

# A Peptide Core Motif for Binding to Heterotrimeric G Protein $\alpha$ Subunits\*

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William W. Ja<sup>†1</sup>, Anirban Adhikari<sup>§</sup>, Ryan J. Austin<sup>†</sup>, Stephen R. Sprang<sup>¶</sup>, and Richard W. Roberts<sup>‡2</sup>

From the <sup>†</sup>Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125 and the <sup>§</sup>Department of Biochemistry and Molecular Biophysics Graduate Program and <sup>¶</sup>The Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75390

Recently, *in vitro* selection using mRNA display was used to identify a novel peptide sequence that binds with high affinity to  $G\alpha_{i1}$ . The peptide was minimized to a 9-residue sequence (R6A-1) that retains high affinity and specificity for the GDP-bound state of  $G\alpha_{i1}$  and acts as a guanine nucleotide dissociation inhibitor (GDI). Here, we demonstrate that the R6A-1 peptide interacts with  $G\alpha$  subunits representing all four G protein classes, acting as a core motif for  $G\alpha$  interaction. This contrasts with the consensus G protein regulatory (GPR) sequence, a 28-mer peptide GDI derived from the GoLoco ( $G\alpha_{i/o}$ -Loco interaction)/GPR motif that shares no homology with R6A-1 and binds only to  $G\alpha_{i1-3}$  in this assay. Binding of R6A-1 is generally specific to the GDP-bound state of the  $G\alpha$  subunits and excludes association with  $G\beta\gamma$ . R6A- $G\alpha_{i1}$  complexes are resistant to trypsin digestion and exhibit distinct stability in the presence of  $Mg^{2+}$ , suggesting that the R6A and GPR peptides exert their activities using different mechanisms. Studies using  $G\alpha_{i1}/G\alpha_s$  chimeras identify two regions of  $G\alpha_{i1}$  (residues 1–35 and 57–88) as determinants for strong R6A- $G\alpha_{i1}$  interaction. Residues flanking the R6A-1 peptide confer unique binding properties, indicating that the core motif could be used as a starting point for the development of peptides exhibiting novel activities and/or specificity for particular G protein subclasses or nucleotide-bound states.

Heterotrimeric guanine nucleotide-binding proteins (G proteins), composed of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits, mediate signaling from transmembrane receptors (GPCRs)<sup>3</sup> to a wide variety of effectors (1, 2). In the inactive state, intracellular  $G\alpha$ -GDP is tightly bound to  $G\beta\gamma$  to form a  $G\alpha\beta\gamma$  heterotrimer. Activation of a GPCR results in GDP exchange with GTP in the  $G\alpha$  subunit, concomitant dissociation of  $G\beta\gamma$ , and subsequent signal transduction through  $G\alpha$ -GTP and/or  $G\beta\gamma$ . The inherent guanosine triphosphatase (GTPase) activity of  $G\alpha$ , which is accelerated by various GTPase-activating proteins, returns the protein to the GDP-bound state, resulting in reassociation with  $G\beta\gamma$  and termination of signaling.

Approximately 50% of currently marketed drugs target cell surface-accessible GPCRs (3, 4). Drug discovery targeting intracellular G proteins directly is inherently difficult due to (i) the broad spectrum of signaling events mediated at the G protein level, (ii) the requirement that drugs must cross the cell membrane to reach intracellular G proteins, and (iii) the high sequence and structural similarities between G protein classes (5, 6). Nevertheless, a number of diseases have been attributed to aberrant G protein activity (7, 8), and direct

G protein ligands will provide new approaches and selectivities for drug treatment (5, 6, 9).

Selection methodologies facilitate the isolation of rare molecules with unique functions from large libraries (10, 11). Selections with combinatorial libraries have already been used to identify peptides that bind to various proteins in the G protein signaling cycle (9). We recently demonstrated that mRNA display, a selection technique where peptides are covalently attached to their encoded RNA, could be used to isolate  $G\alpha_{i1}$ -binding sequences (12). The dominant peptide from the selection, as well as a minimized, active 9-mer sequence (R6A-1), act as guanine nucleotide dissociation inhibitors (GDIs) and compete with  $G\beta\gamma$  for binding to  $G\alpha_{i1}$  (12).

