Introduction of bisecting GlcNAc in N-glycans of adenylyl cyclase III enhances its activity

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Received on January 9, 2007; revised on February 16, 2007; accepted on February 19, 2007

Adenylyl cyclases (ACs) catalyze the synthesis of cAMP in response to extracellular and intracellular signals and are responsible for a wide variety of biological activities including cell growth, differentiation, and metabolism. There are nine, currently known, isoforms of transmembrane ACs, and the primary structure of the catalytic unit and the potential N-glycosylation sites are highly conserved among them. The enzyme β1,4-N-acetylglucosaminyltransferase III (GnT-III) catalyzes the addition of a bisecting N-acetylglucosamine (GlcNAc) to N-glycans. We have been studying the function of GnT-III on signaling molecules. In this study, we report on the effects of a bisecting GlcNAc on AC signaling. We established GnT-III stable transfectants expressing cell lines of Neuro-2a mouse neuroblastoma cells and B16 mouse melanoma cells. Forskolin-induced AC activation and downstream signaling, such as the synthesis of cAMP and the phosphorylation of transcriptional factor CRE-binding protein were upregulated in the GnT-III transfectants compared with mock transfectants or a dominant negative mutant of GnT-III-transfected cells. Since endogenous AC expression levels in Neuro-2a and B16 cells were too low to permit the glycosylation status to be examined, AC type III (ACIII) was overexpressed in a stable expression system using Flp-In-293 cells. The N-glycans of ACIII in the GnT-III transfectants were confirmed to be modified by the introduction of a bisecting GlcNAc, and AC activity was found to be significantly up-regulated in the GnT-III transfectants. Thus, the structure of N-glycans of ACIII regulates its enzymatic activity and downstream signaling.

Key words: adenylyl cyclase/bisecting GlcNAc/ glycosylation/GnT-III/N-glycan

Introduction

Adenylyl cyclases (ACs) catalyze the synthesis of cAMP in response to extracellular and intracellular signals and are involved in wide variety of biological activities including cell growth, differentiation, and metabolism. Nine isoforms of transmembrane ACs and a soluble AC have been isolated and analyzed to date, revealing diverse regulation by G-proteins, Ca2+, protein kinase A (PKA), and protein kinase C (PKC) (Sunahara et al. 1996; Smit and Iyengar 1998; Tang and Hurley 1998; Sunahara and Taussig 2002). All membrane-bound ACs contain two hydrophobic regions that comprise six transmembrane helices and three large cytoplasmic domains, and the primary structure of catalytic unit is conserved among ACs. Although the amino acid sequences in the extracellular regions vary among AC isozymes, potential N-glycosylation sites are highly conserved among them and are located on predicted extracellular loop 5 and/or loop 6, suggesting the importance of glycosylation in the function of ACs.

N-glycans on glycoproteins regulate their function in various manners; for example, N-glycans affect the intracellular transport of membrane proteins, the ligand binding rate of receptors, dimerization status or the endocytosis rate of receptors. The mechanisms of this regulation have been studied, and it was proposed that N-glycans alter the physico-chemical properties of core proteins, such as conformation, flexibility, and hydrophilicity, and thus regulate protein sorting, stability, and protein–protein interactions (Dennis et al. 1999; Rudd et al. 2001; Helenius and Aebl 2004; Freeze and Aebl 2005; Taniguchi et al. 2006). Lectin or lectin-like molecules, which recognize the peripheral structures of N-glycans, are also involved in the regulation of glycoproteins (Partridge et al. 2004; Ohtsubo et al. 2005). N-glycans have a common core structure, and their branching patterns are determined by glycosyltransferase enzymes. β1,4-N-acetylglucosaminyltransferase III (GnT-III) is a glycosyltransferase that catalyzes the addition of a bisecting GlcNAc residue to the β-mannoside of the trimannose core in N-glycans (Narasimhan 1982; Nishikawa et al. 1992; Taniguchi et al. 1999; Stanley 2002). GnT-III is known to suppress the elongation of N-glycans, since other glycosyltransferases such as GnT-IV and GnT-V, both of which are involved in the formation of multiantennary sugar chains, are not able to act on N-glycans that contain a bisecting GlcNAc (Schachter 1986; Gu et al. 1993).

