2.21 ± 0.11 | 2.91 ± 0.16 |
| αβ4γ2 + RGS4 | ND* | ND | ND | ND |
| αβ1γ2 | ND* | ND | 0.22 ± 0.011 | 0.48 ± 0.005 |
| αβ4γ2 | ND | ND | 0.37 ± 0.004 | 0.98 ± 0.031 |

*Heterotrimers (αβγ) contain equimolar concentrations of each subunit. For instance, 1 nM αβ1γ2 contained 1 nM α and 1 nM β1γ2.

DISCUSSION

Because the G protein βγ complex is known to interact directly with and modulate various effectors and the β subunit is known to contact effectors, we first compared the relative abilities of the β1γ2 and β4γ2 complexes to stimulate three common effectors regulated by the βγ complex: PLC-β2, PLC-β3, and AC-II. Both βγ complexes activated each of the effectors with similar potency. This observation is consistent with the conservation of β1 and β4 of 93% of similar amino acids (Fig. 6A). It is unclear whether this result indicates that residues not conserved between β1 and β4 play no significant role in the regulation of effectors examined here. Comparing these results with previous mutational studies of β1, which implicate particular residues in PLC-β regulation, does not resolve this question. β subunit mutants analyzed in three different studies (40–42) did not involve residues that are divergent between β1 and β4. In another study (43), several residues were mutated simultaneously. It is therefore difficult to interpret these results in terms of the divergence in the β1/β4 primary structures. Among the few single residues that were mutated in this study, Asp-303 is the only one that is not conserved between β1 and β4 (Fig. 6A). This mutant β1γ complex stimulated PLC-β2 nor-

ADP-ribosylation by pertussis toxin. As shown in Fig. 5, the βγ complex-stimulated PLC-β activity is inhibited in a dose-dependent manner by the αo subunit. The αo concentration dependence of this inhibition of β1γ2 and β4γ2-stimulated PLC β activity is similar, indicating that both complexes form a heterotrimer with αo with equal effectiveness.
mally. Although very limited, this result is consistent with results presented here.

It has been known for many years that the \( \beta \gamma \) complex is essential for heterotrimeric G protein interaction with a receptor (44). The reasons for this requirement have been less clear. There is increasing evidence to support the interaction of the C-terminal domain of the \( \beta \) subunit with a receptor. This evidence comes from studies of rhodopsin-G \( \alpha \) coupling using peptides and mutant \( \gamma \) subunits (45, 46). It is also supported by the ability of a peptide specific to the \( \beta_4 \) subunit type but not the \( \beta_7 \) or \( \beta_12 \) subunit type to inhibit muscarinic receptor-mediated signaling in superior cervical ganglion neurons (20). More recently, the \( \gamma \) subunit type in a heterotrimer has been shown to influence the M2 receptor-stimulated nucleotide exchange (34). Here we used the same assay to detect a consistent 2–3-fold difference in M2-stimulated GTP hydrolysis between \( \alpha \beta_4 \gamma_2 \) and \( \alpha \beta_1 \gamma_2 \). Because these experiments were performed in the presence of an RGS protein, the difference in activity reflects a difference in receptor-stimulated nucleotide exchange. The measurement of heterotrimer formation between \( \alpha \beta_4 \gamma_2 \) or \( \beta_1 \gamma_2 \) showed that both heterotrimers form with equal effectiveness and ruled out the possibility that the difference in receptor-stimulated nucleotide exchange arose from differences in heterotrimer formation. Receptor-stimulated GTPase assays performed in the absence of the RGS protein showed that the differences arose at the site of receptor interaction and not from differential interaction with the RGS protein.