Here, we examine the specificity of R6A peptides for binding to various *in vitro* translated  $G\alpha$  subunits. Surprisingly, the R6A-1 core motif interacts with  $G\alpha$  subunits representing all four G protein families. Binding of R6A-1 is generally specific for the GDP-bound state of each  $G\alpha$  subunit and appears to exclude heterotrimer formation with  $G\beta\gamma$ . Our findings suggest that the R6A-1 core motif peptide will be useful for the molecular design of  $G\alpha$  ligands with unique specificities.

## EXPERIMENTAL PROCEDURES

**Materials**—Human cDNA clones encoding various G proteins were obtained from the UMR cDNA Resource Center (www.cdna.org) in the pcDNA3.1+ vector (Invitrogen). The  $G\alpha$  subunits used were  $i1$ ,  $i2$ ,  $i3$ ,  $oA$ ,  $q$ ,  $s$  (short form), 12, and 15. All *in vitro* translated  $G\beta$  and  $G\gamma$  subunits refer to  $G\beta_1$  and N-terminal hemagglutinin (HA)-tagged  $G\gamma_2$ , respectively. Rat  $G\alpha_{i1}$ , bovine short form  $G\alpha_s$  and their chimeric constructs were generously provided by Prof. N. Artemyev (University of Iowa). DNA oligonucleotides were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA).

**Peptide/Protein Preparation**—The C-terminal amidated peptides GPR-biotin (TMGEEDFFDL LAKSQSKRLDDQRVDLAGQLRNSYAK, K = biocytin), L19 GPR (TMGEEDFFDLLAKSQSKRLDDQRVDLAGYK), R6A-1-biotin (DQLYWWEYLQLRNSYAK), R6A-1 (DQLYWWEYL), and R6A-4 (SQTKRLLDDQLYWWEYL) were synthesized and purified as described previously (12). R6A-4 lacks an N-terminal methionine that the originally studied “full-length” R6A peptide contained. Biotinylated peptides were immobilized using streptavidin-agarose (immobilized NeutrAvidin on agarose, Pierce). Approximately 500–800 pmol of biotinylated peptide were used per 10  $\mu$ l of agarose.

Full-length R6A (MSQTKRLLDDQLYWWEYL) was expressed as a fusion to maltose-binding protein (MBP) using an *in vivo* biotinylation system (13). Cloning, expression, and purification were performed as described previously (12). R6A-MBP or MBP was immobilized by random amine coupling on CNBr-Sepharose 4B (Amersham Biosciences) as per the manufacturer's instructions at a concentration of  $\sim$ 1 mg/ml hydrated matrix. N-terminal biotinylated  $G\alpha_{i1}$  (Nb- $G\alpha_{i1}$ ) was expressed and purified as described previously (12). A vector for producing biotinylated Nb- $G\alpha_s$  (short form) was constructed analogously, except that an N-terminal His<sub>6</sub> tag was also incorporated to facilitate a primary purification by metal chelation chromatography.

**In Vitro Translation**—All G protein subunits were translated separately in coupled transcription/translation reactions using the TNT reticulocyte lysate system (T7 promoter, Promega, Madison, WI). Typically, 0.3–1.0  $\mu$ g of plasmid DNA and 25  $\mu$ Ci of L-[<sup>35</sup>S]methionine (MP Biomedicals, Irvine, CA) were used in a 25- $\mu$ l reaction. Translation efficiency of  $G\alpha$  subunits was quantitated by trichloroacetic acid precipitation of a 2- $\mu$ l aliquot of each reaction, as per the manufacturer's instructions.  $G\gamma$  reactions were supplemented with 10  $\mu$ M mevalonic acid lactone (Sigma) to ensure complete polyisoprenylation (14). To make  $G\beta\gamma$  heterodimers, independently translated subunits were mixed together (3:1 by volume,  $G\beta$ : $G\gamma$ ) and incubated at 37 °C for 30 min. To reconstitute  $G\alpha\beta\gamma$  heterotrimers, equal volumes of  $G\alpha$  and preformed  $G\beta\gamma$  were mixed and incubated at 37 °C for an additional 30 min. For the heterotrimer immunoprecipitation assays,  $G\beta$  was translated without radioactive labeling due to possible interference in the resolution of  $G\alpha$  subunits by SDS-PAGE. These unlabeled reactions were supplemented with L-methionine (40  $\mu$ M final).

