This paper is available online at www.jbc.org
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 49, pp. 41912–41916, December 9, 2011

Cell Adhesion Receptor GPR133 Couples to G_s Protein*

Received for publication, May 28, 2011, and in revised form, September 24, 2011
Published, JBC Papers in Press, October 24, 2011, DOI 10.1074/jbc.C111.265934

Jens Bohnekamp and Torsten Schöneberg[1]

From the Section of Molecular Biochemistry, Institute of Biochemistry, Medical Faculty, University of Leipzig, 04103 Leipzig, Germany

Adhesion G protein-coupled receptors (GPCR), with their very large and complex N termini, are thought to participate in cell-cell and cell-matrix interactions and appear to be highly relevant in several developmental processes. Their intracellular signaling is still poorly understood. Here we demonstrate that GPR133, a member of the adhesion GPCR subfamily, activates the G_s protein/adenylyl cyclase pathway. The presence of the N terminus and the cleavage at the GPCR proteolysis site are not required for G protein signaling. G_s protein coupling was verified by $G\alpha_s$ knockdown with siRNA, overexpression of $G\alpha_s$, coexpression of the chimeric Gq_{s4} protein that routes GPR133 activity to the phospholipase C/inositol phosphate pathway, and missense mutation within the transmembrane domain that abolished receptor activity without changing cell surface expression. It is likely that not only GPR133 but also other adhesion GPCR signal via classical receptor/G protein-interaction.

Adhesion receptors comprise the second largest subfamily of putatively G protein-coupled receptors (GPCR)[2] with more than 30 members in vertebrates (1, 2). Adhesion GPCR are characterized by long extracellular N termini, which are composed of multiple functional domains, a seven-transmembrane spanning (7TM) domain, and a cytoplasmic tail. Adhesion GPCR are believed to play a role in immune functions (3, 4), angiogenesis (5), cell polarity (6, 7), and development (8, 9). Mutations in some members of the protein family were identified as the cause of inherited developmental defects in humans such as Usher syndrome (VLGR1) (10) and bilateral frontoparietal polymicrogyria (GPR56) (11). Although there is consensus on the fact that this receptor class mediates essential cell-cell and cell-matrix interactions (1, 12), the molecular mechanism of intracellular signal transduction of adhesion GPCR remains obscure.

There are only a few studies on intracellular signaling mechanisms of adhesion GPCR. Latrophilin 1, the prototype of adhesion GPCR, induces intracellular Ca^{2+} signaling upon interaction with the exogenous ligand α-latrotoxin (13, 14). GPR56 appears to activate the $G_{12/13}$ protein/Rho pathway after stimulation with an antibody against the ectodomain (15). BAI1 recognizes phosphatidylserine and can directly recruit a Rac-guanine nucleotide exchange factor (Rac-GEF) complex to mediate the uptake of apoptotic cells (16). The cytoplasmic domain of BAI2 interacts with GA-binding protein γ, and GA-binding protein-α/γ or GA-binding protein-α/β work as transcriptional repressors of VEGF (17). However, clear evidence of intracellular signaling for most adhesion GPCR via G proteins is still missing (12).

Genetic variations in the *GPR133* gene, also a member of the adhesion GPCR family, were associated with adult height (18) and the RR interval duration in electrocardiograms (19). GPR133 is expressed in CNS (20) and other tissues; its endogenous agonists and the signal transduction are unknown. Here we demonstrate that GPR133 is coupled to the G_s protein/adenylyl cyclase pathway. This proves that this adhesion receptor is indeed a G protein-coupled receptor.

EXPERIMENTAL PROCEDURES

Materials

If not stated otherwise, all standard substances were purchased from Sigma-Aldrich (Taufkirchen, Germany), Merck (Darmstadt, Germany), and C. Roth GmbH (Karlsruhe, Germany). Cell culture material and primers were obtained from Invitrogen (Darmstadt, Germany).

Methods

Generation of Wild-type GPR133 and Mutants—Full-length human (NM_198827) and mouse (BC158001) GPR133 sequences were directly cloned from human monocytes and mouse urinary bladder cDNA libraries (primers: human forward, ACTTGGCTCCGAGCTTTGAC, and reverse, CAAAGGTGGGGCATTTCATT; and mouse forward, AGAAGTTCCCTGCAGGCTGT, and reverse, TCTGCTTCAGGGAAGGCACT) and inserted into the mammalian expression vector pcDps. Human and mouse GPR133 (see Fig. 1*A*) were N-terminally tagged after the initial signal peptide at amino acid position 31 with a hemagglutinin (HA) epitope followed by a sequence encoding the N-terminal 20 amino acids of bovine rhodopsin N terminus (as described in Ref. 21) and C-terminally with a FLAG epitope by a PCR-based site-directed mutagenesis and fragment replacement strategy.