To identify the role of N-glycans in the signaling pathway, we employed a promoter–reporter assay and found that the
forskolin-induced activation of the cAMP response element (CRE) was enhanced in the GnT-III transfectants. We observed that the forskolin-induced catalytic activity of AC was significantly increased in GnT-III-transfected Neuro-2a cells and B16 cells, compared with control cells. Exogenously expressed AC type III (ACIII) activity was also up-regulated by introduction of GnT-III, and it was confirmed that the N-glycan of AC was modified by a bisecting GlcNAc. Our findings demonstrate that ACIII activity is up-regulated by GnT-III transfection, and the mechanism by which AC activity is regulated by N-glycans is proposed.

Results

Increase in forskolin-induced downstream signaling in GnT-III-transfected Neuro-2a cells

Previous studies have demonstrated that glycosylation patterns regulate the function of many types of signaling molecules. We examined transcription from the elements of AP-1, CRE, heat shock element (HSE), Myc, NF-κB in several cell lines induced by various types of stimulation using a promoter–reporter assay method. Among those elements, we found that transcription from CRE was elevated by 2-fold in GnT-III-transfected Neuro-2a cells when stimulated with 10 μM forskolin at 37°C for 4 h (data is not shown). We confirmed that the phosphorylation of CRE-binding protein (CREB) at Ser-133 was enhanced in the GnT-III transfectants compared with mock transfectants or the D323A dominant negative mutant of GnT-III-transfected cells (Figure 1A). Since forskolin stimulates ACs that synthesize cAMP, leading to the activation of PKA and the phosphorylation of CREB, we then examined AC activity and cellular cAMP levels in Neuro-2a cells. As shown in Figure 1B, forskolin-induced AC activation was significantly up-regulated in the GnT-III transfectants. It was also observed that cAMP in the GnT-III transfectants was significantly increased compared with control cells (Figure 1C).

To determine whether similar phenomenon could be observed in other cell lines, we examined B16 cells and found that forskolin-induced AC activation and CREB phosphorylation were also elevated in GnT-III-transfected B16 cells (Figure 2A and B).

Identification of AC isoforms up-regulated by GnT-III transfection in Neuro-2a cells and B16 cells

Since there are nine, currently known, membrane-bound AC isoforms and one soluble isoform, we attempted to determine which isoform(s) is (are) affected by GnT-III overexpression. RT–PCR was carried out using Neuro-2a cells and B16 cells, and, as shown in Figure 3, ACIII, -VI, -VII, and -IX are common in those cell lines. As reported previously, ACIII, -VI, and -VIII are glycoproteins, and ACIX is insensitive to forskolin (Premont et al. 1996); thus we assumed that ACIII and/or ACVI might be affected by GnT-III. We examined the function of N-glycan of ACIII in the following experiments.

Establishment of Flp-In 293 cells stably expressing ACIII

Since endogenous AC expression levels in Neuro-2a and B16 cells were too low to examine the glycosylation status, we established ACIII stable expression cell lines using Flp-In
The expression levels of ACIII were confirmed by both western blotting and an activity assay. The ACIII transfectants showed two bands by immunoprecipitation and western blotting using an ACIII antibody, and the molecular masses were about 180 and 120 kDa (Figure 4A), consistent with previously reported data (Wei et al. 1996). As evidenced by the forskolin-treatment, AC activity was about 10 times higher enzymatic compared with the control (Figure 4B). These results indicate that ACIII stable expression cell lines were successfully established.

The profile of ACIII in GnT-III-transfected Flp-In 293 cells stably expressing ACIII

GnT-III, the D323A dominant negative mutant of GnT-III, and vector were then transfected into Flp-In 293 cells stably expressing ACIII, and the effect of GnT-III was investigated. The expression levels of GnT-III were confirmed by western blotting using a GnT-III-specific antibody and a GnT-III activity assay (data not shown). A western blot revealed that the protein levels of ACIII in each transfectant were similar (Figure 5A, left panel). When a lectin blot was performed, E4-PHA reacted with the upper band of ACIII in the GnT-III transfectants but not with ACIII in the mock transfectant and D323A dominant negative mutant of GnT-III transfectant (Figure 5A, right panel), indicating that N-glycans of ACIII in the GnT-III transfectant were modified by the insertion of a bisecting GlcNAc. When the cells were treated with N-glycosidase F (PNGase F), which cleaves N-glycans between the innermost N-acetylglucosamine and asparagines residue, only the lower bands could be detected (Figure 5B), indicating that the upper bands represented the glycosylated form and the lower bands represented the non-glycosylated form, as was suggested in a previous report (Wei et al. 1996). We confirmed that the cell surface expression levels of ACIII in the GnT-III transfectants and mock transfected or D323A dominant negative mutant of GnT-III-transfected cells were nearly the same (Figure 5C), but only the upper bands of the ACIII were detected. Forskolin-induced ACIII activity is increased in GnT-III-transfected Flp-In 293 cells stably expressing ACIII