The differences in receptor-stimulated activity between \( \alpha \beta_4 \gamma_2 \) and \( \alpha \beta_1 \gamma_2 \) therefore indicate that the distinct primary structures of \( \beta_4 \) and \( \beta_1 \) influence the interaction of the heterotrimer with the receptor. Evidence that the \( \gamma \) subunit interacts with receptors and previous evidence that the \( \alpha \) subunit C and N termini interact with receptors (47) help identify the surface

Figure 6. Differences in the amino acid sequences of the \( \beta_1 \) and \( \beta_4 \) subunits mapped on the \( \beta \gamma \) complex three-dimensional structure. A. Primary structures of \( \beta_4 \) and \( \beta_1 \) subunits with the differences highlighted. Residues located in the putative receptor-interacting surface of the \( \beta \gamma \) complex are numbered. Residues in the other \( \beta \) subunit types at these loci are shown. (\( \beta \) subunit types from top to bottom are \( \beta_2 \), \( \beta_3 \), and \( \beta_5 \).) Arrows indicate \( \beta \) strands in the folded \( \beta \) subunit (panel B). Four \( \beta \) strands make up a sheet. Sheets are denoted as lines labeled S1–S7. B. Structure of the G protein subunit complex (\( \alpha \beta_1 \gamma_1 \) from Lambright et al. (50)). Dark gray, \( \alpha \) subunit; light gray, \( \beta \) subunit; and black, \( \gamma \) subunit. Open circles denote residues that are nonconservative changes between the \( \beta_1 \) and \( \beta_4 \) subunit amino acid sequences. The positions of these residues in the primary structure of \( \beta_1 \) and \( \beta_4 \) are: residue 1, –31; residue 2, –35; residue 3, –37; residue 4, –39; residue 5, –302; residue 6, –303; and residue 7, –305. In the phosducin-\( \beta \gamma \) complex the prenyl group, farnesyl (C-15), is buried in the pocket between \( \beta \) sheets S6 and S7 (49). Prepared with Ras Top 1.3 by P. Valadon.
of the G protein that contacts the receptor (Fig. 6B). Inspection of the amino acid sequences of the \( \beta_1 \) and \( \beta_3 \) subunits indicates differences that are distributed over the sequence (Fig. 6A). For these differences to play a role in receptor interaction, they most likely need to be accessible to the receptor and therefore located on the outer surface of the molecule. The location of the different amino acids between the \( \beta_1 \) and \( \beta_3 \) subunits on the three-dimensional structure of the \( \beta \) subunit indicates that two clusters of residues (31–39 and 302–305) are located on parallel strands of the \( \gamma \) subunit, as shown in Fig. 6B, although the two clusters are far apart in the primary structure, located toward the N and C termini of the \( \beta \) subunit (Fig. 6A). These residues are on the outer surface of the molecule. Most strikingly, these residues are on the surface that has been inferred to contact the receptor; note the location of the C termini of the \( \alpha \) subunit and the \( \gamma \) subunit (Fig. 6B). Finally, several of these residues show divergence between the various \( \beta \) subunit types (Fig. 6A).

Antisense oligonucleotides specific to \( \beta_1 \) and \( \beta_3 \) have previously been shown to selectively inhibit somatostatin and muscarinic M4 receptor-mediated Ca\(^{2+} \) channel activity (21). The precise point in the signaling pathway that was perturbed by the introduction of the oligonucleotides has not been elucidated so far. Inferences about the relationship between those results and the differential stimulation of \( \alpha \), in the presence of \( \beta_1, \beta_3 \) cannot be drawn both for this reason and because the physiological effect of the differential M2 stimulation of \( \alpha_{12} \delta \gamma_2 \) versus \( \alpha_{13} \delta \gamma_2 \) is not known.

The recent crystal structure determination of the inactive form of rhodopsin indicates that the intracellular portion of the receptor spans a little over 40 Å. It is unclear whether the activated forms of other receptors will expose intracellular receptor spans a little over 40 Å. It is unclear whether the form of rhodopsin indicates that the intracellular portion of the receptor will expose intracellular protein interaction with the A1 adenosine receptor as measured by the ability of the G proteins to stabilize the high affinity binding state of A1 receptors. It is unclear whether the GDPase assays used here will detect differences between the \( \beta \) subunit mutants and wild type used in that study.

Overall, the importance of these results is that the differential receptor interaction of rhodopsin indicates a direct role for the \( \beta \) subunit in receptor activation of a G protein.

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