Assays to Determine GPR133 Function—COS-7 cells were grown in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C and 7% CO_2 in a humidified atmosphere. Cells were split into 12-well plates (10^5 cells/well, for inositol phosphate (IP) assay) and 48-well plates (3×10^4 cells/well for cAMP assay) and transfected with Lipofectamine™ 2000 (Invitrogen, Paisley, UK) according to the manufacturer's protocol. To measure IP formation, transfected COS-7 cells were incubated with 2 μCi/ml *myo*-[^{3}H]inositol

* This work was supported by grants from the Deutsche Forschungsgemeinschaft (Grant Sfb 610) and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (Integriertes Forschungs- und Behandlungszentrum AdipositasDiseases Leipzig).

[1] To whom correspondence should be addressed: Section of Molecular Biochemistry, Institute of Biochemistry, Medical Faculty, University of Leipzig, Johannisallee 30, 04103 Leipzig, Germany. Tel.: 49-341-9722-151; Fax: 49-341-9722-159; E-mail: schoberg@medizin.uni-leipzig.de.

[2] The abbreviations used are: GPCR, G protein-coupled receptor(s); GPS, GPCR proteolysis site; 7TM, seven-transmembrane spanning domain; CRE-SEAP reporter, cAMP-responsive element-secreted alkaline phosphatase reporter; IP, inositol phosphate; MC4R, melanocortin type 4 receptor; MSH, melanocyte-stimulating hormone; OD, optical density.

(18.6 Ci/mmol, PerkinElmer Life Sciences) for 24 h. Thereafter, cells were washed once with serum-free DMEM containing 10 mM LiCl followed by incubation either solely with serum-free DMEM containing 10 mM LiCl or supplemented with 10 μM α-melanocyte-stimulating hormone (αMSH) for 1 h at 37 °C. Intracellular IP levels were determined by anion-exchange chromatography as described (22). IP accumulation data were analyzed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA). For cAMP measurements, 48 h after transfection, cells were incubated with 3-isobutyl-methyl-xanthine (1 mM)-containing medium for 1 h. Incubation was stopped by washing with ice-cold PBS. Cells were lysed in LI buffer (PerkinElmer Life Sciences, Monza, Italy) and frozen at −20 °C until measurement. To measure cAMP concentration, the Alpha Screen cAMP assay kit (PerkinElmer Life Sciences) was used according to the manufacturer's protocol. The accumulated cAMP was measured in 384-well white OptiPlate microplates (PerkinElmer Life Sciences) with the Fusion AlphaScreen multilabel reader (PerkinElmer Life Sciences). For the CRE-SEAP reporter gene assay, HEK293 cells were grown in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified 7% CO_2 incubator. One day prior to transfection, cells were split into 96-well cell culture plates (2.5×10^4 cells/well), and 24 h later, cells were co-transfected (75 ng of each) with the GPR133 expression plasmid and the CRE-SEAP reporter plasmid (Clontech, Saint-Germain-en-Laye, France). Lipofectamine™ 2000 (Invitrogen) was used for transient transfection. One day after transfection, serum-free DMEM or serum-free DMEM with 5 μM forskolin was added to the HEK293 cells. Cells were incubated for 24 h at 37 °C and then for 2 h at 65–70 °C. An aliquot of the supernatant from each well was then incubated (2–5 min, 21 °C) with an equal volume of 1.2 mM 4-methylumbelliferyl phosphate (Sigma-Aldrich, Seelze, Germany) in 2 M diethanolamine bicarbonate with 1 mM $MgCl_2$ and 4.5 mg/ml L-homoarginine (pH 10) and fluorescence was measured with a Victor 2 1420 Multilabel counter (PerkinElmer LAS, Rodgau-Jügesheim, Germany). For siRNA experiments, the human GPR133 construct was subcloned into the pcDNA3.1 vector (Invitrogen). One day prior to transfection, HEK293 cells were split into 48-well cell culture plates (7×10^4 cells/well), and 24 h later, cells were co-transfected with 600 ng/well plasmid encoding the human GPR133 and 2.5 pmol/well Gα_s siRNA (sc-29328; Santa Cruz Biotechnology, Heidelberg, Germany) or control siRNA (sc-37007; Santa Cruz Biotechnology). For Gα_s co-transfection experiments, the human Gα_s sequence (NM_000516) was directly cloned from a human monocyte cDNA library (primers: forward, ATGGGCTGCCTCGGGAACAGT; and reverse, GTTGCTTTGTTAATCATGCCCTAT) and inserted into the mammalian expression vector pcDps.