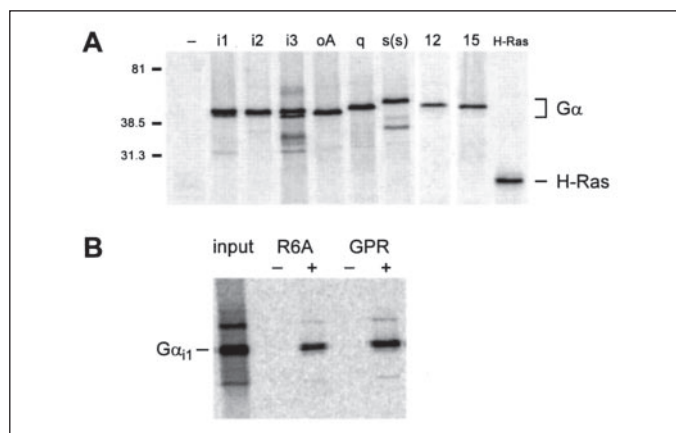
**$G\alpha$  Interaction Assay**— $G\alpha$  translation reactions were desalted and exchanged using MicroSpin G-25 columns (Amersham Biosciences) into buffer (50 mM HEPES-KOH at pH 7.5, 6 mM  $MgCl_2$ , 75 mM sucrose, 1 mM EDTA, 1  $\mu$ M GDP, and 0.05% (v/v) Tween 20 (Bio-Rad)). Equivalent aliquots (2–6  $\mu$ l) of the desalted  $G\alpha$  subunits were used for the *in vitro* binding assays.

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<sup>2</sup> To whom correspondence should be addressed. Tel.: 626-395-2321; Fax: 626-568-9430; E-mail: rroberts@caltech.edu.

<sup>3</sup> The abbreviations used are: GPCR, G protein-coupled receptor; GDI, guanine nucleotide dissociation inhibitor; GPR, G protein regulatory; HA, hemagglutinin; MBP, maltose-binding protein; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; EPPS, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid; GTP $\gamma$ S, guanosine 5'-O-(3-thiotriphosphate).



**FIGURE 1. Binding analysis with *in vitro* translated G proteins.** *A*, autoradiograph of indicated  $G\alpha$  subunits or H-Ras, directly translated from human cDNA vectors in coupled transcription/translation reactions with [ $^{35}$ S]methionine labeling. A blank reaction (-) did not contain vector. The slightly lower molecular weight bands (seen clearly for  $G\alpha_{11}$  and  $G\alpha_{12}$ ) correspond to translation initiation at alternate methionine codons. *B*, pull down of radiolabeled  $G\alpha_{11}$  on matrix with (+) or without (-) the indicated peptide (L19 GPR or full-length R6A).

