THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 273, No. 23, Issue of June 5, pp. 14533–14537, 1998
Printed in U.S.A.


# Analysis of the $G_s$/Mitogen-activated Protein Kinase Pathway in Mutant S49 Cells*

(Received for publication, February 11, 1998, and in revised form, March 30, 1998)

**Yong Wan and Xin-Yun Huang‡**

*From the Department of Physiology, Cornell University Medical College, New York, New York 10021*

**Heterotrimeric G protein-coupled receptors can activate the mitogen-activated protein kinase (MAPK) cascade. Recent studies using pharmacological inhibitors or dominant-negative mutants of signaling molecules have advanced our understanding of the pathways from G protein-coupled receptors to MAPK. However, molecular genetic analysis of these pathways is inadequate in mammalian cells. Here, using the well characterized $G_s\alpha$- and protein kinase A-deficient S49 mouse lymphoma cells, we provide the molecular genetic evidence that $G_s\alpha$ is responsible for transducing the β-adrenergic receptor signal to MAPK in a protein kinase A-dependent pathway involving Rap1 and Raf (but not Ras) molecules.**

G proteins serve their physiological roles by transducing signals from a broad class of cell-surface receptors to specific effector proteins (1–4). A variety of intracellular signal transduction pathways are regulated by G proteins, including the mitogen-activated protein kinase (MAPK)[1] pathway (5). Although the activation mechanism of the MAPK cascade by receptors with intrinsic tyrosine kinase activity has been well studied, the route from G proteins to the MAPK cascade in mammalian cells is less understood (6, 7).

Recent studies in cultured cell lines with pharmacological inhibitors and dominant-negative mutants of certain signaling molecules have revealed the participation of some molecular components in the regulation of MAPK by G protein-coupled receptors (for review, see Ref. 5). Although the detailed biochemical steps are far from clear, these studies have shown that G protein-coupled receptors use pathways very similar to those utilized by receptor tyrosine kinases to activate the prototype Raf/MEK/MAPK cascade. $G_q$- and $G_i$-coupled receptors transmit the signals to MAPK through a pathway involving tyrosine kinase, adapter proteins Shc and Grb2, guanine nucleotide exchange factor Sos, Ras, and Raf in most cases (for review, see Ref. 5). Phosphatidylinositol 3-kinase has been implicated to act upstream of tyrosine kinases in the $G_i$/MAPK pathway in some cells (8–12).

For receptors coupled to $G_s$, overexpressing $G\beta\gamma$ or $G_s\alpha$ subunits in COS-7 cells has shown that whereas $G\beta\gamma$ subunits have the capacity to stimulate MAPK, the ability of $G_s\alpha$ to stimulate MAPK is controversial (13, 14). It is also unclear if cAMP and protein kinase A (PKA) participate in the $G_s$-coupled receptor/MAPK pathway. Whereas one group reported that cAMP, forskolin, and $G_s$-coupled receptors can stimulate MAPK in COS-7 cells (13), another reported that cAMP and PKA do not mediate activation of MAPK by $G_s$-coupled receptors in COS-7 cells (14). It was proposed that the $G_s$-coupled β-adrenergic receptor used the $G\beta\gamma$ subunit to activate the MAPK pathway through Ras and used the $G_s\alpha$ subunit to inhibit MAPK activation through cAMP and PKA (14). These contradictory results regarding whether the α-subunit or the βγ-subunits of $G_s$ protein mediate the receptor stimulation of MAPK and whether PKA is involved in the $G_s$/MAPK pathway in mammalian cells prompted us to address this question genetically. For the most part, signaling by heterotrimeric G proteins has not been studied genetically in mammalian cells.

S49 mouse lymphoma cells have played an important historical role in G protein research (15). A variant of S49 cells lacking $G_s\alpha$ was instrumental in defining the function of and characterizing $G_s\alpha$ (15). Elevation of intracellular cAMP levels results in growth arrest in the $G_1$ phase of the cell cycle and later (after several days) in cell death (16, 17). Mutants have been selected that are resistant to cytolysis. These mutants include *cyc*$^-$ (which lacks $G_s\alpha$) (18), *UNC* (which has a mutation of arginine at position 372 of $G_s\alpha$ and thus uncouples the interaction of $G_s\alpha$ with the receptors) (19), and *kin*$^-$ (which lacks protein kinase A activity) (20).

These $G_s\alpha$ and PKA mutant S49 cells should be very useful in a molecular genetic study to understand the role of $G_s\alpha$ and PKA in the β-adrenergic receptor/MAPK signaling system. In this study, using these mutant S49 cells, we demonstrate that $G_s\alpha$ transduces the β-adrenergic receptor signal to MAPK in a protein kinase A-dependent pathway involving Rap1 and Raf (but not Ras) molecules.

