

Activation of p38 Mitogen-activated Protein Kinase by Signaling through G Protein-coupled Receptors

INVOLVEMENT OF $G\beta\gamma$ AND $G\alpha_{q/11}$ SUBUNITS*

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Various extracellular stimuli activate three classes of mitogen-activated protein kinases (MAPKs): extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 MAPK. In mammalian cells, p38 MAPK is activated by endotoxins, inflammatory cytokines, and environmental stresses. We show here that p38 MAPK is also activated upon stimulation of G protein-coupled receptors (G_q/G_{11} -coupled m1 and G_i -coupled m2 muscarinic acetylcholine and G_s -coupled β -adrenergic receptors) in human embryonal kidney 293 cells. The activation of p38 MAPK through the m2 and β -adrenergic receptors was completely inhibited by coexpression of $G\alpha_o$, whereas the activation by the m1 receptor was only partially inhibited. Furthermore, we show that overexpression of $G\beta\gamma$ or a constitutively activated mutant of $G\alpha_{q/11}$, but not $G\alpha_s$ and $G\alpha_i$, can stimulate p38 MAPK. These results suggest that the signal from the m2 and β -adrenergic receptors to p38 MAPK is mediated by $G\beta\gamma$, whereas the signal from the m1 receptor is mediated by both $G\beta\gamma$ and $G\alpha_{q/11}$.

A variety of extracellular stimuli cause the sequential protein phosphorylation leading to the activation of mitogen-activated protein kinases (MAPKs),¹ which are divided into three major classes: MAPK/ERK, JNK/SAPK, and p38 MAPK (1, 2). MAPK/ERK is stimulated by growth factors and cytokines and phosphorylates various target proteins (including transcription factors, protein kinases, and cytoskeletal proteins) to transmit signals directed toward cell growth and differentiation (3–5). On the other hand, stresses such as osmotic shock and ultraviolet radiation or inflammatory cytokines including tumor necrosis factor- α and interleukin-1 stimulate the activities of JNK/SAPK and p38 MAPK (6). JNK/SAPK phosphorylates and activates transcription factors such as c-Jun (7, 8) and ATF2 (9), whereas p38 MAPK phosphorylates not only transcription factors such as ATF2 (10), CHOP (11), and Max (12), but also MAPK-activated protein kinases, which phosphorylate the

small heat shock protein HSP25/27 (13–15). The physiological significance of p38 MAPK is not yet fully understood.

Heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins) are composed of α , β , and γ subunits and mediate a variety of extracellular signals from seven transmembrane receptors to effector molecules (16–20). Several G protein-coupled receptors are known to stimulate the MAPK/ERK pathway through different G protein subunits. In the case of G_q/G_{11} -coupled m1 muscarinic acetylcholine and α_1 -adrenergic receptors, the activation of MAPK/ERK is mediated primarily by α subunits (21, 22). On the other hand, G_i -coupled m2 muscarinic acetylcholine and α_2 -adrenergic receptors (21–24) and G_s -coupled β -adrenergic receptor (25) induce MAPK/ERK activation mainly through $G\beta\gamma$. It has been demonstrated that JNK/SAPK is activated also by stimulation of m1 and m2 muscarinic acetylcholine receptors (26, 27). Involvement of $G\beta\gamma$ (28) as well as $G\alpha_{q/11}$, $G\alpha_{12}$, and $G\alpha_{13}$ has also been reported in JNK/SAPK activation (29–31). On the other hand, much less is known about the activation of p38 MAPK by G protein-coupled receptors.

In this study, we demonstrate that p38 MAPK is activated by the stimulation of m1 and m2 muscarinic acetylcholine and β -adrenergic receptors. Furthermore, we examine the involvement of G protein α , β , and γ subunits in p38 MAPK activation.

MATERIALS AND METHODS

Antibodies—Rabbit polyclonal antibodies (RM/1 and AS/7) against C-terminal segments of $G\alpha_s$ and $G\alpha_{12}$, respectively, were purchased from NEN Life Science Products. Rabbit polyclonal anti- $G\alpha_{11}$ antibody raised against amino acids 13–29 of $G\alpha_{11}$ was from Santa Cruz Biotechnology, Inc. Rabbit anti- $G\alpha_o$ antibody was produced as described previously (44). Rabbit polyclonal antibody (06-238) against a peptide corresponding to amino acids 127–139 identical in $G\beta_1$ and $G\beta_2$ was from Upstate Biotechnology, Inc. Mouse monoclonal antibody (M2) against the FLAG epitope (8 amino acids, EYKEEEEK) was purchased from Eastman Kodak Co. Rabbit anti-mouse Ig antibody (55480) was obtained from Cappel. Anti-mouse (NA931) and anti-rabbit (NA934) Ig antibodies conjugated with horseradish peroxidase were from Amersham Life Science, Inc.