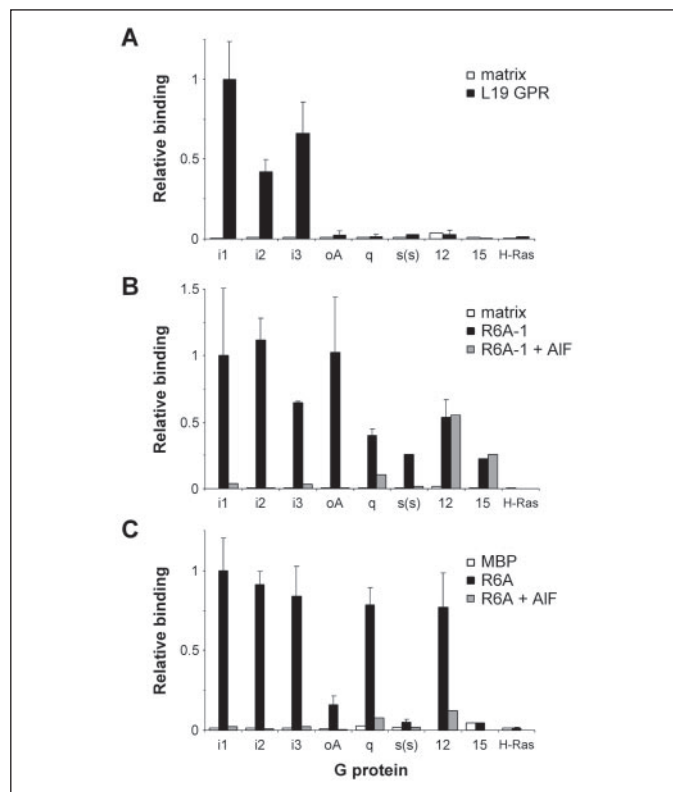
$G\alpha$  was added to 0.6 ml of binding buffer (25 mM HEPES-KOH at pH 7.5, 5 mM  $MgCl_2$ , 1 mM EDTA, 150 mM NaCl, 0.05% Tween 20, 0.05% (w/v) bovine serum albumin, 1 mM  $\beta$ -mercaptoethanol, and 10  $\mu$ M GDP) containing  $\sim 10$ - $\mu$ l matrix with or without immobilized target. After rotating at 4  $^{\circ}C$  for 1 h, samples were briefly centrifuged and the supernatant was removed. The matrix was transferred to a 0.45- $\mu$ m cellulose acetate spin filter (CoStar Spin-X, Corning, Inc., Corning, NY) and washed with 3  $\times$  0.6 ml of binding buffer at 4  $^{\circ}C$  (1500  $\times$  g,  $\sim 40$  s). The washed matrix was then removed from the spin filter for scintillation counting or analysis by SDS-PAGE. An approximation of the fraction of bound G protein was calculated (bound cpm divided by input protein counts, as determined by trichloroacetic acid precipitation). Assays with aluminum fluoride were performed identically, except that the binding buffer was supplemented with 10 mM NaF and 25  $\mu$ M  $AlCl_3$ .

**$G\alpha\beta\gamma$  Heterotrimer Immunoprecipitation**—Equivalent aliquots (10  $\mu$ l) of reconstituted  $G\alpha\beta\gamma$  heterotrimer were added to 0.6 ml of binding buffer containing  $\sim 10$ - $\mu$ l matrix or 1- $\mu$ l anti-HA monoclonal antibody (Sigma, clone HA-7). After rotating at 4  $^{\circ}C$  for 1 h,  $\sim 10$   $\mu$ l of protein G-Sepharose 4B Fast Flow (Sigma) was added to the monoclonal antibody-containing samples. After an additional 30 min of rotating at 4  $^{\circ}C$ , immobilization matrices were washed in 0.45- $\mu$ m spin filters (3  $\times$  0.6 ml of binding buffer) as described above. A fourth wash was performed in batch, after transferring the matrices to new tubes, to prevent contamination from the spin filter membrane. The samples were resuspended in 2  $\times$  SDS-loading buffer, incubated at 90  $^{\circ}C$  for 5 min, and analyzed by Tricine SDS-PAGE. Gels were fixed, dried *in vacuo*, and imaged by autoradiography (Storm PhosphorImager, Amersham Biosciences).

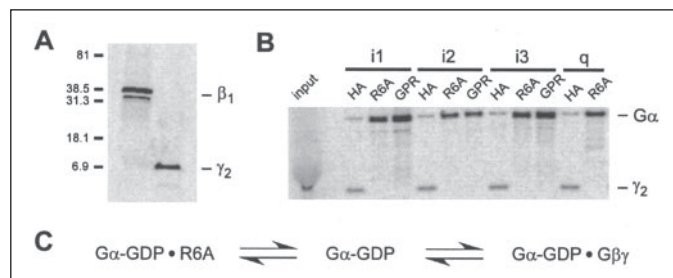
**Trypsin Protection Assay**— $G\alpha_{11}$  ( $\sim 25$   $\mu$ M) in digest buffer (50 mM EPPS at pH 8.0, 10 mM  $MgCl_2$ , 2 mM dithiothreitol, 1 mM EDTA, and 10  $\mu$ M GDP or GTP $\gamma$ S) was incubated with and without 1.5-fold excess peptide (R6A-4; R6A-M1, identical to R6A-1 with the addition of two C-terminal lysines to enhance solubility; and GPR1, the first GPR motif of AGS3 (15)) and digested with trypsin (1:50 trypsin: $G\alpha_{11}$ ) at 4  $^{\circ}C$  for the indicated time. Reactions were analyzed by SDS-PAGE.