To estimate cell surface expression of receptors carrying an N-terminal HA tag, we used an indirect cellular enzyme-linked immunosorbent assay (ELISA) (23). To assess the amounts of full-length HA/FLAG double-tagged GPR133 constructs and to demonstrate that the reduction of cell surface expression levels is not due to a decrease in receptor expression in general, a sandwich ELISA was used and performed as described previously (24).

RESULTS AND DISCUSSION

GPR133 Displays Increased Basal Activity in the G_s Protein/Adenylyl Cyclase Pathway—According to the current model of GPCR function (25, 26), receptor overexpression can result in a constitutive activation of signaling pathways, which are normally activated after agonist stimulation (27–30). Thus, the coupling abilities of several receptors, including "orphan" receptors, have been characterized by overexpression in the absence of an agonist (31–33). For example, the wild-type adenylate cyclase constitutive activator (ACCA), an orphan GPCR, stimulates the G_s protein/adenylyl cyclase system to some extent when expressed in COS-7 cells (31). Following this strategy, the human and mouse GPR133 were transiently expressed in HEK293 cells and tested in AP1-, nuclear factor of activated T-cells (NFAT)-, serum responsive element (SRE)-, and CRE-SEAP reporter gene assays. Interestingly, GPR133-transfected cells displayed elevated basal activities when compared with mock-transfected cells in the CRE-SEAP reporter assay (Fig. 1*B*), indicating that GPR133 activity may increase intracellular cAMP levels. After the initial screen in reporter gene assays, we focused on the signal transduction of the human GPR133 in further functional experiments. First, to verify the basal activation of the G_s pathway, we directly measured cAMP formation in COS-7 cells transfected with the human GPR133. Indeed, cAMP formation was significantly increased in cells transfected with GPR133 (~4-fold) when compared with mock-transfected COS-7 cells (Fig. 1, *C* and *D*). Second, because constitutive GPCR activity directly correlates with cell surface expression levels (34), we performed receptor amount titration experiments. As shown in Fig. 1*C*, increasing amounts of transfected GPR133 plasmid produced increased cell surface expression and cAMP levels, proving that cAMP levels directly depend on expression of the constitutively active GPR133.

Structural Requirements for G Protein Coupling of GPR133—To control whether the high basal activity of the human GPR133 is specific, several GPR133 mutants were generated and tested in functional assays. Mutations were generated at amino acid positions highly conserved during GPR133 evolution, and we therefore speculated that mutation of these residues may have an effect on receptor function. In contrast to several other mutations that abolished GPR133 basal activity (data not shown), L808T did not significantly affect cell surface expression (Fig. 1, *D* and *E*). This indicates that elevated cAMP levels were directly linked to the properly folded structure of the 7TM domain of GPR133. It was shown for many rhodopsin-like GPCR that mutations can induce structural changes in the 7TM domain modulating the constitutive G protein-coupling activity of the receptor (34, 35).

Many adhesion GPCR undergo a self-catalytic cleavage at the GPCR proteolysis site (GPS) to form a heterodimeric complex containing the N terminus and 7TM domain (36–39). To test whether GPS cleavage is required for G protein-signaling of GPR133, we mutated position −2 of the cleavage site (···HL ↓ T···) to an arginine. This change has been shown to abolish autocatalytic activity without disrupting protein