## EXPERIMENTAL PROCEDURES

*S49 Mouse Lymphoma Cells*—S49 mouse lymphoma T cells were obtained from the Cell Culture Facility at the University of California at San Francisco and were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated horse serum as described (16, 17, 21). Transient transfection was done with 2 μg of plasmid DNA and LipofectAMINE (Life Technologies, Inc.) in six-well culture plates as described previously (22–25). Transfection efficiency was ~20%.

*Immunoprecipitation and Immunoblot Analysis*—S49 whole cell extracts were prepared as follows. Cells were harvested from 10-cm plates and washed twice with cold phosphate-buffered saline, and pellets were resuspended in 0.8 m1 of extraction buffer (150 mM NaCl, 10 mM Tris, pH 7.4, l mM EDTA, l mM EGTA, 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100, 0.5% Nonidet P-40, 0.2 mM phenylmethylsulfonyl fluoride, 0.2 mM sodium orthovanadate, 0.02 mg/m1 tosylphenylalanyl chloromethyl ketone, and 0.03 mg/m1 leupeptin). Resuspended pellets were passed five times through a 26-gauge needle and centrifuged at 5000 rpm for 5 min at 4 °C to remove insoluble material, and the supernatant was saved as the whole cell extract. For immunoprecipitation, 10 μl of protein G-agarose was added to the whole cell lysate to

* This work was supported by grants from NIH, the National Science Foundation, and the American Heart Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Beatrice F. Parvin Investigator of the American Heart Association, New York City Affiliate. To whom correspondence should be addressed: Dept. of Physiology, Cornell University Medical College, 1300 York Ave., New York, NY 10021. Tel.: 212-746-6362; Fax: 212-746-8690; E-mail: xyhuang@mail.med.cornell.edu.

[1] The abbreviations used are: MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated protein kinase kinase; PKA, protein kinase A.

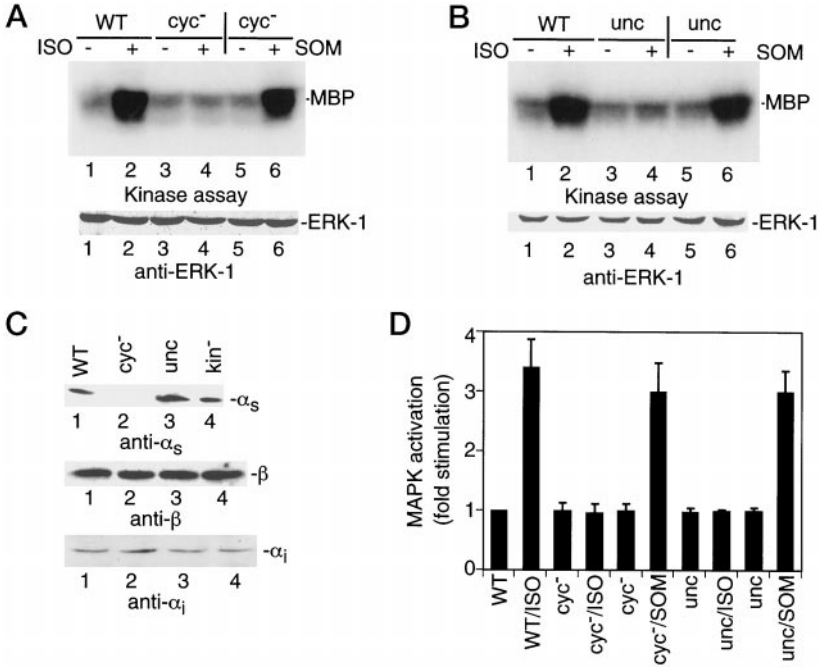


FIG. 1. **Stimulation of MAPK by $G_s$- and $G_i$-coupled receptors in wild-type and $G_s\alpha$ mutant S49 cells.** *A*, in wild-type (*WT*) S49 cells, the agonist isoproterenol (*ISO*) activated the endogenous $G_s$-coupled β-adrenergic receptor, leading to the stimulation of MAPK activity. Cells were treated for 5 min with isoproterenol (100 μM) or somatostatin (*SOM;* 1 μM). MAPK activity was assayed with myelin basic protein (*MBP*) as substrate. In *cyc*⁻ cells, stimulation of the β-adrenergic receptor failed to increase the activity of MAPK, although the stimulation of MAPK by the endogenous $G_i$-coupled somatostatin receptor was normal. The isoproterenol effect could be blocked by the β-receptor antagonist propranolol (data not shown). *B*, in *UNC* cells, stimulation of the β-adrenergic receptor failed to increase the activity of MAPK, although the stimulation of MAPK by the $G_i$-coupled somatostatin receptor was normal. *C*, shown are the results of Western blot analysis of the expression of $G_s\alpha$, Gβ, and $G_i\alpha$ in wild-type and mutant S49 cells. *D*, shown are the results from the quantification of $G_s$- and $G_i$-coupled receptor-stimulated MAPK activity. Data represent the means ± S.D. of four to six experiments.