AC activity in the GnT-III transfectants was examined. When cells were treated with 100 μM forskolin, the GnT-III transfectants showed an increased AC activity compared with mock transfectants and the D323A dominant negative mutant of GnT-III transfectants, as expected (Figure 6). Furthermore, when cells were treated with PMA, a calmodulin-dependent
Discussion

AC isoforms are tightly controlled by various signals and therefore contribute to the complexity of cellular signaling mechanisms. ACIII is expressed in several tissues including brain, spinal cord, adrenal medulla, adrenal cortex, heart, atrium, aorta, lung, retina (Xia et al. 1992), testis and spermatozoa (Defer et al. 1998; Gautier-Courteille et al. 1998; Livera et al. 2005), but it is particularly abundant in olfactory tissue, where it may play a major role in coupling olfactory receptors to cAMP and ion channel regulation (Bakalyar and Reed 1990). It has been demonstrated that ACIII-deficient mice fail several olfaction-based behavioral tests and lack electro-olfactogram responses elicited by either cAMP or inositol triphosphate, despite the presence of other AC isoforms in the olfactory cilia (Wong et al. 2000). Point mutations in the promoter region of the ACIII-encoding gene associated with the restoration of insulin release by forskolin have been reported in a type 2 diabetes model rat (Abdel-Halim et al. 1998). Thus, ACIII is possibly involved in various crucial biological events.

In this study, we reported that glycosyltransferase GnT-III participates in the regulation of the AC signaling pathway. We found that the overexpression of GnT-III up-regulates AC activity in Neuro-2a mouse neuroblastoma cells and B16 mouse melanoma cells (Figures 1B and 2A). In these GnT-III transfectants, downstream signaling such as the formation of cellular cAMP and CREB phosphorylation, is also enhanced (Figures 1A, C and 2B). To elucidate the mechanism by which AC activity is enhanced in the GnT-III transfectants, we constructed a stable expression system of ACIII and GnT-III using Flp-In 293 cells. We confirmed that neither the total expression nor the cell surface expression levels of ACIII were changed in the GnT-III transfectants (Figure 5A and C). Experiments with PNGase F suggest that ACIII is glycosylated in Flp-In 293 cells, and that the upper band represents the glycosylated form and the lower band the unglycosylated form (Figure 5B). The results of lectin blotting indicated that N-glycan of the upper band of ACIII is modified by the insertion of a bisecting GlcNAc in the GnT-III transfectants (Figure 5A). It appears likely that the modification of N-glycan affects the function of ACIII itself, since activity was measured in the membrane fraction preparations, which might be free from AC-regulating molecules (Figure 6), although we cannot completely rule out the possibility that the addition of bisecting GlcNAc to other glycoproteins in addition to ACIII affects AC activity.

The function of the N-glycan of ACs has been studied by several groups; it was proposed that glycosylation is involved in the Ca\(^{2+}\)-induced phosphorylation of ACIII (Wei et al. 1996). The glycosylation of ACVI significantly affects its catalytic activity in an activator-dependent manner and also alters the sensitivity of ACVI to the inhibition induced by a Gαi-coupled receptor or by PKC (Wu et al. 2001). The glycosylation state of ACVIII affects the catalytic activity without altering its \(K_m\) values or its dependence on calmodulin (Cali et al. 1996). These results strongly suggest that the N-glycan on ACs regulates AC activity. In the present study, we examined the effects of the modification of N-glycan structure of ACIII.
We assume that other AC isoforms might also be involved in the up-regulation of forskolin-induced CREB phosphorylation in GnT-III. The detailed mechanisms of how bisecting GlcNAc affects ACIII activity remain to be elucidated, but the possibility that ACIII with a bisecting GlcNAc becomes less responsive to inhibitory signals cannot be excluded since N-glycans regulate protein–protein interactions. In the case of ACVI, sensitivity toward the Goi-coupled dopamine D2 receptor decreases by the deletion of glycans. Since GnT-III is a key enzyme that inhibits the extension and modification of N-glycans by introducing a bisecting GlcNAc, it is possible that GnT-III transfection reduces the interaction between certain residues of the N-glycan of ACIII and inhibitory molecules.