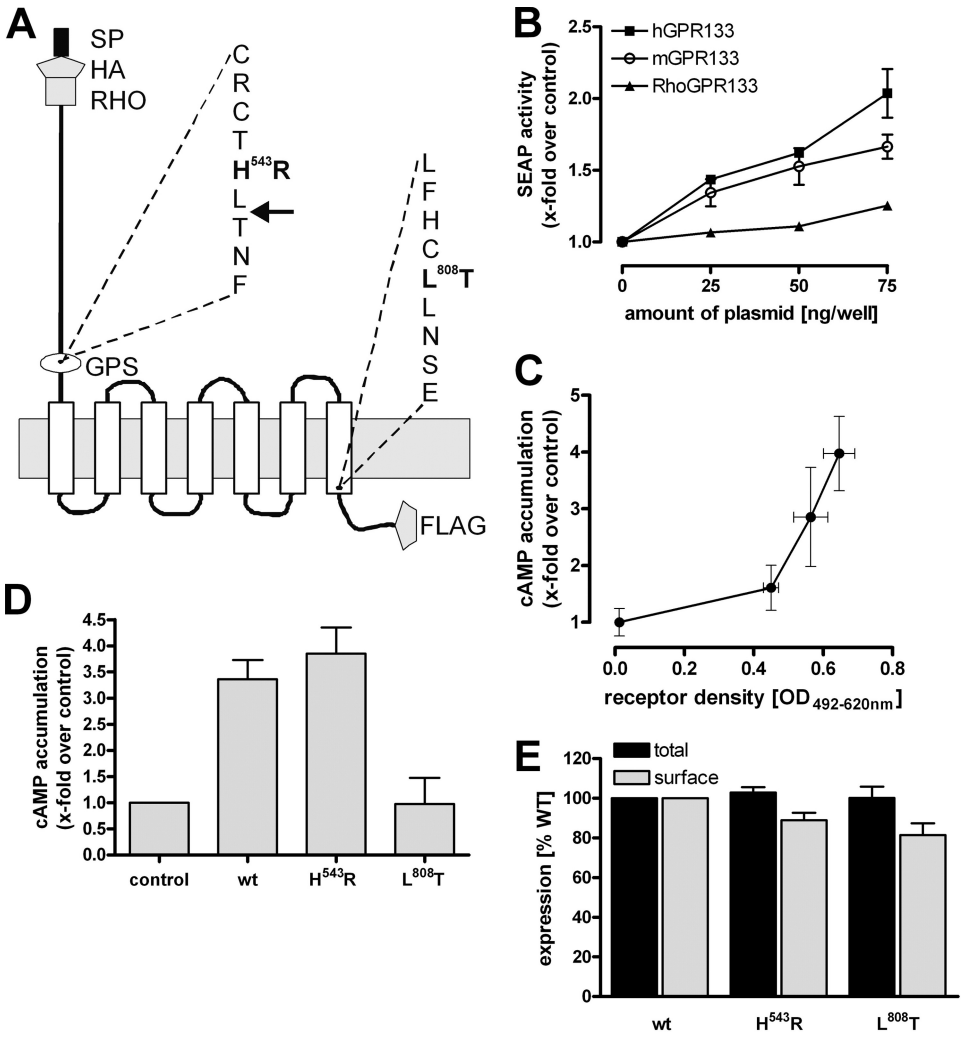

FIGURE 1. **GPR133 increases cAMP accumulation in HEK293 and COS-7 cells.** *A*, the domain architecture of the human GPR133 is shown. To monitor GPR133 expression, the receptor was N-terminally epitope-tagged downstream of the signal peptide (*SP*) with an HA epitope followed by a sequence encoding the N-terminal 20 amino acids of bovine rhodopsin N terminus (*RHO*) and C-terminally with a FLAG epitope (*FLAG*). *B*, HEK293 cells were transfected with the human and mouse GPR133 and a construct where the N terminus of the human GPR133 was replaced by the bovine rhodopsin N terminus (human RhoGPR133), and CRE-SEAP assays were performed as described under "Experimental Procedures." Basal (transfected pcDps vector) SEAP activity was 155,634 ± 8,949 arbitrary units/well, and stimulation of cells with 5 μM forskolin resulted in a 4.89 ± 2.84-fold increase in SEAP activity. Data are given as means ± S.D. of two independent experiments performed in quadruplicates. *C*, COS-7 cells were transfected with increasing amounts (0, 200, 300, 400 ng/well) of plasmid encoding the human GPR133. After 2 days, intracellular cAMP levels and cell surface expression levels were determined as described under "Experimental Procedures." The pcDps vector served as negative control showing a cAMP level of 6.3 ± 1.5 nM/well and an optical density (OD) value of 0.012 ± 0.001 $OD_{492-620\ nm}$. Data of a representative assay are given as means ± S.D. (-fold over negative control) performed in triplicate. *D*, COS-7 cells were transfected with the wild-type (*wt*) and human GPR133 mutants. Two days after transfection, intracellular cAMP levels (*D*) and cellular expression levels (*E*) were determined as described under "Experimental Procedures." Cyclic AMP levels were referred to the negative control (pcDps; cAMP level: 21.9 ± 3.2 nM/well). Data are given as means ± S.E. (-fold over negative control) of three independent experiments each performed in triplicate. For expression studies, cell surface and sandwich ELISA were used to measure cell surface and total cellular expression levels, respectively. Specific OD readings (OD value of double HA/FLAG-tagged GPR133 constructs minus OD value of mock-transfected cells) are given as the percentage of double HA/FLAG-tagged WT GPR133. For the cell surface ELISA, the nonspecific OD value (pcDps) was 0.040 ± 0.003 (set as 0%), and the OD value of WT GPR133 was 1.091 ± 0.323 (set as 100%). OD readings of 0.022 ± 0.002 (set as 0%) and 1.149 ± 0.042 (set as 100%) were found in sandwich ELISA (total expression) for the negative control vector (pcDps) and the WT GPR133, respectively. Data are given as means ± S.E. of three independent experiments each performed in triplicate.

expression on the cell surface (40). As expected, GPR133 containing H543R was expressed normally and yet displayed increased basal activity (Fig. 1, *D* and *E*). Experiments with a GPR133 where the N terminus is completely exchanged by the N terminus of bovine rhodopsin showed increased basal activity in the CRE-SEAP assay (Fig. 1*B*). Therefore, the presence of the N-terminal domain and the cleavage at GPS are not required for G protein coupling. These features are very similar to the group of glycoprotein hormone receptors, such as thyrotropin and lutropin receptors. Mutagenesis studies with these glycoprotein hormone receptors have shown that the large ectodomain is not required for ligand- and mutation-induced signaling of the 7TM domain (41, 42).