## RESULTS AND DISCUSSION

**R6A-1 Is a Core Motif for  $G\alpha$  Binding**—Previously, *in vitro* selection with an mRNA display library was used to isolate novel peptide sequences that act as GDIs for  $G\alpha_{11}$  (12). The minimal 9-mer peptide, R6A-1, retained high affinity and competed with  $G\beta\gamma$  for binding to  $G\alpha_{11}$ . To investigate the binding specificity of the R6A-1 peptide, a pull-down assay was developed using radiolabeled, *in vitro* translated G protein subunits (Fig. 1A). Cell-free coupled transcription/translation offered a rapid method for screening multiple G proteins (16–18). R6A-1 and L19 GPR peptides were synthesized with a C-terminal biotin-containing linker peptide derived from the constant region used in the original selection (12). The full-length R6A peptide was also expressed as an N-terminal fusion to MBP, which was subsequently immobilized by random amine coupling. [ $^{35}$ S]methionine-labeled  $G\alpha_{11}$  was first tested against immobilized L19 GPR and full-length R6A, demonstrating specific pull-down of



**FIGURE 2. Binding of various *in vitro* translated G proteins to L19 GPR (A), R6A-1 (B), or R6A-MBP (C).** Binding has been scaled relative to the interaction to  $G\alpha_{11}$ . For comparison,  $K_D$  values of  $G\alpha_{11}$  binding to L19 GPR, R6A-1, and R6A are 82, 200, and 60 nM, respectively (12). The negative control matrices used in the assay were streptavidin-agarose (A and B) and MBP-Sepharose (C).  $G\alpha_{12}$  consistently exhibited higher nonspecific binding which was especially noticeable on the MBP-Sepharose in C, which was background subtracted to more clearly show the binding activity. In B and C, binding assays were also performed in the presence of aluminum fluoride (AlF) except for  $G\alpha_{15}$  (C) and H-Ras (B and C).

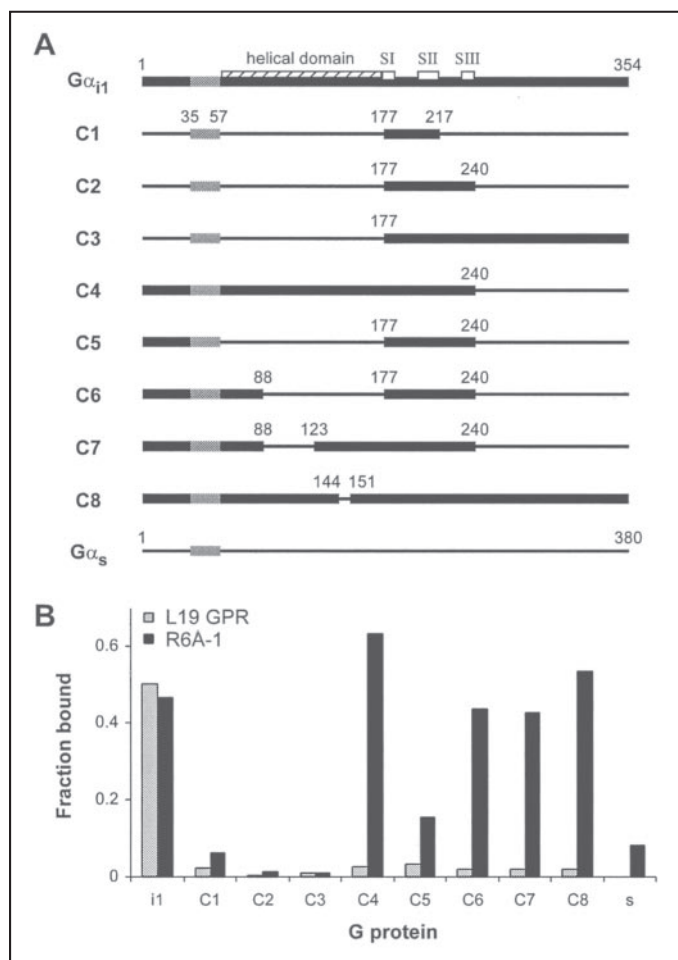
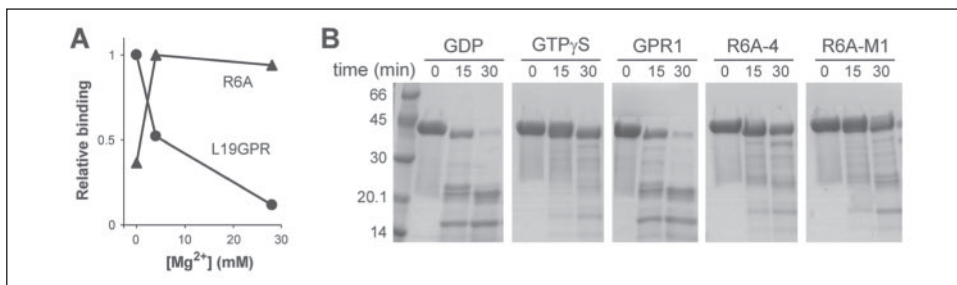