Construction of Plasmids—Complementary DNA for CSBP1 (32), a human homologue of p38 MAPK, was isolated from total RNA of HeLa cells by reverse transcription-polymerase chain reaction. The FLAG epitope was inserted between Met-1 and Ser-2 of p38 MAPK using synthetic polymerase chain reaction primers. The DNA of FLAG epitope-tagged p38 MAPK was subcloned into the *EcoRI* restriction site of the pCMV5 mammalian expression vector (33). cDNA for the N terminus (amino acids 1–96) of ATF2 (9, 10, 34) was amplified from a rat brain cDNA library (CLONTECH) and subcloned into the *EcoRI* site of the pGEX-2T *Escherichia coli* expression vector. Plasmids of human m1 and m2 muscarinic acetylcholine receptors (35) were kindly provided by Dr. E. M. Ross (University of Texas Southwestern Medical Center). Rat $G\alpha_o$ ($G\alpha_{o1}$) cDNA (36, 37) was ligated with *SmaI*-digested pCMV5. Bovine $G\alpha_s$ ($G\alpha_{s4}$) cDNA (38) was subcloned into *KpnI/HindIII* sites of pCMV5. Rat $G\alpha_{12}$ cDNA (36, 39) was inserted into *EcoRI/XbaI* sites of pCMV5. Mouse $G\alpha_{11}$ cDNA (40) was isolated by reverse transcription-polymerase chain reaction from total RNA of S49 cells and ligated with *HindIII*-digested pCMV5. DNAs for constitutively acti-

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¹ The abbreviations used are: MAPKs, mitogen-activated protein kinases; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; G protein, heterotrimeric guanine nucleotide-binding regulatory protein; $G\alpha$, G protein α subunit; $G\beta\gamma$, G protein $\beta\gamma$ subunit; HEK, human embryonal kidney; GST, glutathione S-transferase; [³H]QNB, [³H]quinuclidinyl benzilate.

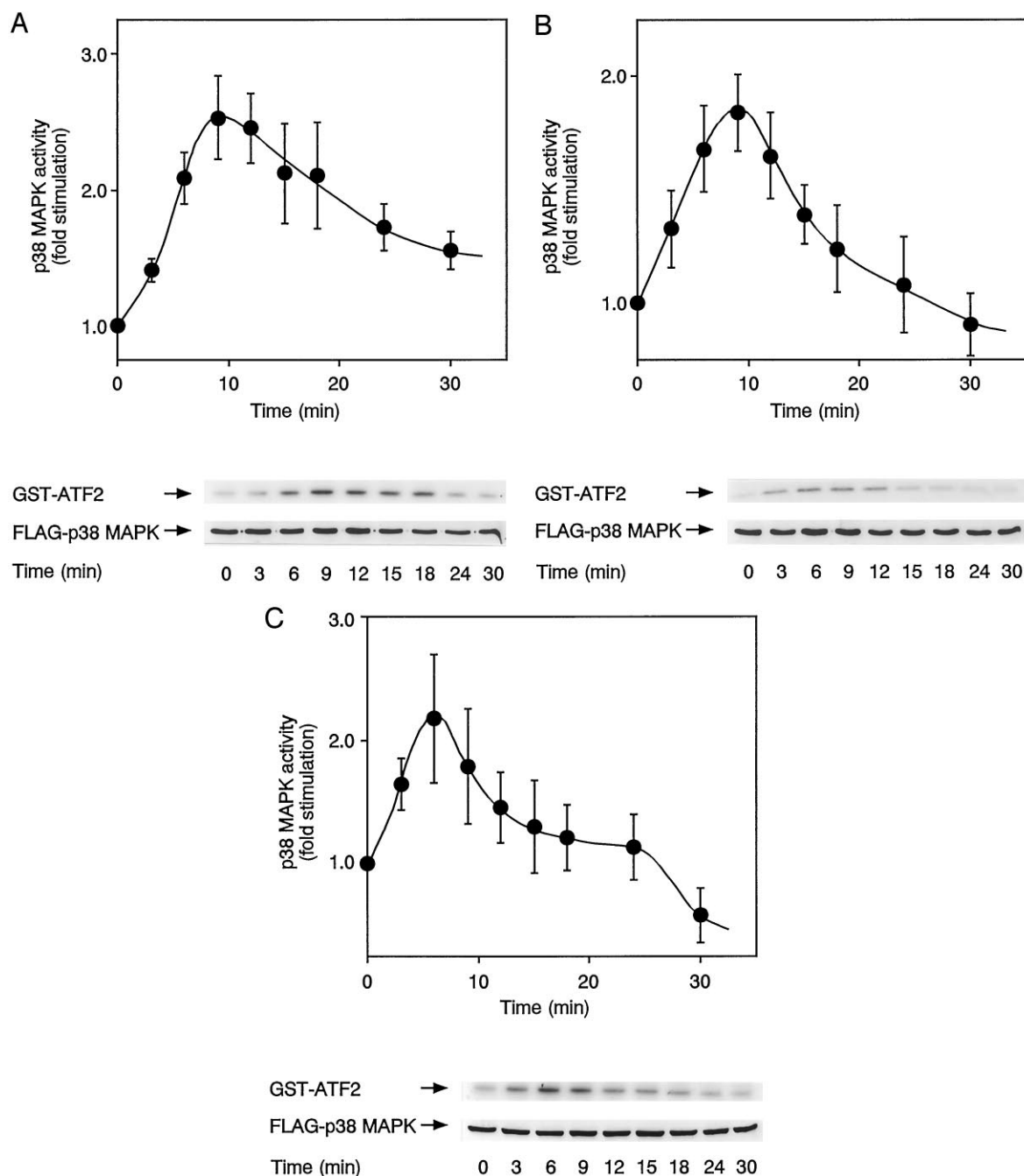


FIG. 1. Time course of p38 MAPK activation upon stimulation of m1 and m2 muscarinic acetylcholine and β -adrenergic receptors. HEK 293 cells were transfected with plasmids carrying cDNAs for FLAG-p38 MAPK (A–C) and m1 (A) and m2 (B) muscarinic acetylcholine receptors. p38 MAPK activity was measured at different time points after the addition of 10 μ M carbachol (A and B) or 10 μ M isoproterenol (C) as described under “Materials and Methods.” The values shown represent the means \pm S.E. from three separate experiments. The phosphorylation of GST-ATF2 and the expression of FLAG-p38 MAPK in the cell lysate are shown.