G Protein Coupling Specificity of GPR133—It has been demonstrated that replacement of the four or five C-terminal amino acids of $G\alpha_q$ with the corresponding residues of other $G\alpha$ subunits confers the ability to stimulate the phospholipase C-β pathway onto *e.g.* G_i-coupled receptors using a $G\alpha_{qi4}$ construct (43). We co-expressed GPR133 with a $G\alpha_{qs4}$ construct (kindly provided by Prof. Dr. Evi Kostenis, Institute of Pharmaceutical

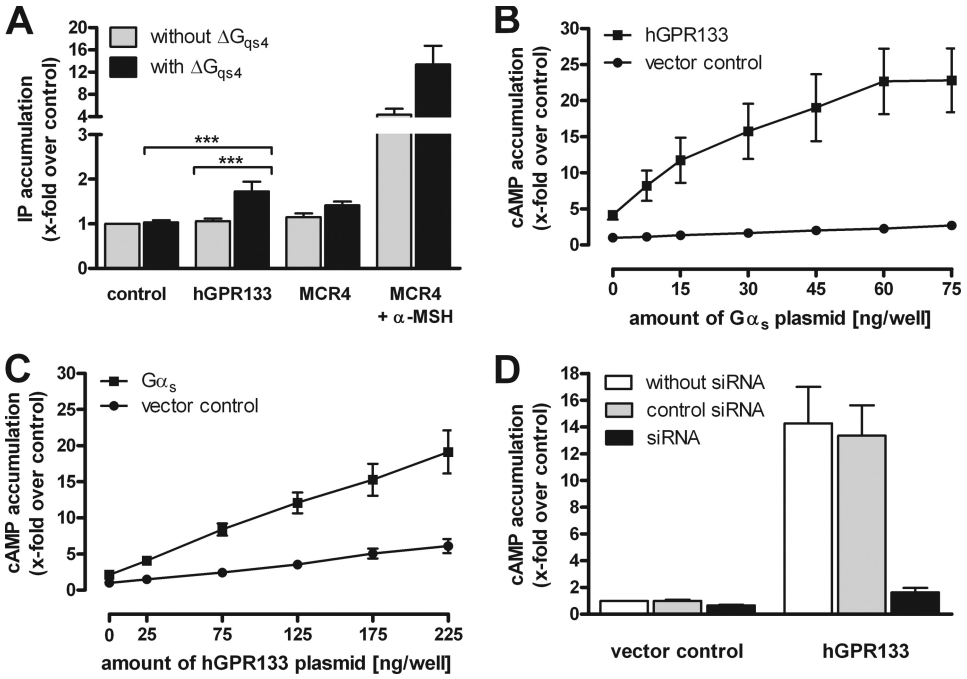

FIGURE 2. **GPR133 is coupled to the G_s protein.** *A*, to evaluate the signaling specificity of GPR133, COS-7 cells were co-transfected with the indicated receptor construct and the chimeric $G\alpha_{qs4}$ protein (see "Experimental Procedures"). The $G\alpha_s$-coupled MC4R served as positive control and was stimulated with 10 μM αMSH. IP assays were performed 48 h after transfection as described under "Experimental Procedures." Basal IP formation is expressed as *X*-fold over basal levels of mock-transfected cells (204 ± 52 cpm/well). Data are presented as means ± S.D. of four (GPR133) and two (MC4R) independent experiments, each carried out in triplicate. ***, $p < 0.001$. *B*, COS-7 cells were co-transfected with 225 ng/well of plasmid encoding the human GPR133 or vector control (pcDps) and increasing amounts (0, 7.5, 15, 30, 45, 60, 75 ng/well) of plasmid encoding the human $G\alpha_s$. Data are given as means ± S.E. (-fold over negative control) of three independent experiments each performed in triplicate. *C*, in a second setup, COS-7 cells were co-transfected with 60 ng/well of plasmid encoding human $G\alpha_s$ or vector control (pcDps) and increasing amounts (0, 25, 75, 125, 175, 225 ng/well) of plasmid encoding the human GPR133. After 2 days, intracellular cAMP levels were determined as described under "Experimental Procedures." The empty pcDps vector served as negative control (cAMP level: 3.1 ± 0.9 nM/well). Data are given as means ± S.E. (-fold over negative control) of three independent experiments each performed in triplicate. *D*, HEK293 cells were co-transfected with 600 ng/well of plasmid encoding the human GPR133 in pcDNA3.1 or vector control (pcDNA3.1) and 2.5 pmol/well $G\alpha_s$ siRNA or control siRNA. The human cell line HEK293 was used to meet the species specificity of the siRNA against the human $G\alpha_s$ subunit. After 2 days, intracellular cAMP levels were determined as described under "Experimental Procedures." The vector pcDNA3.1 served as negative control (cAMP level: 6.1 ± 0.6 nM/well). Data are given as means ± S.E. (-fold over negative control) of three independent experiments each performed in triplicate.