preclear. Then, 5 μl of primary antibody was added and continuously incubated at 4 °C for 30 min. After another 2-h incubation with 30 μl of protein G-agarose beads, the immunocomplex was washed three times with extraction buffer and three times with wash buffer (10 mM Tris, pH 7.4, and 1 mM EDTA). The immunocomplex was then subjected to SDS-polyacrylamide gel electrophoresis. Western blotting with anti-ERK-1, anti-$G_s\alpha$, anti-Gβ, and anti-$G_i\alpha$ was done as described (22–25). Anti-G protein antibodies were from Santa Cruz Biotechnology. Membrane filters were incubated in 1× Tris-buffered saline/5% milk for 1 h and then incubated in primary antibody for 2 h at room temperature. Blots were washed three times with Tris-buffered saline/Tween-20 and one time with Tris-buffered saline and then incubated with secondary antibody for 2 h at room temperature. Blots were washed again, and signal was detected with ECL (NEN Life Science Products).

*MAPK Assay*—For treatments, cells were stimulated with 100 μM isoproterenol or 1 μM somatostatin for 5 min. This brief treatment (5 min) with isoproterenol did not cause cell death. Whole cell lysate was prepared, and immunocomplex MAPK assay was performed as described previously using myelin basic protein (10 μg) as substrate (23, 24). ERK-1 immunoprecipitation was done with a monoclonal antibody to ERK-1 (Transduction Laboratories). Kinase assay buffer contained 30 mM Tris-HCl, pH 8, 20 mM $MgC1_2$, 2 mM $MnC1_2$, and 10 μM ATP. The mixture was preincubated for 3 min before 10 μCi of [γ-$^{32}$P]ATP was added. After 10 min at 30 °C, samples were separated by 12% SDS-polyacrylamide gel electrophoresis. Gels were transferred to nitrocellulose membrane filters and exposed for autoradiography. Quantitation was performed using a Molecular Dynamics PhosphorImager.

## RESULTS

*$G_s\alpha$ Is Required for Transmitting the β-Adrenergic Receptor Signal to MAPK*—In wild-type S49 mouse lymphoma cells, the agonist isoproterenol activates the endogenous $G_s$-coupled β-adrenergic receptor, leading to the stimulation of MAPK activity (Fig. 1*A*). Isoproterenol-induced increase in MAPK activity was not sensitive to pertussis toxin and could be blocked by the β-adrenergic receptor-specific antagonist propranolol (data not shown). To genetically determine whether $G_s\alpha$ or Gβγ subunits transduce the receptor signal to MAPK, we have taken advantage of the availability of mutant S49 cells that have genetic defects in β-receptor signaling.

In *cyc*⁻ S49 cells that lack $G_s\alpha$ proteins (a null mutant), stimulation of the β-adrenergic receptor failed to activate adenylyl cyclase or to increase intracellular cAMP (18). As shown in Fig. 1 (*A* and *D*), in these $G_s\alpha$-deficient cells, stimulation of the β-adrenergic receptor failed to increase the activity of MAPK, although the stimulation of MAPK by the endogenous $G_i$-coupled somatostatin receptor was normal. As expected, the $G_i$-mediated somatostatin-induced increase in MAPK activity was blocked by pertussis toxin (data not shown). This result indicates that $G_s\alpha$ is positively required for transmitting the β-adrenergic receptor signal to MAPK.

To further confirm the necessity of $G_s\alpha$ for this signal transduction pathway, we tested another allele of the $G_s\alpha$ mutant, the *UNC* S49 cells. The *UNC* mutant of $G_s\alpha$ has a single amino acid change at position 372 (from arginine to proline), six residues from the carboxyl terminus (19, 26). This mutant $G_s\alpha$ fails to couple to the β-adrenergic receptor. Thus, the β-adrenergic receptor signal is unable to be delivered to downstream targets *in vivo*. As shown in Fig. 1 (*B* and *D*), in *UNC* mutant S49 cells, stimulation of the β-adrenergic receptor failed to stimulate MAPK activity. Again, stimulation of MAPK by the endogenous $G_i$-coupled somatostatin receptor was normal. These data reaffirm that $G_s\alpha$ is necessary for β-adrenergic receptor/MAPK signaling. The inability of *cyc*⁻ and *UNC* mutant S49 cells to respond to isoproterenol in stimulating MAPK was not due to gross abnormality of G protein expression in these mutants since we found that expression of $G_s\alpha$, Gβ, and $G_i\alpha$ proteins in all cell lines was similar, except that $G_s\alpha$ was missing in *cyc*⁻ cells (Fig. 1*C*).