vated GTPase-deficient mutants of $G\alpha_s$, $G\alpha_{i2}$, and $G\alpha_{i11}$ (α_s Q213L, α_{i2} Q205L, and α_{i11} Q209L) were generated by primer-mediated mutagenesis and inserted into pCMV5. cDNAs of bovine $G\beta_1$ (41) and $G\gamma_2$ (42) were generously provided by Dr. M. I. Simon (California Institute of Technology) and Dr. T. Nukada (Tokyo Institute of Psychiatry), respectively. The $G\beta_1$ and $G\gamma_2$ cDNAs were subcloned into pCMV5 as described previously (43). DNA sequence was confirmed by the dideoxy method using Sequenase Version 2.0 (Amersham Life Science, Inc.).

Cell Culture—Human embryonic kidney (HEK) 293 cells (ATCC CRL 1573) were maintained in Dulbecco's modified Eagle's medium (Sigma) containing 1 mg/ml kanamycin (Sigma) with 10% heat-inactivated fetal bovine serum (Life Technologies, Inc.). The cells were cultured at 37 °C in a humidified atmosphere containing 10% CO₂.

Transfection—Plasmid DNAs were transfected into HEK 293 cells by calcium phosphate precipitation. The final amount of transfected DNA for a 60-mm dish was adjusted to 30 μ g with the empty vector pCMV5.

Three μ g of pCMV5-FLAG-p38 MAPK was transfected together with 0.3 μ g of pCMVm1R, 0.9 μ g of pCMVm2R, 5 μ g of pCMVG α_s , 5 μ g of pCMVG β_1 , 5 μ g of pCMVG γ_2 , 10 μ g of pCMVG α_{i2} , 10 μ g of pCMVG α_{i11} , 10 μ g of pCMVG α_s Q213L, 10 μ g of pCMVG α_{i2} Q205L, 10 μ g of pCMVG α_{i11} Q209L. The medium was replaced 24 h after transfection, and the cells were starved in the serum-free medium containing 1 mg/ml bovine serum albumin for 24 h.

Immune Complex p38 MAPK Assay—After 24 h of starvation, the transfected cells were treated with or without carbachol, isoproterenol, or anisomycin at 37 °C and lysed in 600 μ l of lysis buffer A (20 mM HEPES-NaOH (pH 7.5), 3 mM MgCl₂, 100 mM NaCl, 1 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride, 1 μ g/ml leupeptin, 1 mM EGTA, 1 mM Na₃VO₄, 10 mM NaF, 20 mM β -glycerophosphate, and 0.5% Nonidet P-40) on ice. The lysate was centrifuged at 14,000 rpm for 10 min at 4 °C. Aliquots of the supernatants were mixed with mouse anti-FLAG antibody (0.3 μ g). FLAG-p38 MAPK was precipitated with