Previous studies have shown that GnT-III affects a number of intracellular signaling pathways (Yoshimura et al. 1996; Ihara et al. 1997; Kitada et al. 2001; Sato et al. 2001; Bhattacharjya et al. 2002; Shibukawa et al. 2003; Yang et al. 2003; Zhao et al. 2006). For example, when GnT-III is overexpressed in PC12 cells, Trk A, a nerve growth factor receptor, is modified with a bisecting GlcNAc, which suppresses its dimerization (Ihara et al. 1997). GnT-III overexpression in HeLaS3 cells leads to the up-regulation of endocytosis of epidermal growth factor receptor (Sato et al. 2001). Furthermore, GnT-III transfection in HeLaS3 cells suppressed H2O2-induced activation of the PKCδ–JNK1 pathway, resulting in the inhibition of apoptosis (Shibukawa et al. 2003). Since cAMP is involved in a variety of physiological activities, the up-regulation of AC activity in GnT-III transfectants might be involved in the mechanisms of the above-observed phenomena.

Our results suggest that N-glycans on ACIII play an important role in regulating its activity. In a future study, we plan to analyze the structures of AC-linked N-glycans and to conduct detailed studies of the mechanisms by which N-glycans affect AC function.

Materials and methods

Promoter–reporter assay

To determine the pathway that is up-regulated in the GnT-III transfectants, we utilized the Pathway Profiling Systems (Clontech, Palo Alto, CA). Briefly, Neuro-2a cells were plated in six-well plates and transfected with promoter–reporter plasmids using Lipofectamine 2000 as described in the manufacturer’s protocol. These plasmids contained the luciferase reporter gene downstream of several copies of specific transcription factor-binding sequences such as AP-1, CRE, HSE, Myc, and NF-κB. After incubation at 37 °C for 18 h, the cells were starved for 7 h and then treated with different chemicals. Cells were lysed and luciferase activities were measured using the Dual-Luciferase Reporter Assay (Promega, Madison, WI) following the manufacturer’s protocol.

Construction of GnT-III and ACIII expression vectors

Rat GnT-III was subcloned in pCXN2 (Niwa et al. 1991), which contains a neo gene, and is regulated by the β-actin promoter. A D232A dominant negative mutant of GnT-III was constructed as reported previously. D323 in GnT-III is supposed to be involved in the coordination of a divalent cation such as Mn2+ along with a phosphoryl group of the donor nucleotide sugar and thus crucial for the enzymatic activity (Ihara et al. 2002). Human ACIII cDNA was cloned from human brain RNA; the full-length of human ACIII was amplified by RT–PCR with the following primers: CAGCAACATG TCATGGTATT (sense); CTTCAGCTGAATTGTGGCT (antisense). The coding region of ACIII was subcloned into an eukaryotic expression vector, pcDNA5/FRT (Invitrogen, Carlsbad, CA), driven by the cytomegalovirus promoter, for a stable high expression Flp-In system.

Cell culture and transfection

Neruo-2a mouse neuroblastoma cells and B16 mouse melanoma cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, low glucose), and Flp-In 293 cells (Invitrogen) were maintained in DMEM (high glucose) supplemented with 10% fetal bovine serum in an incubation chamber supplied with 5% CO2 at 37 °C. The cells were transfected with the desired construct using Lipofectamine 2000 (Invitrogen) following the manufacturer’s protocol. For GnT-III transfection, cells were selected by treatment with 1000 μg/mL of neomycin for 2 weeks, and for ACIII transfection, the cells were selected by treatment with 200 μg/mL of hygromycin B (Calbiochem, La Jolla, CA) for 2 weeks. Antibiotic-resistant colonies were isolated and cloned by serial dilution to ensure clonality. To confirm the expression of GnT-III or ACIII, western blotting and an enzyme activity assay were performed.