*$G_s\alpha$ Is the Signal Transducer in the β-Adrenergic Receptor/MAPK Pathway*—The requirement for $G_s\alpha$ may be due to its signaling role or to its requirement in maintaining the structural integrity of trimeric G proteins or both. To distinguish between the signaling *versus* structural role, we introduced into *cyc*⁻ cells (a null $G_s\alpha$ mutant background) a $G_s\alpha$ mutant that still complexes with the Gβγ subunit and is still able to couple to the β-adrenergic receptor (that is, the structural role is still fulfilled), but is unable to stimulate its downstream target adenylyl cyclase (that is, the signaling role is impaired). If such a mutant is unable to rescue the *cyc*⁻ mutant response to β-adrenergic receptor stimulation of MAPK, then $G_s\alpha$ is very likely the signal transducer. If such a mutant is able to rescue the *cyc*⁻ response to β-adrenergic receptor stimulation of MAPK, $G_s\alpha$ is probably needed for structural integrity. Therefore, we tested two $G_s\alpha$ mutants with amino acid changes in the effector contact region of $G_s\alpha$, previously described to be defec-

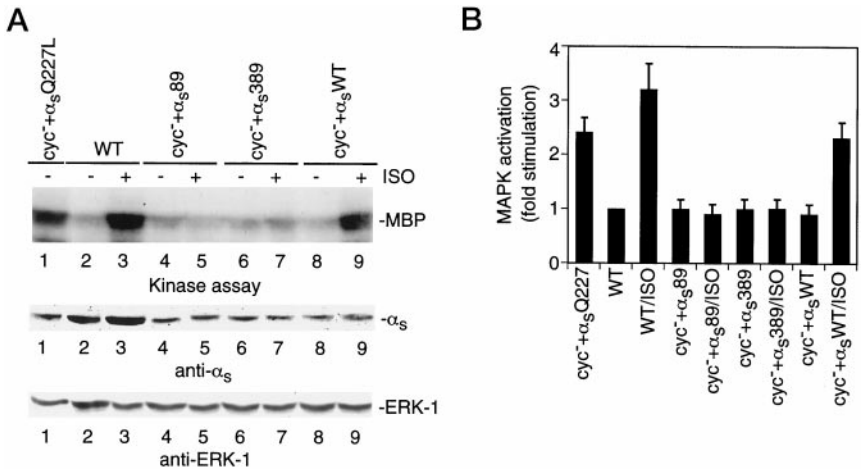


FIG. 2. **Rescue of the stimulation of MAPK by $G_s$-coupled receptors in *cyc*$^-$ cells by mutant $G_s\alpha$.** *A*, transfection of constitutively active $G_s\alpha$ ($\alpha_s$Q227L) leads to the activation of MAPK (*lane 1*). Transfection of $G_s\alpha$ mutants ($\alpha_s$89 (*lanes 4* and *5*; $\alpha_s$389 (*lanes 6* and *7*)) failed to rescue the activation of MAPK in response to isoproterenol (*ISO*) stimulation. In mutant $\alpha_s$89, $Leu^{268}$ and $Arg^{269}$ were changed to phenylalanine and threonine, respectively. In mutant $\alpha_s$389, $Trp^{263}$, $Leu^{268}$, and $Arg^{269}$ were changed to cysteine, phenylalanine, and threonine, respectively. These mutants have a markedly reduced ability to activate adenylyl cyclase (27). Transfection of wild-type (*WT*) $G_s\alpha$ (*lanes 8* and *9*) restored the response of MAPK to β-receptor stimulation. *B*, quantification of receptor-stimulated MAPK activity. The values shown represent the means ± S.D. of four experiments.

tive in stimulating adenylyl cyclase, but still interacting with Gβγ and the β-adrenergic receptor (27, 28). As shown in Fig. 2, wild-type $G_s\alpha$ rescues the *cyc*$^-$ cell response to β-adrenergic stimulation of MAPK, whereas neither of the two mutants could rescue the response. Furthermore, expression of a constitutively activated $G_s\alpha$ mutant ($\alpha_s$Q227L, with $Gln^{227}$ changed to Leu) (29) leads to stimulation of MAPK, indicating that $G_s\alpha$ is not only required but also sufficient to activate the MAPK pathway. Thus, we conclude that $G_s\alpha$ is the signal transducer in the β-adrenergic receptor/MAPK pathway.