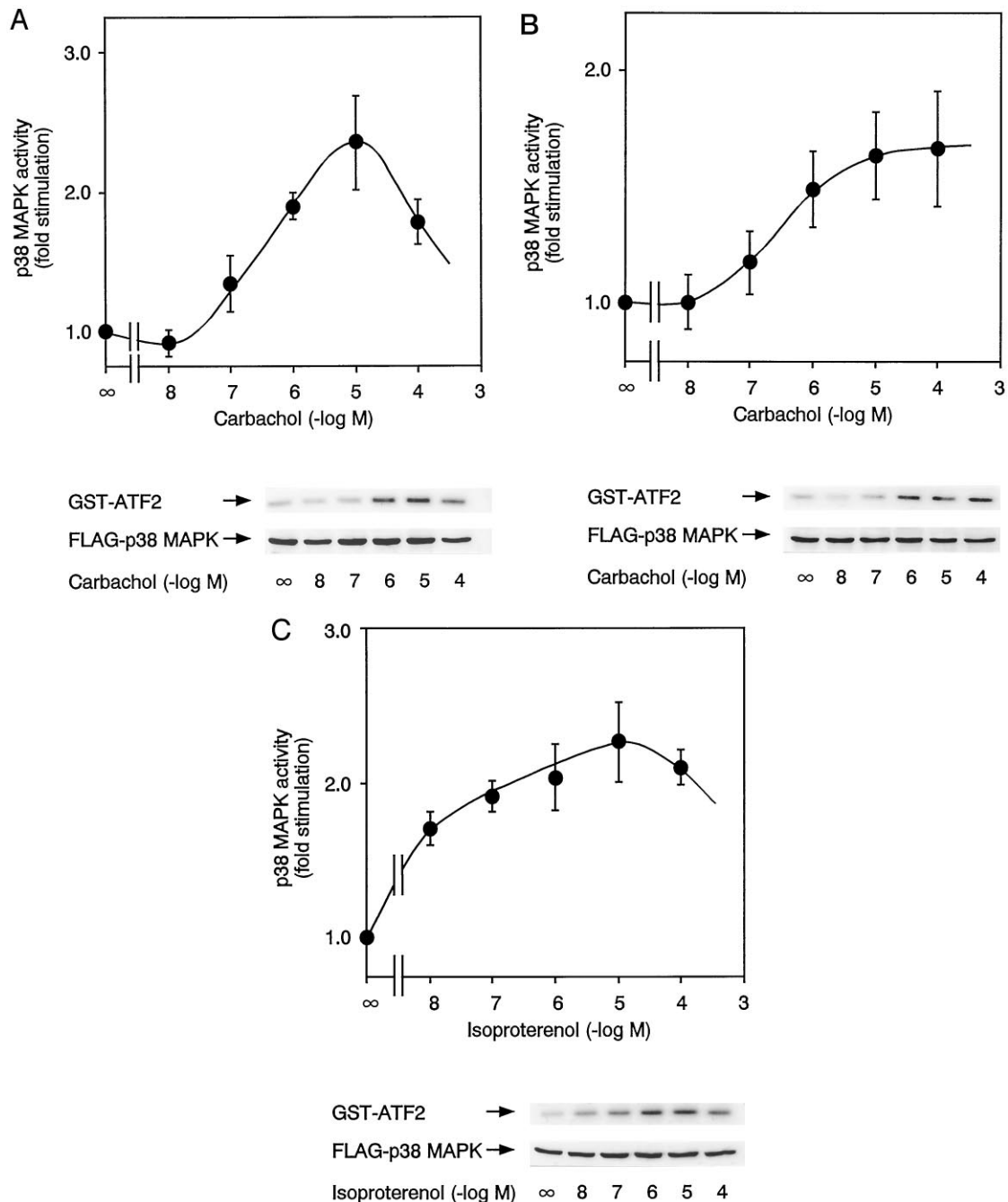


FIG. 2. Dose-dependent p38 MAPK activation upon stimulation of m1 and m2 muscarinic acetylcholine and β -adrenergic receptors. Cells were transfected with plasmids carrying cDNAs for FLAG-p38 MAPK (A–C) and m1 (A) and m2 (B) muscarinic acetylcholine receptors. p38 MAPK activity was measured at 10 min (A and B) or at 5 min (C) after the addition of increasing concentrations of carbachol (A and B) or isoproterenol (C) as described under “Materials and Methods.” The values shown represent the means \pm S.E. from three separate experiments. The phosphorylation of GST-ATF2 and the expression of FLAG-p38 MAPK in the cell lysate are shown.

protein A-Sepharose CL-4B (Pharmacia Biotech Inc.) preabsorbed with rabbit anti-mouse Ig (1 μ g) and washed twice with lysis buffer A and twice with reaction buffer A (20 mM HEPES-NaOH (pH 7.5), 10 mM MgCl₂, 0.1 mM phenylmethanesulfonyl fluoride, 0.1 μ g/ml leupeptin, 0.1 mM EGTA, 0.1 mM Na₃VO₄, and 0.2 mM β -glycerophosphate). The washed immunoprecipitates were incubated in 30 μ l of reaction buffer A containing 1 μ g of affinity-purified GST-ATF2 (amino acids 1–96), 20 μ M ATP, and 5 μ Ci of [γ -³²P]ATP (ICN Pharmaceuticals, Inc.) at 30 °C for 15 min, and the reaction was stopped by adding 10 μ l of 4 \times Laemmli sample buffer (Laemmli sample buffer = 50 mM Tris-HCl (pH 6.8), 2% SDS, 30 mM dithiothreitol, and 10% glycerol). The boiled samples were subjected to SDS-polyacrylamide gel electrophoresis, and the radioactivity incorporated into GST-ATF2 was measured by an imaging analyzer (Fuji BAS 2000) and detected by autoradiography.

Immunoblotting—Cell lysates were boiled in Laemmli sample buffer.

The boiled samples were separated by SDS-polyacrylamide gel electrophoresis, and the proteins were transferred to nitrocellulose membranes. After the membranes were blocked, the separated proteins were immunoblotted with mouse anti-FLAG antibody or rabbit antibody of various G protein subunits. The bound antibodies were visualized by an enhanced chemiluminescence detection system using anti-Ig antibody conjugated with horseradish peroxidase as a secondary antibody.

Ligand Binding Assays—Binding studies were carried out with crude membranes and [³H]QNB (NEN Life Science Products), a muscarinic receptor antagonist. The transfected cells were harvested and disrupted by a Teflon homogenizer in HME buffer (20 mM HEPES (pH 8.0), 2 mM MgCl₂, and 1 mM EDTA). After unbroken cells and nuclei were removed by centrifugation at 700 \times g for 5 min, crude membranes were collected by centrifugation at 100,000 \times g for 30 min and resuspended in HME buffer. [³H]QNB binding was determined by incubating