Immunoprecipitation and western blotting

Cell cultures were washed with ice-cold phosphate-buffered saline (PBS) and harvested in lysis buffer [20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% (w/v) Nonidet P-40, 10% (w/v) glycerol, 5 mM sodium pyrophosphate, 10 mM NaF, 1 mM sodium orthovanadate, 10 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 μg/mL aprotinin, 5 μg/mL leupeptin, and 1 mM dithiothreitol]. Cell lysates were centrifuged at 15 000 rpm for 15 min to obtain supernatants, and protein concentrations were determined using a protein assay CBB kit (Nacalai Tesque, Kyoto, Japan). For immunoprecipitation with ACIII, the lysate was incubated with a rabbit polyclonal antibody against ACIII (C-20) (Santa Cruz Biotechnology, Santa Cruz, CA) for 3 h at 4 °C and then with 15 μL of protein G-Sepharose for 2 h at 4 °C. For Western blotting, whole cell lysates or immunoprecipitates were subjected to SDS polyacrylamide gel electrophoresis (PAGE), and transferred to a PVDF membrane (Amersham Pharmacia Biotech, Piscataway, NJ). The membrane was blocked with 5% (w/v) skimmed milk in Tris-buffered saline containing 0.1% (v/v) Tween-20 (TBST, pH 7.5) for 2 h at room temperature and incubated with antibodies against CREB (Cell signaling), phospho-CREB (Cell signaling), ACIII (C-20) (Santa Cruz) or GnT-III (Fuji rebio Inc., Tokyo, Japan) overnight at 4 °C. After washing with TBST, the membranes were treated with the second antibodies for 1 h at room temperature. The membranes were washed and immunoreactive bands were visualized using an ECL kit (Amersham Pharmacia), according to the manufacturer’s instructions.
Measurement of total cellular cAMP
Cells were cultured overnight until 80% confluent in 96-well dishes (10^3 to 10^5 cells/well). After treatment with the indicated chemicals at 37 °C for 20 min, the cells were lysed and total cellular cAMP levels were determined using a Biotrak cAMP enzyme immunoassay kit (Amersham Pharmacia), following the manufacturer’s protocol.

Membrane fraction preparation for AC activity assay
Cells were harvested, resuspended in buffer A (25 mM Tris–HCl, 0.4 mM EDTA, 1 mM EGTA, 250 mM sucrose, 0.1 mM leupeptin, and 40 μM PMSF, pH 7.4), and homogenized using a glass Dounce homogenizer. The homogenate was centrifuged at 1500 rpm for 5 min to remove nuclei and broken cell pieces, and the supernatant centrifuged again at 50 000 g for 30 min to collect the membrane fractions. The pelleted membrane fraction was resuspended in buffer B (10 mM Tris–HCl, pH 7.4, 10 mM EDTA, 5 mM MgCl₂), and the protein concentrations determined using a protein assay CBB kit (Nacalai Tesuque, Japan).

AC activity assay
The catalytic activities of AC were measured as described previously, with minor modifications (Onda et al. 2001). The reaction mixtures contained 20 mM HEPES (pH 8.0), 0.1 mM ATP containing [α-^32P] ATP (1 × 10⁶ cpm) (Amersham Pharmacia), 0.1 mM cAMP, 1 mM creatine phosphate, 8 U/mL creatine phosphokinase, 5 mM MgCl₂, and 10 μg of membrane protein in a final volume of 100 μL. Reactions were performed at 30 °C for 20 min in the presence or absence of 100 μM forskolin and terminated by adding 10 μL of ice-cold 2.2 M HCl. The [^32P] cAMP produced was separated on acidic alumina columns (Alvarez and Daniels 1992). Briefly, the samples were applied to an acidic alumina column (ICN Pharmaceuticals, Inc., Costa Mesa, CA), which was washed with 5 mM HCl to remove any unbound contaminants, and the bound cAMP was eluted with 6 mL of 0.1 M ammonium acetate (pH 7.0). The radioactivity of the eluted samples was determined by scintillation counting.

RT–PCR analysis
Total RNA was prepared from Neuro-2a and B16 cells using the TRIzol reagent (Invitrogen), and the cDNAs were synthesized by Superscriptase II (Gibco BRL, Gaithersburg, MD) with an oligo (dT) primer. To determine the expression of each AC isoform, PCR was performed with the specific primers listed in Table I. The reaction conditions were as follows; 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Amplified cDNA fragments were confirmed by DNA electrophoresis on 1% agarose gel.