*PKA Is Required for Transmitting the β-Adrenergic Receptor Signal to MAPK*—Binding of isoproterenol to the β-adrenergic receptor results in the activation of $G_s\alpha$, leading to stimulation of adenylyl cyclase and elevation of the intracellular levels of cAMP. Most cAMP-mediated intracellular responses are mediated through protein kinase A in mammalian cells (30). As shown in Fig. 3, stimulation of MAPK by the β-adrenergic receptor is blocked in *kin*$^-$ mutant S49 cells that lack protein kinase A activity (20), whereas stimulation of MAPK by the $G_i$-coupled somatostatin receptor is normal in *kin*$^-$ cells. This result further demonstrates that the $G_s\alpha$/adenylyl cyclase/cAMP/protein kinase A cascade links the β-adrenergic receptor to MAPK in S49 mouse lymphoma cells.

*Downstream Components of the PKA/MAPK Pathway in S49 Cells*—Activation of MAPK by PKA in some cells has been proposed to act through a Ras-independent but B-Raf- and Rap1-dependent signaling pathway (31). To examine the role of Ras, Raf, and Rap1 in the PKA/MAPK pathway in S49 cells, we tested the effects of dominant-negative mutants of Ras, Raf, and Rap1 (Fig. 4). Expression of a dominant-negative Ras mutant (RasN17) (32) in S49 cells had no effect on the stimulation of MAPK by $G_s$-coupled β-receptors (Fig. 4*A*), whereas it inhibited the MAPK stimulation by $G_i$-coupled somatostatin receptors (Fig. 4*B*). Transfection of a dominant-negative Raf mutant (a truncated Raf mutant with the conserved region 1, which interferes with the activation of endogenous Raf including B-Raf) (33–36) into S49 cells blocked the MAPK stimulation by both $G_s$- and $G_i$-coupled receptors (Fig. 4). While there was no effect on the stimulation of MAPK by the $G_i$-coupled somatostatin receptor, transfection of a dominant-negative Rap1 mutant (Rap1N17) (31) blocked the MAPK stimulation by the $G_s$-coupled β-receptor (Fig. 4*C*). These data suggest that in S49 cells, as in some other mammalian cells, the PKA/MAPK pathway requires Rap1 and Raf, but not Ras (31, 37).

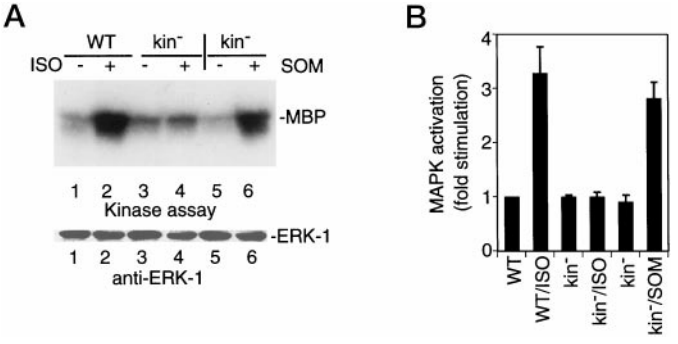


FIG. 3. **Stimulation of MAPK by $G_s$-coupled and $G_i$-coupled receptors in *kin*$^-$ S49 cells.** *A*, in *kin*$^-$ cells, stimulation of the β-adrenergic receptor failed to increase the activity of MAPK, whereas the stimulation of MAPK by the $G_i$-coupled somatostatin (*SOM*) receptor was normal. *B*, shown are the results from the quantification of $G_s$- and $G_i$-coupled receptor-stimulated MAPK activity. Data represent the means ± S.D. of four experiments. *WT*, wild-type S49 cells; *ISO*, isoproterenol; *MBP*, myelin basic protein.

## DISCUSSION

In summary, using various mutant S49 mouse lymphoma cells, we have provided the first genetic evidence for $G_s\alpha$ and protein kinase A transducing the β-adrenergic receptor signal to MAPK. In *cyc*$^-$ mutant S49 cells that lack $G_s\alpha$ proteins, stimulation of the β-adrenergic receptor failed to activate MAPK. In *UNC* mutant S49 cells that $G_s\alpha$ is unable to couple to the β-adrenergic receptor, MAPK could not be stimulated by the β-receptor. Two $G_s\alpha$ mutants that can complex with the Gβγ subunit and β-receptor, but are unable to stimulate adenylyl cyclase, failed to rescue the *cyc*$^-$ mutant response to β-receptor stimulation of MAPK. Wild-type $G_s\alpha$ rescued the *cyc*$^-$ cell response. Furthermore, in *kin*$^-$ mutant S49 cells that lack protein kinase A activity, stimulation of MAPK by the β-receptor was blocked. Moreover, dominant-negative mutants of Rap1 or Raf, but not Ras, suppressed the β-receptor-induced MAPK stimulation. These data collectively demonstrate that the $G_s$-coupled adrenergic receptor uses $G_s\alpha$, transducing the signal to a PKA-, Rap1-, and Raf-dependent, but Ras-independent, pathway leading to MAPK activation in S49 mouse lymphoma cells.