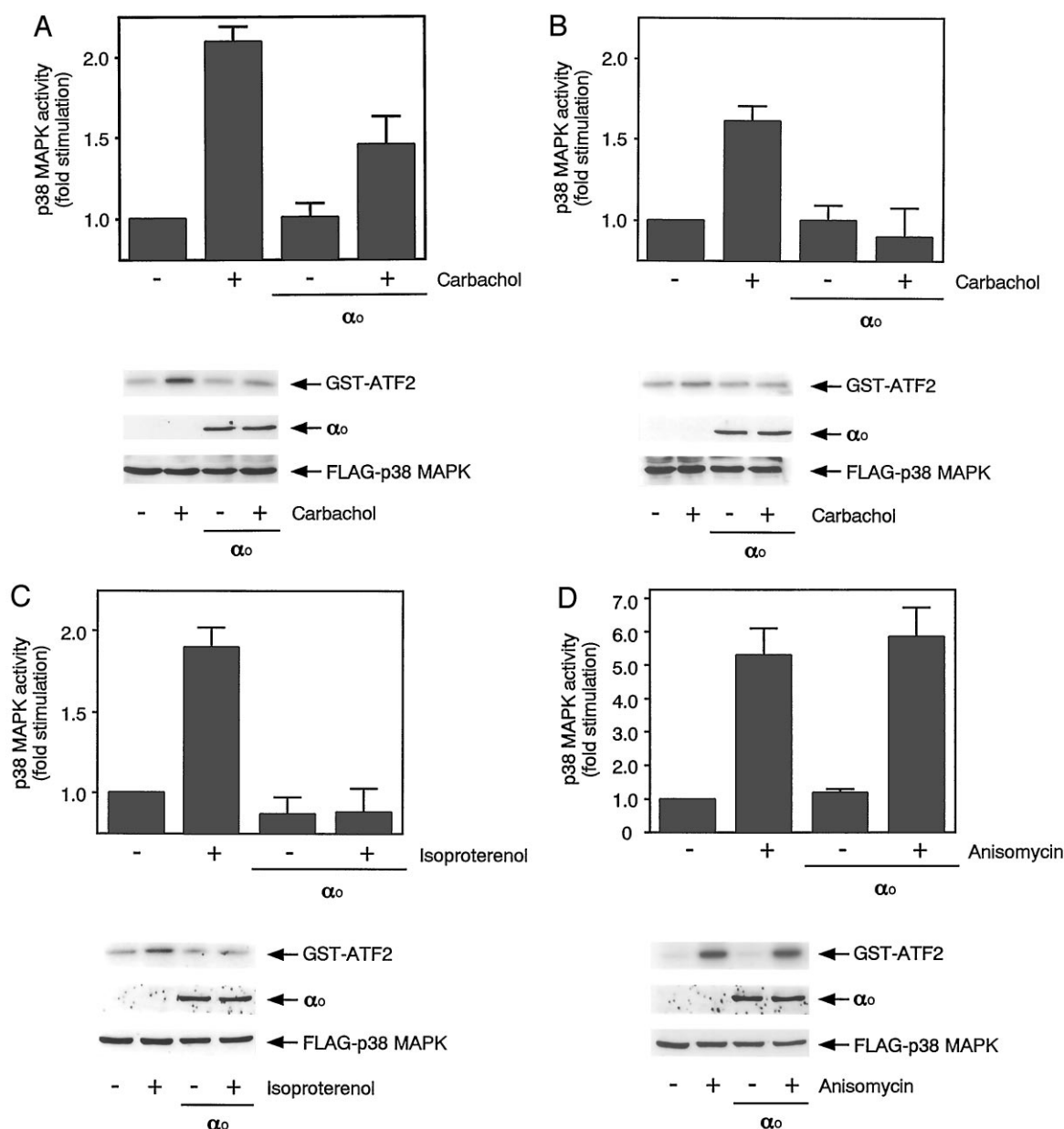


FIG. 3. **p38 MAPK activation by stimulation of muscarinic acetylcholine and β -adrenergic receptors is blocked by coexpression of $G\alpha_o$.** Cells were transfected with plasmids carrying cDNAs for FLAG-p38 MAPK (A–D), m1 (A) and m2 (B) muscarinic acetylcholine receptors, and $G\alpha_o$ (α_o) (A–D) as indicated. The cells were treated with (+) or without (–) 10 μ M carbachol (A and B), 10 μ M isoproterenol (C), or 20 mg/ml anisomycin (D) for 10 min (A and B), 5 min (C), or 30 min (D). p38 MAPK activity was measured as described under “Materials and Methods.” The values shown represent the means \pm S.E. from three separate experiments. The phosphorylation of GST-ATF2 and the expression of $G\alpha_o$ and FLAG-p38 MAPK in the cell lysate are shown.

50 μ g of the membrane protein with [3 H]QNB in a final volume of 50 μ l at 30 $^{\circ}$ C for 30 min. Nonspecific binding was determined in the presence of 10 μ M atropine and was always <5% of total [3 H]QNB binding. Reactions were terminated by the addition of ice-cold wash buffer (10 mM Tris-HCl (pH 7.5) and 5 mM $MgCl_2$), followed immediately by filtration with glass-fiber filters (Whatman GF/C). Filters were rinsed with 4 ml of wash buffer and air-dried. The radioactivity of the filters was measured by a liquid scintillation counter (Beckman LS6500). The data represent the means \pm S.E. from three experiments.

RESULTS

p38 MAPK Is Activated by Stimulation of Muscarinic Acetylcholine and β -Adrenergic Receptors—To examine the regulation of p38 MAPK activity through G protein-coupled receptors, HEK 293 cells were transiently transfected with cDNA encoding p38 MAPK that was tagged with the FLAG epitope (8 amino acids) at the N-terminal position. Using a monoclonal anti-FLAG antibody, epitope-tagged p38 MAPK was detected

and immunoprecipitated from the transfected cell lysate, and the *in vitro* kinase activity was assayed using a specific substrate (GST-ATF2) containing the activation domain (amino acids 1–96) of ATF2 (9, 10). The expression level of FLAG epitope-tagged p38 MAPK was investigated in each experiment to compare the transfection efficiency.