Biology, University of Bonn, Bonn, Germany) where G_s-coupled receptors can be rerouted to the phospholipase C-β/inositol phosphate pathway. As expected, αMSH stimulation (10 μM αMSH) of the G_s-coupled melanocortin type 4 receptor (MC4R) co-transfected with $G\alpha_{qs4}$ resulted in a robust increase in intracellular IP levels (Fig. 2). Note that MC4R-transfected cells alone produce some IP response via G_i coupling (44), which is, however, much lower when compared with MC4R/$G\alpha_{qs4}$-co-transfecetd cells. Transfection of either GPR133 or $G\alpha_{qs4}$ alone did not increase basal IP levels when compared with mock-transfected COS-7 cells. However, GPR133 co-transfected with $G\alpha_{qs4}$ led to a significant increase in IP levels (Fig. 2*A*). This clearly indicates that GPR133 must functionally interact with a G protein to mediate intracellular IP formation.

Because increased activity of cAMP-dependent protein kinase is only one of several ways to activate cAMP-response element-binding protein (CREB) (45) and some adenylyl cyclase isoforms can be activated not only by $G\alpha_s$ but also by $\beta\gamma$ subunits (46, 47), we more directly addressed the involvement of $G\alpha_s$ in GPR133 signal transduction. As shown in Fig. 2, *B* and *C*, co-expression of $G\alpha_s$ with GPR133 increased cAMP levels in a concentration-dependent manner. This experiment provides strong evidence that adenylyl cyclase activity is not driven by $\beta\gamma$ subunits from other G proteins. Finally, siRNA was used to specifically knock down the $G\alpha_s$ subunit. Co-transfection of siRNA and GPR133 almost abolished GPR133-induced cAMP levels, whereas control siRNA had no effect (Fig. 2*D*). This experiment clearly showed that increased cAMP formation in cells transfected with GPR133 is due to $G\alpha_s$ coupling.

Conclusion—Taking advantage of increased basal activity, we demonstrated that GPR133, a member of the adhesion GPCR family, signals via the G_s protein/adenylyl cyclase pathway. The coupling specificity was verified by co-expression with a chimeric G protein and $G\alpha_s$ subunit as well as by co-transfection with siRNA specific to $G\alpha_s$. Phylogenetic analysis found that in the adhesion GPCR family, GPR133 and GPR144 are the closest relatives to secretin family members, which are well known to couple to the G_s protein/adenylyl cyclase pathway (48). The association of GPR133 variants with heart rate (19) is very consistent with the known role of cAMP as a regulator of this function. Furthermore, known occurrences of activating $G\alpha_s$ mutations in growth hormone-secreting pituitary tumors may provide a rationale for the association between genetic variations of GPR133 and adult height reported previously (18). The high expression of GPR133 in the pituitary gland (20) is consistent with this notion. Recently, an orphan receptor of the adhesion GPCR family, GPR126, has been shown to play an essential role in the myelination of peripheral nerves by neural crest-derived Schwann cells in the zebrafish *Danio rerio* (49).

Interestingly, elevation of cAMP by forskolin in GPR126 mutants could restore myelination. It needs therefore to be tested in future experiments whether not only GPR133 but also other adhesion GPCR signal via classical receptor/G protein-interaction.

Acknowledgments—We are very grateful to Petra Krumbholz for technical assistance. We are thankful to Michael Stumvoll and Peter Kovacs for bringing the physiological relevance of GPR133 to our attention (18).