**FIGURE 3. R6A-MBP and L19 GPR compete with  $G\beta\gamma$  for binding to  $G\alpha$  subunits.** *A*, *in vitro* translated, [ $^{35}$ S]methionine-labeled  $G\beta_1$  and HA-tagged  $G\gamma_2$  subunits. *B*, reconstituted  $G\alpha\beta\gamma$  heterotrimers, with the indicated  $G\alpha$  subunit, were pulled down with an anti-HA antibody, R6A-MBP, or L19 GPR. Only the  $G\alpha$  and  $G\gamma_2$  subunits were radiolabeled. Immunoprecipitation with anti-HA confirmed the presence of reconstituted heterotrimers in the reaction mix.  $G\gamma$  was not co-precipitated when  $G\alpha$  subunits were pulled down by R6A-MBP or L19 GPR (B), suggesting that binding of  $G\alpha$ -GDP to  $G\beta\gamma$  and R6A are exclusive events (C). Results were similar for  $G\alpha_{12}$  (data not shown).

full-length  $G\alpha_{11}$ , as well as a slightly higher mobility band that corresponds to an alternate translation initiation site (Fig. 1B).

The R6A-1 minimal peptide exhibited strong pull-down for all  $G\alpha$  subunits tested (Fig. 2B). There was negligible interaction with H-Ras (a member of the monomeric or small G protein family), suggesting that the R6A-1 peptide is a core motif for binding to  $G\alpha$  subunits in the heterotrimeric G protein family. Conversely, the L19 GPR peptide was highly specific for  $G\alpha_{11-3}$  and showed negligible interaction with other  $G\alpha$  subunits (Fig. 2A). This contrasts with previous results that show the GPR consensus exhibits significantly lower, but detectable, affinity and GDI activity for  $G\alpha_o$  compared with  $G\alpha_i$  subunits (19, 20). The determined affinities of R6A-1 to  $G\alpha_{11}$  and  $G\alpha_s$  ( $K_D = \sim 200$  nM and  $\sim 50$   $\mu$ M, respectively; data not shown) suggest that the sensitivity of the binding assay was sufficient to detect weak interactions.

**FIGURE 4. Binding and structural properties of R6A- and GPR-derived peptides.** A, influence of  $Mg^{2+}$  on binding of L19 GPR and R6A-MBP to  $G\alpha_{11}$ . Binding was measured as described for Fig. 2, with data for each peptide scaled to the maximum amount of bound  $G\alpha$ . B, trypsin digest of  $G\alpha_{11}$ -GDP,  $G\alpha_{11}$ -GTP $\gamma$ S, or  $G\alpha_{11}$ -GDP in complex with the indicated peptide. After binding of nucleotide and/or peptide,  $G\alpha_{11}$  complexes were digested with trypsin at 4 °C for the indicated time and analyzed by SDS-PAGE. Molecular masses of a protein ladder (lane 1) are shown in kilodaltons.



**FIGURE 5. R6A interaction mapping using  $G\alpha_{11}/G\alpha_s$  chimeras.** A, schematic representation of  $G\alpha_{11}/G\alpha_s$  chimeras (29). The helical domain (residues 60–175) and switch regions (residues 176–184, 201–215, and 233–241 for SI, SII, and SIII, respectively) are marked. Residues 35–57 (gray) are highly conserved among  $G\alpha$  subunits: between  $G\alpha_{11}$  and  $G\alpha_s$ , 20 residues are identical, and the remaining 3 residues are conserved differences (Lys to Arg or Ile to Leu). Residue numbering is based on the  $G\alpha_{11}$  sequence. B, binding of radiolabeled  $G\alpha_{11}/G\alpha_s$  chimeras to immobilized L19 GPR or R6A-1. Binding to the immobilization matrix for each protein was  $<0.05$ , and the results shown have been background subtracted. The R6A full-length peptide and R6A-1 core motif demonstrated similar binding results (data not shown).