Fig. 1 (A and B) shows the time course of p38 MAPK activation following the stimulation by carbachol, an agonist, in the cells transfected with m1 and m2 muscarinic acetylcholine receptors, respectively. Mock-transfected cells did not respond to carbachol (data not shown). The peak of p38 MAPK activation was observed \sim 10 min after the stimulation of both receptors. p38 MAPK activation upon stimulation of the endogenous β -adrenergic receptor was also observed upon the addition of isoproterenol, a β -adrenergic agonist (Fig. 1C). The activation of p38 MAPK induced by the m1 receptor was slightly higher

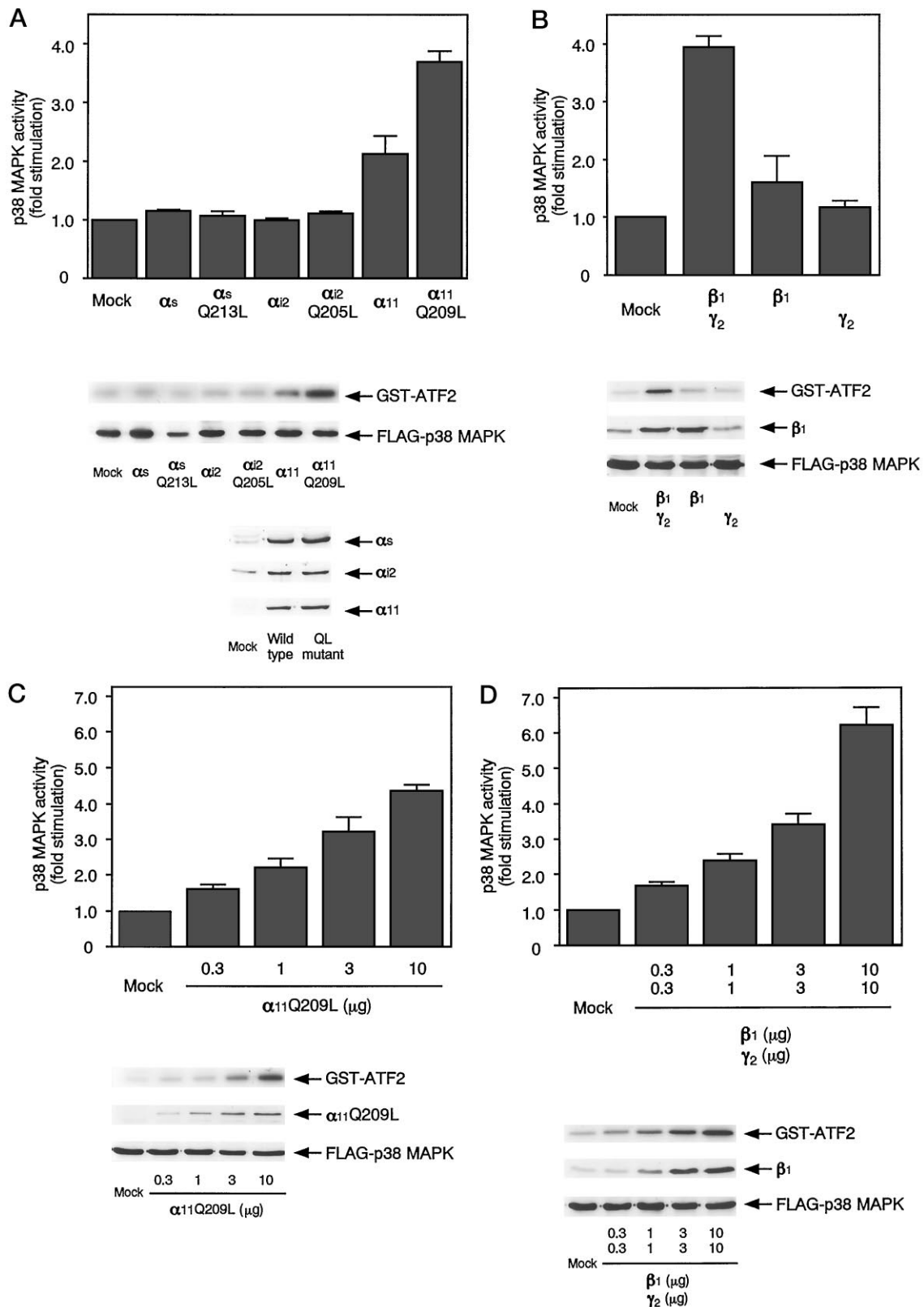


FIG. 4. Transfection of $G\alpha_{11}$ Q209L and $G\beta_1\gamma_2$, but not $G\alpha_s$ Q213L and $G\alpha_{i2}$ Q205L, stimulates p38 MAPK activity. Cells were transfected with a plasmid carrying cDNA for FLAG-p38 MAPK plus vector alone (*Mock*) or plasmids of wild-type $G\alpha_s$ (*as*), $G\alpha_s$ Q213L (*as*Q213L), wild-type $G\alpha_{i2}$ (*ai2*), $G\alpha_{i2}$ Q205L (*ai2*Q205L), wild-type $G\alpha_{11}$ (*11*), $G\alpha_{11}$ Q209L (*11*Q209L), $G\beta_1$ (*11*), and $G\gamma_2$ (*2*) as indicated (A–D). p38 MAPK activity was measured as described under “Materials and Methods.” The values shown represent the means \pm S.E. from three or five separate experiments. The phosphorylation of GST-ATF2 and the expression of FLAG-p38 MAPK and G protein subunits in the cell lysate are shown.

than that induced by the m2 and β -adrenergic receptors.