REFERENCES

1. Yona, S., Lin, H. H., Siu, W. O., Gordon, S., and Stacey, M. (2008) *Trends Biochem. Sci.* **33,** 491–500
2. Bjarnadóttir, T. K., Fredriksson, R., and Schiöth, H. B. (2007) *Cell Mol. Life Sci.* **64,** 2104–2119
3. Yona, S., Lin, H. H., Dri, P., Davies, J. Q., Hayhoe, R. P., Lewis, S. M., Heinsbroek, S. E., Brown, K. A., Perretti, M., Hamann, J., Treacher, D. F., Gordon, S., and Stacey, M. (2008) *FASEB J.* **22,** 741–751
4. Veninga, H., Becker, S., Hoek, R. M., Wobus, M., Wandel, E., van der Kaa, J., van der Valk, M., de Vos, A. F., Haase, H., Owens, B., van der Poll, T., van Lier, R. A., Verbeek, J. S., Aust, G., and Hamann, J. (2008) *J. Immunol.* **181,** 6574–6583
5. Kuhnert, F., Mancuso, M. R., Shamloo, A., Wang, H. T., Choksi, V., Florek, M., Su, H., Fruttiger, M., Young, W. L., Heilshorn, S. C., and Kuo, C. J. (2010) *Science* **330,** 985–989
6. Lawrence, P. A., Struhl, G., and Casal, J. (2007) *Nat. Rev. Genet* **8,** 555–563
7. Langenhan, T., Prömel, S., Mestek, L., Esmaeili, B., Waller-Evans, H., Hennig, C., Kohara, Y., Avery, L., Vakonakis, I., Schnabel, R., and Russ, A. P. (2009) *Dev. Cell* **17,** 494–504
8. Tissir, F., Bar, I., Jossin, Y., De Backer, O., and Goffinet, A. M. (2005) *Nat. Neurosci.* **8,** 451–457
9. Waller-Evans, H., Prömel, S., Langenhan, T., Dixon, J., Zahn, D., Colledge, W. H., Doran, J., Carlton, M. B., Davies, B., Aparicio, S. A., Grosse, J., and Russ, A. P. (2010) *PLoS One* **5,** e14047
10. Weston, M. D., Luijendijk, M. W., Humphrey, K. D., Möller, C., and Kimberling, W. J. (2004) *Am. J. Hum. Genet.* **74,** 357–366
11. Piao, X., Hill, R. S., Bodell, A., Chang, B. S., Basel-Vanagaite, L., Straussberg, R., Dobyns, W. B., Qasrawi, B., Winter, R. M., Innes, A. M., Voit, T., Ross, M. E., Michaud, J. L., Déscarie, J. C., Barkovich, A. J., and Walsh, C. A. (2004) *Science* **303,** 2033–2036
12. Kwakkenbos, M. J., Kop, E. N., Stacey, M., Matmati, M., Gordon, S., Lin, H. H., and Hamann, J. (2004) *Immunogenetics* **55,** 655–666
13. Silva, J. P., Lelianova, V., Hopkins, C., Volynski, K. E., and Ushkaryov, Y. (2009) *J. Biol. Chem.* **284,** 6495–6506
14. Volynski, K. E., Silva, J. P., Lelianova, V. G., Atiqur Rahman, M., Hopkins, C., and Ushkaryov, Y. A. (2004) *EMBO J.* **23,** 4423–4433
15. Iguchi, T., Sakata, K., Yoshizaki, K., Tago, K., Mizuno, N., and Itoh, H. (2008) *J. Biol. Chem.* **283,** 14469–14478
16. Park, D., Tosello-Trampont, A. C., Elliott, M. R., Lu, M., Haney, L. B., Ma, Z., Klibanov, A. L., Mandell, J. W., and Ravichandran, K. S. (2007) *Nature* **450,** 430–434
17. Jeong, B. C., Kim, M. Y., Lee, J. H., Kee, H. J., Kho, D. H., Han, K. E., Qian, Y. R., Kim, J. K., and Kim, K. K. (2006) *FEBS Lett.* **580,** 669–676
18. Tönjes, A., Koriath, M., Schleinitz, D., Dietrich, K., Böttcher, Y., Rayner, N. W., Almgren, P., Enigk, B., Richter, O., Rohm, S., Fischer-Rosinsky, A., Pfeiffer, A., Hoffmann, K., Krohn, K., Aust, G., Spranger, J., Groop, L., Blüher, M., Kovacs, P., and Stumvoll, M. (2009) *Hum. Mol. Genet* **18,** 4662–4668
19. Marroni, F., Pfeufer, A., Aulchenko, Y. S., Franklin, C. S., Isaacs, A., Pichler, I., Wild, S. H., Oostra, B. A., Wright, A. F., Campbell, H., Witteman, J. C., Kääb, S., Hicks, A. A., Gyllensten, U., Rudan, I., Meitinger, T., Pattaro, C., van Duijn, C. M., Wilson, J. F., and Pramstaller, P. P. (2009) *Circ. Cardiovasc. Genet* **2,** 322–328
20. Vanti, W. B., Nguyen, T., Cheng, R., Lynch, K. R., George, S. R., and O'Dowd, B. F. (2003) *Biochem. Biophys. Res. Commun.* **305,** 67–71