The full-length R6A peptide, compared with the minimal R6A-1 sequence, demonstrated significantly less binding to  $G\alpha_o$  and  $G\alpha_s$  and negligible interaction with  $G\alpha_{15}$  (Fig. 2C). It is not clear whether the differences between R6A and R6A-1 in relative binding to the various G proteins was due to the N-terminal flanking region of the full-length R6A sequence, the altered immobilization scheme (random amine coupling *versus* C-terminal biotinylation on the R6A-1 peptide), or steric effects from the comparatively large MBP fusion. However, assuming that the various G proteins are structurally homologous and that the R6A and R6A-1 peptides bind to  $G\alpha$  subunits analogously, the differences in relative binding would most likely be a direct result of the R6A flanking residues.

Previously, it was shown that R6A was highly specific for the GDP state of  $G\alpha_{11}$  and did not bind to  $G\alpha_{11}$ -GDP- $AlF_4^-$  or  $G\alpha_{11}$ -GTP $\gamma$ S (12). To establish

the nucleotide state specificity of R6A-derived peptides to various  $G\alpha$  subunits, radiolabeled G proteins were assayed for binding to immobilized R6A-1 and R6A-MBP in the presence and absence of aluminum fluoride. The minimal peptide was specific for the GDP state of all G proteins except for  $G\alpha_{12}$  and  $G\alpha_{15}$ , where binding was observed in the presence of aluminum fluoride (Fig. 2B). Full-length R6A-MBP demonstrated strong specificity for the GDP state of all G proteins, including  $G\alpha_s$ , confirming the weak but specific interaction (Fig. 2C). These findings suggest that residues flanking the 9-mer core motif play a strong role in modulating the binding properties of the peptide, modifying  $G\alpha$  class as well as nucleotide state specificity.

**R6A Competes with  $G\beta\gamma$  for Binding to  $G\alpha$  Subunits**—Previously, GPR- and R6A-derived peptides had been shown to compete with  $G\beta\gamma$  heterodimers for binding to  $G\alpha_{11}$  (12, 21–24). To determine whether R6A would exclude  $G\beta\gamma$  binding to other  $G\alpha$  subunits, *in vitro* translated  $G\beta_1$  and HA-tagged  $G\gamma_2$  (Fig. 3A) were reconstituted with various  $G\alpha$  subunits and pulled down by immobilized L19 GPR, R6A-MBP, or an anti-HA monoclonal antibody. The tested  $G\alpha$  subunits all appeared to couple to  $G\beta\gamma$  heterodimers (Fig. 3B). Co-precipitation of  $G\gamma$  subunits was not seen when heterotrimers were pulled down by L19 GPR or R6A-MBP (Fig. 3B), clearly indicating that binding to these motifs excludes  $G\beta\gamma$  interaction (Fig. 3C). Results for R6A-MBP with reconstituted  $G_{12}\alpha\beta_1\gamma_2$  heterotrimers were similar (data not shown).

**R6A and GPR Peptides Exert Distinct Modes of Activity**—Previous analyses of the R6A and GPR peptides suggested that their binding modes and mechanisms of action differ (12). Here, additional assays suggest that R6A binds and stabilizes a GDP-bound conformation of  $G\alpha_{11}$  unlike the conformation of the inactive  $G\alpha$ -GDP subunit.  $Mg^{2+}$  binds with high affinity and stabilizes the GTP-bound state of  $G\alpha$  subunits (25). In contrast,  $Mg^{2+}$  binds  $G\alpha_{11}$ -GDP with weak affinity (25). Measurements of L19 GPR and R6A binding to  $G\alpha_{11}$ -GDP as a function of  $Mg^{2+}$  concentration revealed distinct trends (Fig. 4A). While R6A showed higher relative binding with increasing  $Mg^{2+}$  concentrations, L19 GPR exhibited the highest binding in the absence of  $Mg^{2+}$ . The original isolation of the R6A peptide, from a doped library based on the GPR motif, most likely resulted from the presence of  $Mg^{2+}$  in the selection buffer (12). Crystal structures of a  $G\alpha_{11}$ -GDP- $Mg^{2+}$  complex indicate that binding of  $Mg^{2+}$  induces switch I on  $G\alpha_{11}$  to assume a conformation similar to that observed in the active  $G\alpha_{11}$ -GTP $\gamma$ S- $Mg^{2+}$  complex (26). Hence, the R6A peptide may stabilize a conformation of  $G\alpha_{11}$ -GDP that is similar to the structure of an active, GTP-bound subunit.