Lectin blotting
The immunoprecipitated ACIII was electrophoresed on 8% SDS–PAGE and transferred to PVDF membranes, as described above. The membrane was blocked with 3% bovine serum albumin (BSA) (w/v) in TBST and then incubated with 1 μg/mL of biotinylated erythroagglutinating phytohemagglutinin (E₄-PHA) (Seikagaku Corp., Tokyo, Japan) in TBST for 30 min at room temperature. After washing with TBST for three times, lectin-reactive proteins were detected by using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and an ECL kit.

PNGase F digestion
Whole-cell lysates of human ACIII transfectants were boiled with 0.1 M 2-mercaptoethanol and 0.1% SDS for 10 min. After boiling, the cell lysates were incubated with 60 mM Tris–HCl (pH 8.6), 0.8% Nonidet P-40, and 40 mU/mL of PNGase F (Roche Applied Science, Indianapolis, IN) at 37 °C, 16 h. The samples were then subjected to western blotting using an anti-ACIII antibody, as described above.

Cell surface biotinylation
Cells were cultured overnight until 80% confluence was reached. After washing three times with PBS supplemented with 0.1 mM CaCl₂ and 1 mM MgCl₂, the cells were incubated with 200 μg/mL of EZ-Link™ Sulfó-NHS-Biotin (Pierce, Rockford, IL) at room temperature for 30 min. The reaction was quenched with 25 mM Tris (pH 8.0) containing 150 mM NaCl. After washing three times with PBS, the cells were solubilized with 1 mL of lysis buffer, and the cell lysates were centrifuged at 15 000 rpm for 15 min to obtain the supernatant. Immunoprecipitation was performed using an anti-ACIII antibody and the immunoprecipitates were separated by 8% SDS–PAGE and then transferred to a PVDF membrane as described above. After blocking the membrane

Table I. Primers used in the RT–PCR of AC isoforms

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Sense</th>
<th>Antisense</th>
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<tr>
<td>ACI</td>
<td>ATGACGCAACAGGGGAGGGCATT</td>
<td>CAAGGATGTAAGGAAGTACAGGCC</td>
</tr>
<tr>
<td>ACII</td>
<td>AGACACCCCTCCGACTCGCAAT</td>
<td>GACGCCAGCTATTACAGGTTCA</td>
</tr>
<tr>
<td>ACIII</td>
<td>CTGAGCTTTTTACTTACTTCGCG</td>
<td>GAAGCGGTACTCTCCAAGGATGA</td>
</tr>
<tr>
<td>ACIV</td>
<td>CTGCTCCTTTTTCTGACACATGAG</td>
<td>TTTGCTCTTGACCTGTAGCTGCC</td>
</tr>
<tr>
<td>ACV</td>
<td>ACGAGGCAAAGCCCTCACAATAAG</td>
<td>ATAAAGTCTTACTCGGGGACCATT</td>
</tr>
<tr>
<td>ACVI</td>
<td>ACTGTCGCCCTTCTTCTGAGG</td>
<td>AGTACCCGCTTCGACCTGCTT</td>
</tr>
<tr>
<td>ACVII</td>
<td>CTGGACCAAGCGGACACAAAG</td>
<td>CTGGAGGATAGCGTCTCTTC</td>
</tr>
<tr>
<td>ACVIII</td>
<td>CTAACTCGTCTGCTGAGGCT</td>
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</tr>
<tr>
<td>ACIX</td>
<td>ATCCGAGGAGCAGGAGGACAGG</td>
<td>CGTCTCTGAGGGACACAGAG</td>
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with 3% (w/v) BSA in TBST overnight, a Vectastain ABC kit and an ECL kit were used to detect the biotinylated protein.

Acknowledgments

Grant support was provided by the 21st Century COE Program from the Japan Society for the promotion of Science; Core Research for Evolutional Science and Technology from Japan Science and Technology Agency; and Special Coordination Funds for Promoting Science and Technology, the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Conflict of interest statement

None declared.

Abbreviations

AC, adenylyl cyclase; ACIII, adenylyl cyclase type III; BSA, bovine serum albumin; CRE, cAMP response element; CREB, CRE-binding protein; DMEM, Dulbecco’s modified Eagle’s medium; GlcNAC, N-acetylgalactosamine; GnT-III, β1, 4-N-acetylgalactosaminyltransferase III; HSE, heat shock element; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PKA, cAMP-dependent protein kinase A; PKC, protein kinase C; PMSF, phenylmethylsulfonyl fluoride; PNGase, N-glycosidase F; TBST, Tris-buffered saline with Tween-20.

References