21. Liberles, S. D., and Buck, L. B. (2006) *Nature* **442,** 645–650
22. Berridge, M. J. (1983) *Biochem. J.* **212,** 849–858
23. Schöneberg, T., Schulz, A., Biebermann, H., Grüters, A., Grimm, T., Hübschmann, K., Filler, G., Gudermann, T., and Schultz, G. (1998) *Hum. Mutat.* **12,** 196–205
24. Römpler, H., Yu, H. T., Arnold, A., Orth, A., and Schöneberg, T. (2006) *Genomics* **87,** 724–732
25. Lefkowitz, R. J. (1993) *Nature* **365,** 603–604
26. Lefkowitz, R. J., Cotecchia, S., Samama, P., and Costa, T. (1993) *Trends Pharmacol. Sci.* **14,** 303–307
27. Böselt, I., Römpler, H., Hermsdorf, T., Thor, D., Busch, W., Schulz, A., and Schöneberg, T. (2009) *PLoS One* **4,** e5573
28. Sánchez-Más, J., Hahmann, C., Gerritsen, I., García-Borrón, J. C., and Jiménez-Cervantes, C. (2004) *Pigment Cell Res.* **17,** 386–395
29. Biebermann, H., Schöneberg, T., Schulz, A., Krause, G., Grüters, A., Schultz, G., and Gudermann, T. (1998) *FASEB J.* **12,** 1461–1471
30. Preuss, H., Ghorai, P., Kraus, A., Dove, S., Buschauer, A., and Seifert, R. (2007) *J. Pharmacol. Exp. Ther.* **321,** 983–995
31. Eggerickx, D., Denef, J. F., Labbe, O., Hayashi, Y., Refetoff, S., Vassart, G., Parmentier, M., and Libert, F. (1995) *Biochem. J.* **309,** 837–843
32. Römpler, H., Schulz, A., Pitra, C., Coop, G., Przeworski, M., Pääbo, S., and Schöneberg, T. (2005) *J. Biol. Chem.* **280,** 31068–31075
33. Schulz, A., and Schöneberg, T. (2003) *J. Biol. Chem.* **278,** 35531–35541
34. Zhang, M., Mizrachi, D., Fanelli, F., and Segaloff, D. L. (2005) *J. Biol. Chem.* **280,** 26169–26176
35. Schmidt, C., Li, B., Bloodworth, L., Erlenbach, I., Zeng, F. Y., and Wess, J. (2003) *J. Biol. Chem.* **278,** 30248–30260
36. Okajima, D., Kudo, G., and Yokota, H. (2010) *J. Recept. Signal Transduct. Res.* **30,** 143–153
37. Hsiao, C. C., Chen, H. Y., Chang, G. W., and Lin, H. H. (2011) *FEBS Lett.* **585,** 313–318
38. Chiang, N. Y., Hsiao, C. C., Huang, Y. S., Chen, H. Y., Hsieh, I. J., Chang, G. W., and Lin, H. H. (2011) *J. Biol. Chem.* **286,** 14215–14225
39. Krasnoperov, V., Deyev, I. E., Serova, O. V., Xu, C., Lu, Y., Buryanovsky, L., Gabibov, A. G., Neubert, T. A., and Petrenko, A. G. (2009) *Biochemistry* **48,** 3230–3238
40. Lin, H. H., Chang, G. W., Davies, J. Q., Stacey, M., Harris, J., and Gordon, S. (2004) *J. Biol. Chem.* **279,** 31823–31832
41. Sangkuhl, K., Schulz, A., Schultz, G., and Schöneberg, T. (2002) *J. Biol. Chem.* **277,** 47748–47755
42. Jäschke, H., Neumann, S., Moore, S., Thomas, C. J., Colson, A. O., Costanzi, S., Kleinau, G., Jiang, J. K., Paschke, R., Raaka, B. M., Krause, G., and Gershengorn, M. C. (2006) *J. Biol. Chem.* **281,** 9841–9844
43. Conklin, B. R., Farfel, Z., Lustig, K. D., Julius, D., and Bourne, H. R. (1993) *Nature* **363,** 274–276
44. Büch, T. R., Heling, D., Damm, E., Gudermann, T., and Breit, A. (2009) *J. Biol. Chem.* **284,** 26411–26420
45. Johannessen, M., Delghandi, M. P., and Moens, U. (2004) *Cell. Signal.* **16,** 1211–1227
46. Feldman, R. D., and Gros, R. (2007) *Life Sci.* **81,** 267–271
47. Dessauer, C. W. (2009) *Mol. Pharmacol.* **76,** 935–941
48. Nordström, K. J., Lagerström, M. C., Wallér, L. M., Fredriksson, R., and Schiöth, H. B. (2009) *Mol. Biol. Evol.* **26,** 71–84
49. Monk, K. R., Naylor, S. G., Glenn, T. D., Mercurio, S., Perlin, J. R., Dominguez, C., Moens, C. B., and Talbot, W. S. (2009) *Science* **325,** 1402–1405