Trypsin digestions were performed to further probe the peptide-bound  $G\alpha_{11}$  complex. GTP-bound  $G\alpha$  is highly resistant to protease digestion, compared with the GDP-bound subunit (27, 28). R6A peptides protected  $G\alpha_{11}$ -GDP from trypsin similarly to  $G\alpha_{11}$ -GTP (Fig. 4B). Conversely, a GPR peptide- $G\alpha_{11}$  complex showed similar proteolysis as  $G\alpha_{11}$ -GDP (Fig. 4B). The R6A and GPR peptides did not appear to inhibit trypsin directly (data not shown). These results suggest that the R6A-bound complex is structurally analogous to  $G\alpha_{11}$ -GTP and further support distinct binding modes and mechanisms of activity for the R6A and GPR peptides.

**Mapping of the R6A- $G\alpha_{11}$  Interaction Surface**—A series of  $G\alpha_{11}/G\alpha_s$  chimeric proteins, C1–C8 (Fig. 5A), was used to determine the binding site of the R6A-1 peptide. The chimeras were previously shown to be correctly folded and functional in GTP $\gamma$ S binding assays (29). Based on the binding seen for chimeras C4, C6, C7, and C8 for immobilized R6A-1, two regions of  $G\alpha_{11}$  are required for strong interaction (Fig. 5B). Substitution of residues  $G\alpha_{11}$ -58–88 for the corresponding residues in  $G\alpha_s$  (comparing chimeras C5 and C6) resulted in substantially reduced binding. This region, which includes the linker between the Ras-like and helical domains of  $G\alpha_{11}$ , might be involved in determining the binding specificity of the R6A peptides. The N-terminal 35 residues of  $G\alpha_{11}$  also play an important role (comparing chimeras C2 and C5), most likely by affecting global protein stability and/or conformation.

Pcp2, a protein containing a single GPR motif, exhibited binding to the G protein chimeras C4 and C7 (data not shown), agreeing with previous studies (29). In contrast, the consensus GPR sequence appears to be highly optimized for specific interaction with G $\alpha_i$ , as none of the chimeras showed substantial binding to the L19 GPR peptide (Fig. 5B). Previous studies of different GPR motif-containing proteins have mapped key binding interactions that promote class specificity to regions in the G $\alpha$  helical domain (29–31). The GPR consensus region itself, however, binds primarily to the G $\alpha$  Ras-like domain (30). Hence, it appears that the determinants for G $\alpha_i$  class specificity can be contained within the Ras-like domain, despite the high sequence and structural conservation of the GPR binding site on G $\alpha$  subunits of different classes (9). The GPR consensus peptide may be highly sensitive to the global conformation of G $\alpha$  subunits rather than specific for particular amino acid contacts, perhaps making the motif easily adaptable for binding to specific G $\alpha$  classes (e.g. by the addition of flanking residues, as in Pcp2 (30)).

We have shown that the R6A-1 peptide, previously identified by *in vitro* selection against G $\alpha_{i1}$ , acts as a core motif for interaction with G $\alpha$  subunits and appears to compete with G $\beta\gamma$  for binding. It will be of interest to determine whether R6A-1 also retains its GDI activities for different G $\alpha$  family members. The R6A-1 core motif may serve as a template for the molecular design of G $\alpha$  ligands with unique class and/or nucleotide state specificities. Additionally, as the core motif appears to stabilize a distinct conformation of G $\alpha$ -GDP, it may be possible to develop peptides with unique effects on G protein-effector interaction, GDP exchange, and GTP hydrolysis.

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