Previously, Faure *et al.* (13) have shown that overexpressing Gβγ or constitutively activated $G_s\alpha$ could lead to activation of MAPK and that cAMP, forskolin, and $G_s$-coupled receptors could stimulate MAPK in COS-7 cells. On the other hand, Crespo *et al.* (14) reported that only overexpression of Gβγ subunits, but not the activated $G_s\alpha$ subunit, could increase MAPK activity in COS-7 cells. It was proposed that in COS-7 cells, whereas Gβγ transduces a positive signal to increase MAPK activity, $G_s\alpha$, through protein kinase A, inhibits the MAPK stimulation (14). The reason for this discrepancy is not

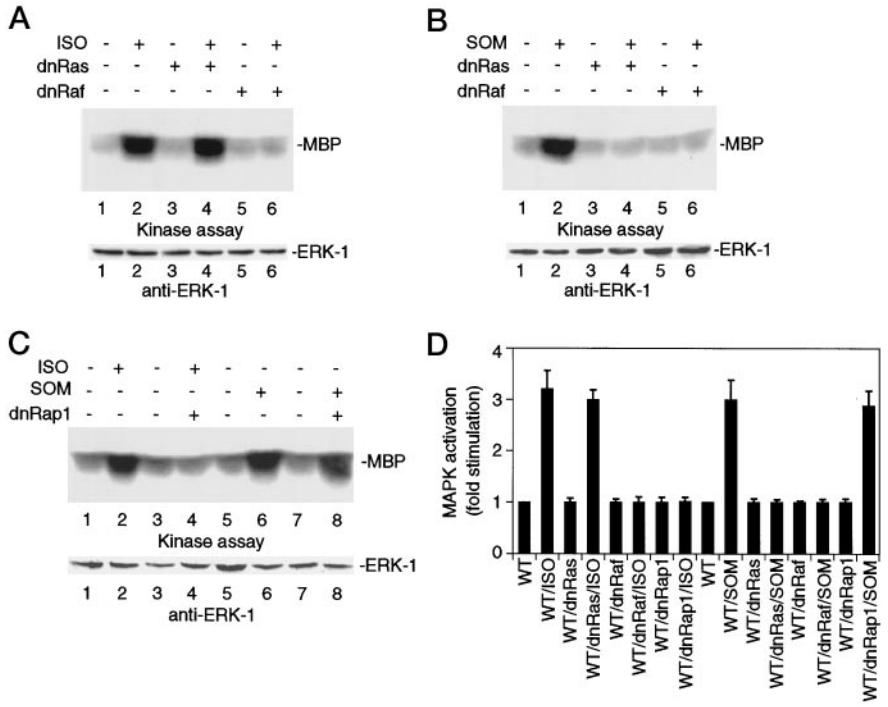


FIG. 4. **Effects of dominant-negative mutants of Ras, Raf, and Rap1 on the stimulation of MAPK by $G_s$- and $G_i$-coupled receptors in S49 cells.** *A*, whereas a dominant-negative Ras mutant (*dnRas*) has no effect on the stimulation of MAPK by $G_s$-coupled receptors, a dominant-negative Raf mutant (*dnRaf*) inhibits the MAPK activation by $G_s$-coupled receptors. *B*, both a dominant-negative Ras mutant and a dominant-negative Raf mutant inhibit the MAPK stimulation by $G_i$-coupled receptors. *C*, a dominant-negative Rap1 mutant (*dnRap1*) blocks the stimulation of MAPK by $G_s$-coupled receptors, but has no effect on the stimulation by $G_i$-coupled receptors. *D*, quantification of receptor-stimulated MAPK activity. The values shown represent the means ± S.D. of four experiments. *WT*, wild-type S49 cells; *ISO*, isoproterenol; *MBP*, myelin basic protein; *SOM*, somatostatin.

clear. The suggestion that G$\beta\gamma$ mediates the $\beta$-adrenergic receptor signal to MAPK is based on two types of experiments (14). One is that, as mentioned above, overexpression of G$\beta\gamma$ could lead to increased MAPK activity. Another is that overexpression of a G$\beta\gamma$-binding fragment from the $\beta$-adrenergic receptor kinase protein or $G_t\alpha$ could attenuate the stimulation of MAPK by $\beta$-adrenergic receptors.