The p38 MAPK activation by m1 and m2 muscarinic acetylcholine and β -adrenergic receptors was dependent on the con-

centrations of carbachol and isoproterenol, respectively, with maximum response at $\sim 10 \mu\text{M}$ (Fig. 2). Pretreatment of the cells with atropine and propranolol (muscarinic and β -adrener-

gic antagonists) completely abolished the p38 MAPK activation by carbachol and isoproterenol, respectively (data not shown).

p38 MAPK Activation by G Protein-coupled Receptors Is Mediated by $G\alpha$ and/or $G\beta\gamma$ —Next, we examined whether $G\beta\gamma$ is involved in the p38 MAPK activation by these receptors. Cotransfection of $G\alpha_o$ is thought to remove free $G\beta\gamma$, dissociated from $G\alpha$ upon receptor stimulation. It has been previously demonstrated that ERK2 activation induced by $G\beta\gamma$ and stimulated by G protein-coupled receptors was blocked by cotransfection of $G\alpha_t$ (23) or $G\alpha_o$ (43). It has been shown that $G\alpha_o$ forms a complex with $G\beta\gamma$ in HEK 293 cells (44). The activation of p38 MAPK by the G_q/G_{11} -coupled m1 muscarinic acetylcholine receptor was reduced to ~50% by cotransfection with $G\alpha_o$, whereas the activation by G_i -coupled m2 and G_s -coupled β -adrenergic receptors was completely blocked (Fig. 3, A–C).

To examine whether the coexpression of $G\alpha_o$ does not affect the expression of exogenous muscarinic receptors, we carried out the ligand binding assays using a specific radiolabeled antagonist, [3H]QNB. The numbers of expressed muscarinic receptors were determined by Scatchard analysis of [3H]QNB saturation binding, and the B_{max} values from three experiments are as follows: the m1 receptor without $G\alpha_o$, 3850 ± 570 fmol/mg of protein; the m1 receptor with $G\alpha_o$, 4360 ± 440 fmol/mg of protein; the m2 receptor without $G\alpha_o$, 187 ± 30 fmol/mg of protein; and the m2 receptor with $G\alpha_o$, 156 ± 36 fmol/mg of protein. It was reported that the numbers of muscarinic receptors in cerebral cortex and heart were 2600 and 110 fmol/mg of protein, respectively (61, 62). Since the muscarinic receptors in cerebral cortex and heart appear to be mainly m1 and m2 receptors, respectively (35), the level of receptor expression in the transfected cells seems to be physiological.

These results suggest that both $G\beta\gamma$ and $G\alpha_{q/11}$ may mediate the signal from the m1 receptor to p38 MAPK, whereas the p38 MAPK activation by the m2 and β -adrenergic receptors may be predominantly mediated by $G\beta\gamma$. On the other hand, the p38 MAPK activation by anisomycin was not blocked by the cotransfection of $G\alpha_o$ (Fig. 3D), indicating that the blockage of the p38 MAPK activation by $G\alpha_o$ appears to be specific for G protein-coupled receptors.

We then investigated whether the transfection of G protein α , β , and γ subunits induces p38 MAPK activation. The cells were transfected with wild-type or constitutively activated mutants of $G\alpha_s$, $G\alpha_{i2}$, and $G\alpha_{11}$, and $G\beta_1$ and/or $G\gamma_2$ (Fig. 4). Expression of FLAG epitope-tagged p38 MAPK was comparable when each subunit was transfected into HEK 293 cells. Transfection of the activated $G\alpha_{11}$ mutant stimulated p38 MAPK activity by ~4-fold, whereas transfection of the activated mutant of $G\alpha_s$ or $G\alpha_{i2}$ failed to activate p38 MAPK (Fig. 4A). Cotransfection of $G\beta_1$ and $G\gamma_2$ enhanced the kinase activity by ~4-fold, but transfection of either $G\beta_1$ or $G\gamma_2$ alone was not effective (Fig. 4B). Fig. 4 (C and D) shows the dose-dependent activation of p38 MAPK by the $G\alpha_{11}$ mutant and $G\beta\gamma$. A good correlation between p38 MAPK activation and expression of the activated $G\alpha_{11}$ mutant and $G\beta_1$ was observed.

To examine whether inhibition of the carbachol-induced p38 MAPK activation by $G\alpha_o$ (Fig. 3) was due to the sequestration of $G\beta\gamma$, $G\alpha_o$ was cotransfected with the activated $G\alpha_{11}$ mutant or $G\beta_1\gamma_2$ into HEK 293 cells (Fig. 5). The p38 MAPK activation by $G\beta_1\gamma_2$, but not by the $G\alpha_{11}$ mutant, was completely blocked by cotransfection of $G\alpha_o$. Expression of exogenous $G\beta\gamma$ was higher than that of endogenous $G\beta\gamma$. It is likely that the coexpression of $G\alpha_o$ fully inhibits the function of endogenous $G\beta\gamma$.

DISCUSSION

p38 MAPK was identified as a tyrosine-phosphorylated protein with an apparent molecular mass of 38 kDa in lipopolysaccharide-treated cells (45, 46). A variety of extracellular stimuli,