We were unable to perform a genetic analysis of the role of G$\beta\gamma$ subunits due to the lack of null mutants of G$\beta$ or G$\gamma$ subunits in S49 cells. G$\beta\gamma$ is likely required in a structural role for the integrity of G protein function, but is unlikely to play a major signaling role for the following reasons. First, in *cyc*$^-$ mutant cells, the basal activity of MAPK is similar to that in wild-type cells. If G$\beta\gamma$ is the major signal transducer, as in *Saccharomyces cerevisiae*, then in *cyc*$^-$ cells, MAPK could be constitutively active, as in G$\alpha$ null mutant cells in *S. cerevisiae* (38, 39). Second, two mutant $G_s\alpha$ ($\alpha_s$89 and $\alpha_s$389) subunits did not rescue the *cyc*$^-$ cell response despite being able to release G$\beta\gamma$ upon receptor stimulation. Third, in S49 cells, the isoforms of adenylyl cyclases can be stimulated by $G_s\alpha$, but not by G$\beta\gamma$ or calcium/calmodulin (40). These data suggest that if G$\beta\gamma$ is needed, it is for structural reasons only, not for activating downstream targets. Therefore, in S49 mouse lymphoma cells, $G_s\alpha$, not G$\beta\gamma$, transduces the receptor signal to MAPK.

In the fission yeast *Schizosaccharomyces pombe*, the $\alpha$-subunit of G protein carries the signal to the MAPK pathway (41). In the budding yeast *S. cerevisiae*, the $\beta\gamma$-subunit of G protein couples the receptor to the MAPK cascade (38, 39). Given that the $\alpha$-subunit of $G_s$ transduces $G_s$-coupled receptor signal to MAPK in S49 cells and that the $\beta\gamma$-subunit of $G_i$ likely conveys the message from $G_i$-coupled receptors to MAPK (13, 42, 43), these different usages of the $\alpha$ and $\beta\gamma$ subunits might be reminiscent of *S. pombe versus S. cerevisiae* MAPK signaling pathways. Thus, mammalian cells have both yeast pathways that are utilized by different families of G proteins.

The cAMP and protein kinase A effect on the MAPK pathway depends on cell type: in some cells, they are stimulatory to the MAPK pathway, whereas in other cells, they are inhibitory (44, 45). A recent study has determined that these stimulatory or inhibitory effects are dictated by the expression of B-Raf (31). Protein kinase A directly activates the small G protein Rap1, which in turn, selectively and directly activates B-Raf, leading to the activation of MAPK. We found that B-Raf is expressed in S49 cells and that isoproterenol could stimulate B-Raf activity in S49 cells. Also, we have examined the activation of MEK, an upstream activator of MAPK, and obtained results similar to those for MAPK activation. Thus, we propose the activation sequence as $\beta$-adrenergic receptor/$G_s\alpha$/adenylyl cyclase/cAMP/PKA/Rap1/B-Raf/MEK/MAPK. This $G_s\alpha$/MAPK pathway represents the first complete biochemical pathway for G protein/MAPK signaling.

*Acknowledgments*—We thank Drs. T. Kozasa, A. Gilman, and P. Stork for providing plasmids. We are grateful to Drs. R. Iyengar, L. Levin, T. Maack, and the members of our laboratory for reading the manuscript.