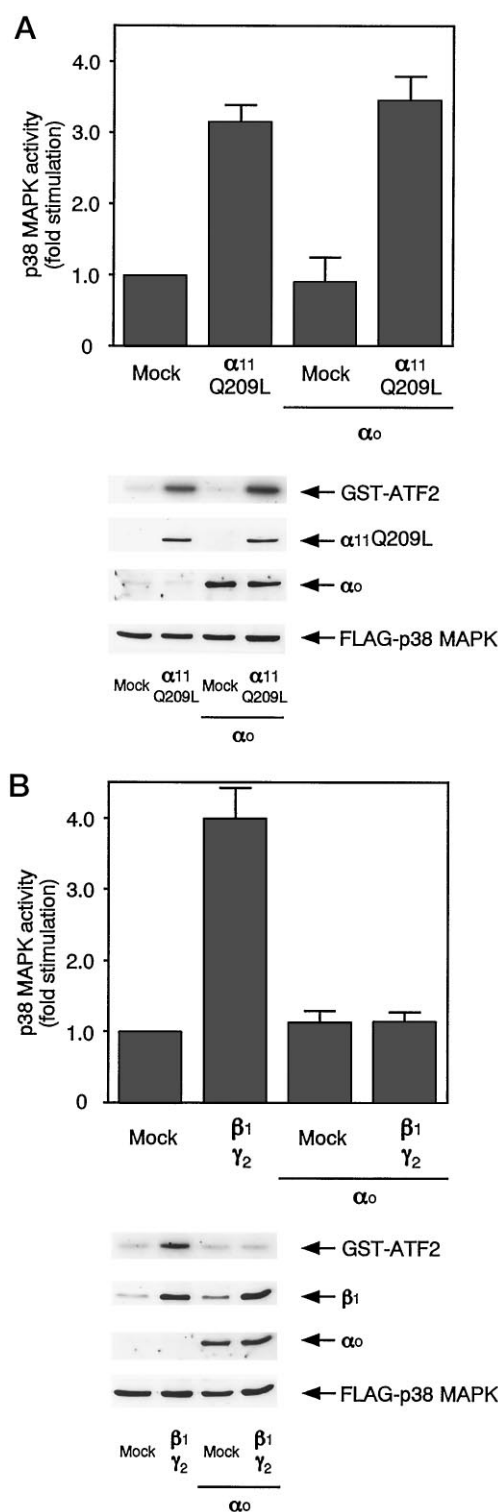


FIG. 5. $G\beta\gamma$ -induced p38 MAPK activation is blocked by coexpression of $G\alpha_o$. Cells were transfected with a plasmid carrying cDNA for FLAG-p38 MAPK plus vector alone (mock) or plasmids of $G\alpha_{11}$ Q209L (α_{11} Q209L), $G\beta_1$ (β_1), $G\gamma_2$ (γ_2), and $G\alpha_o$ (α_o) as indicated (A and B). p38 MAPK activity was measured as described under "Materials and Methods." The values shown represent the means \pm S.E. from three separate experiments. The phosphorylation of GST-ATF2 and the expression of FLAG-p38 MAPK and G protein subunits in the cell lysate are shown.

such as endotoxins, inflammatory cytokines, and stresses, activate p38 MAPK in mammalian cells (10). Recently, Krammer *et al.* (47, 48) showed that thrombin induces the activation of p38 MAPK in human platelets. Saklatvala *et al.* (49) reported

that a specific inhibitor of p38 MAPK inhibits platelet aggregation and secretion induced by a thromboxane analogue. More recently, Krump *et al.* (50) also reported that the chemotactic peptide (formylmethionylleucylphenylalanine) induces p38 MAPK activation, leading to the activation of MAPK-activated protein kinase-2 in human neutrophils. Although thrombin, thromboxane, and formylmethionylleucylphenylalanine receptors are known to be members of the G protein-coupled receptor family, it remains to be shown whether each G protein subunit mediates the signals from the receptors to p38 MAPK.

In this study, we showed that p38 MAPK is activated by the stimulation of m1 and m2 muscarinic acetylcholine and β -adrenergic receptors in HEK 293 cells. The activation of p38 MAPK by the β -adrenergic and m2 receptors was completely inhibited by coexpression of $G\alpha_s$, whereas the activation by the m1 receptor was partially inhibited (Fig. 3). Expression of a constitutively activated mutant of $G\alpha_{11}$, but not $G\alpha_s$ and $G\alpha_{12}$, activated p38 MAPK, and overexpression of $G\beta_1\gamma_2$ also induced activation (Fig. 4). It seems likely that the p38 MAPK activation by the m1 muscarinic acetylcholine receptor is mediated by both $G\beta\gamma$ and $G\alpha_{q/11}$, whereas the activation by the β -adrenergic and m2 receptors is mainly mediated by $G\beta\gamma$, but not by $G\alpha_s$ and $G\alpha_i$.

Recently, some upstream protein kinases involved in the activation of p38 MAPK were identified in mammalian cells. It has been shown that MKK3 and MKK6 are specific activators of p38 MAPK in the signaling pathway caused by endotoxins, inflammatory cytokines, and stresses (51–55). TAK1 (56) and ASK1 (57) were also proposed to function as a mitogen-activated protein kinase kinase of p38 MAPK in the signaling pathway from the transforming growth factor- β and tumor necrosis factor- α receptors, respectively. The molecules farther upstream of these kinases have not been fully investigated, although the small guanine nucleotide-binding proteins Rac1 and Cdc42 have been shown to regulate PAK1, which may be involved in the p38 MAPK pathway (58–60). It remains to be clarified whether these molecules participate in G protein-mediated p38 MAPK activation.

The physiological role of p38 MAPK activation by G protein-coupled receptors also remains to be clarified. It has been suggested that the p38 MAPK pathway may be involved in the inhibition of cell growth and the promotion of cell death (6). Further studies on the molecular mechanism of p38 MAPK activation induced by two distinct G protein components, $G\beta\gamma$ and $G\alpha_{q/11}$, may provide new insight into the functions of p38 MAPK. We are currently investigating the molecular connections that lead to p38 MAPK activation.

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