REFERENCES

1. Gilman, A. (1987) *Annu. Rev. Biochem.* **56,** 615–649
2. Bourne, H. R., Sanders, D. A., and McCormick, F. (1991) *Nature* **349,** 117–127
3. Simon, M. I., Strathmann, M. P., and Gautam, N. (1991) *Science* **252,** 802–808
4. Clapham, D. E., and Neer, E. J. (1993) *Nature* **365,** 403–406
5. Post, G. R., and Brown, J. H. (1996) *FASEB J.* **10,** 741–749
6. Seger, R., and Krebs, E. G. (1995) *FASEB J.* **9**, 726–735
7. Herskowitz, I. (1995) *Cell* **80,** 187–197
8. Ferby, I. M., Waga, I., Sakanaka, C., Kume, K., and Shimizu, T. (1994) *J. Biol. Chem.* **269,** 30485–30488
9. Pace, A. M., Faure, M., and Bourne, H. R. (1995) *Mol. Biol. Cell* **6,** 1685–1695
10. Hawes, B. E., Luttrell, L. M., van Biesen, T., and Lefkowitz, R. J. (1996) *J. Biol. Chem.* **271,** 12133–12136
11. Cowen, D. S., Sowers, R. S., and Manning, D. R. (1996) *J. Biol. Chem.* **271,** 22297–22300
12. Lopez-Ilasaca, M., Crespo, P., Pellici, P. G., Gutkind, J. S., and Wetzker, R. (1997) *Science* **275,** 394–397
13. Faure, M., Voyno-Yasenetskaya, T. A., and Bourne, H. R. (1994) *J. Biol. Chem.* **269,** 7851–7854
14. Crespo, P., Cachero, T. G., Xu, N., and Gutkind, J. S. (1995) *J. Biol. Chem.* **270,** 25259–25265
15. Gilman, A. G. (1995) *Biosci. Rep.* **15,** 65–97
16. Coffino, P., Bourne, H. R., and Tomkins, G. M. (1975) *J. Cell. Physiol.* **85,** 603–610
17. Bourne, H. R., Coffino, P., and Tomkins, G. M. (1975) *J. Cell. Physiol.* **85,** 611–620
18. Bourne, H. R., Coffino, P., and Tomkins, G. M. (1975) *Science* **187,** 750–752
19. Haga, T., Ross, E. M., Anderson, H. J., and Gilma, A. G. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 2016–2020
20. Insel, P. A., Bourne, H. R., Coffino, P., and Tomkins, G. M. (1975) *Science* **190,** 896–897
21. Hadcock, J. R., Ros, M., and Malbon, C. C. (1989) *J. Biol. Chem.* **264,** 13956–13961

22. Langhans-Rajasekaran, S. A., Wan, Y., and Huang, X.-Y. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 8601–8605
23. Wan, Y., Kurosaki, T., and Huang, X.-Y. (1996) *Nature* **380,** 541–544
24. Wan, Y., Bence, K., Hata, A., Kurosaki, T., Veillette, A., and Huang, X.-Y. (1997) *J. Biol. Chem.* **272,** 17209–17215
25. Bence, K., Ma, W., Kozasa, T., and Huang, X.-Y. (1997) *Nature* **389,** 296–299
26. Sullivan, K. A., Miller, R. T., Master, S. B., Beiderman, B., Heideman, W., and Bourne, H. R. (1987) *Nature* **330,** 758–760
27. Itoh, H., and Gilman, A. G. (1991)*J. Biol. Chem.* **266**, 16226–16231
28. Berlot, C. H., and Bourne, H. R. (1992) *Cell* **68,** 911–922
29. Graziano, M. P., and Gilman, A. G. (1989) *J. Biol. Chem.* **264,** 15475–15482
30. Walsh, D. A., and Van Patten, S. M. (1994) *FASEB J.* **8,** 1227–1236
31. Vossler, M. R., Yao, H., York, R. D., Pan, M.-G., Rim, C. S., and Stork, P. J. S. (1997) *Cell* **89,** 73–82
32. Stacy, D. W., Feig, L. A., and Gibbs, J. B. (1991) *Mol. Cell. Biol.* **11,** 4053–4064
33. Kolch, W., Heidecker, G., Lloyd, P., and Rapp, U. R. (1991) *Nature* **349,** 426–428
34. Cai, H., Erhardt, P., Troppmair, J., Diaz-Meco, M. T., Sithanandam, G., Rapp, U. R., Moscat, J., Cooper, G. M. (1993) *Mol. Cell. Biol.* **13,** 7645–7651
35. Shibuya, E. K., Morris, J., Rapp, U. R., and Ruderman, J. V. (1996) *Cell Growth Differ.* **7,** 235–241
36. Cosgaya, J. M., and Aranda, A. (1996) *Oncogene* **12,** 2651–2660
37. Verheijen, M. H. G., and Defize, L. H. K. (1997) *J. Biol. Chem.* **272,** 3423–3429
38. Dietzel, C., and Kurjan, J. (1987) *Cell* **50,** 1001–1010
39. Miyajima, I., Nakafuku, M., Nakayama, N., Brenner, C., Miyajima, A., Kaibuchi, K., Arai, K., Kaziro, Y., and Matsumoto, K. (1987) *Cell* **50,** 1011–1019
40. Tang, W.-J., and Gilman, A. G. (1991) *Science* **254,** 1500–1503
41. Obara, T., Nakafuku, M., Yamamoto, M., and Kaziro, Y. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 5877–5881
42. Crespo, P., Xu, N., Simonds, W. F., and Gutkind, J. S. (1994) *Nature* **369,** 418–420
43. Koch, W. J., Hawes, B. E., Allen, L. F., and Lefkowitz, R. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 12706–12710
44. Faure, M., and Bourne, H. R. (1995) *Mol. Biol. Cell* **6,** 1025–1035
45. Burgering, B. M. T., and Bos, J. L. (1995) *Trends Biol. Sci.* **20,** 18–22