Protein Kinase C Phosphorylates G_{12\alpha} and Inhibits Its Interaction with G_{\beta\gamma}*

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Tohru Kozasa and Alfred G. Gilman†
From the Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Of nine G protein \(\alpha\) subunits examined, only \(\alpha_{12}\) and \(\alpha_{2}\) served as substrates for phosphorylation by various isoforms of protein kinase C in vitro. A close homolog of \(\alpha_{12}^g\), \(\alpha_{13}^g\), was not phosphorylated. Exposure of NIH 3T3 cells that stably express \(\alpha_{12}\) to phorbol 12-myristate 13-acetate also resulted in phosphorylation of the protein. Phosphorylation in vitro occurred near the amino terminus (probably Ser\(^{\text{39}}\)), and approximately 1 mol of phosphate was incorporated per mol of \(\alpha_{12}\). Although G protein heterotrimers containing either \(\alpha_{12}^g\) or \(\alpha_{2}\) were poor substrates for phosphorylation, the isolated \(\alpha\) subunits were phosphorylated equally well in their GDP- or GTP\(S\)-bound forms. The guanine nucleotide binding properties of purified \(\alpha_{12}\) and \(\alpha_{2}\) were unaltered by phosphorylation, as was the capacity of \(\alpha_{2}\) to inhibit type V adenyl cyclase. However, phosphorylation of either protein greatly reduced its affinity for G protein \(\beta\gamma\) subunits, consistent with the newly determined crystal structure of a G protein heterotrimer. We suggest that protein kinase C regulates \(\alpha_{12}\) and \(\alpha_{2}\)-mediated signaling pathways by preventing their association with \(\beta\gamma\).

Heterotrimeric guanine nucleotide-binding proteins (G proteins)\(^1\) transduce regulatory signals from cell surface receptors to effectors such as adenyl cyclases, phosphodiesterases, phospholipases, and ion channels (1–3). Each G protein oligomer contains a guanine nucleotide-binding \(\alpha\) subunit and a high-affinity dimer of \(\beta\) and \(\gamma\) subunits. There are many isoforms of each subunit and thus a very large number of distinct G protein oligomers. G protein \(\alpha\) subunits are commonly described as members of four subfamilies: \(\alpha_g\) and \(\alpha_{\text{af}}\) (stimulators of adenyl cyclases); \(\alpha_{11}\), \(\alpha_{12}\), \(\alpha_{13}\), \(\alpha_{14}\), \(\alpha_{15}\), and \(\alpha_{16}\) (functionally diverse group of pertussis toxin substrates, with the exception of \(\alpha_{12}\)); \(\alpha_{20}\), \(\alpha_{21}\), \(\alpha_{24}\), and \(\alpha_{15/16}\) (activators of phospholipase C-\(\beta\)); and \(\alpha_{12}\) and \(\alpha_{13}\).

The two members of the \(\alpha_{12}\) and \(\alpha_{13}\) subfamily, discovered most recently, are expressed ubiquitously (4) and share interesting biochemical characteristics, including relatively slow guanine nucleotide exchange and hydrolysis (5, 6). Although the receptors and effectors that interact with these G proteins have not yet been identified, overexpression of wild-type or mutationally activated \(\alpha_{12}\) or \(\alpha_{13}\) transforms fibroblasts (7–9). Furthermore, overexpression of constitutively activated \(\alpha_{12}\) or \(\alpha_{13}\) stimulates Na\(^+/H^+\) exchange activity (10, 11). Of interest, Dhanasekaran et al. (10) showed that this stimulatory effect of \(\alpha_{12}\), but not that of \(\alpha_{13}\), is lost after prolonged exposure of cells to PMA. These results suggest that \(\alpha_{12}\) and \(\alpha_{13}\) transduce similar regulatory signals related to cell growth or transformation and that there is further regulation of the \(\alpha_{12}\) pathway by PKC.

There are other interactions between G protein-regulated pathways and PKC. Treatment of cells with PMA has a variety of often confusing effects on their capacity to synthesize cyclic AMP in response to various activators or inhibitors; certain adenyl cyclases are activated following phosphorylation by PKC in vitro (12, 13). Activation of phospholipase C by muscarinic or \(\alpha_g\)-adrenergic agonists is blocked by treatment of astrocytoma cells or hepatocytes, respectively, with PMA (14, 15). The inhibitory effects of substance P on an inward rectifier K\(^+\) channel appear to be mediated by a pertussis toxin-insensitive G protein and protein kinase C (16). With regard to direct effects of PKC on G protein subunits, there are descriptions of phosphorylation of \(G_s\) and \(G_v\), both in vitro and in vivo (17–20), but the functional significance of such modification has been unclear. We describe here the phosphorylation of \(\alpha_{12}\) by PKC in vitro and, in addition, in cells exposed to PMA. We further demonstrate that phosphorylated \(\alpha_{12}\) and \(\alpha_{13}\) have reduced affinity for G protein \(\beta\gamma\) subunits compared to the unmodified \(\alpha\) subunits. Similar results with \(\alpha_{12}\) have just been reported by Fields and Casey (21).

**EXPERIMENTAL PROCEDURES**

Purification of G Protein Subunits from Sf9 Cells—\(\alpha_{12}\) and \(\alpha_{13}\) were purified from Sf9 cells infected with appropriate baculoviruses after their coexpression with \(\beta_1\) and His\(\beta\gamma\) subunits as described by Kozasa and Gilman (5), with the following modifications. A novel His\(\beta\gamma\)-encoding virus (amino acid sequence MAHHHHHG\(\beta\gamma\)-\(12\)) (7–13) was utilized. The resulting protein binds with higher apparent affinity to Ni-NTA than does the protein without the two glycine residues inserted after the hexahistidine tag. After application of the Sf9 cell membrane extract to the Ni-NTA column (Qiagen), the resin was washed extensively with buffer containing 15 (instead of 5) mM imidazole, \(\alpha_{12}\) was eluted from the Ni-NTA column with buffer containing AMF (30 \(\mu\)M AICl\(_n\), 50 mM MgCl\(_n\), and 10 mM NaF) and was purified further on a Mono S HR/55 column (Pharmacia Biotech Inc.) with solutions containing 10% glycerol to prevent precipitation during concentration of peak fractions. \(\alpha_{12}\) and \(\alpha_{13}\) were purified as described previously (5); \(\alpha_{12}\) and \(\alpha_{13}\) were purified by the same procedure used for \(\alpha_{12}\) and \(\alpha_{13}\), and then according to Singer et al. (6). His\(\gamma\)C68S was purified as described (22) and generously provided by Dr. Bruce Posner (this laboratory).

Purification of PKC from Sf9 Cells—Sf9 cells (1 liter, 1.5 \(\times\) 10\(^{6}\) cells/ml) were infected with a recombinant baculovirus encoding rabbit PKC\(\alpha\) (23). Cells were harvested 48 h later and suspended in 120 ml of ice-cold lysis buffer (20 mM Tris-HCl (pH 7.5), 5 mM EDTA, 1 mM EGTA, 1 mM iodoacetate).
10 mM β-mercaptoethanol, and protease inhibitors). After cell lysis (nitrogen cavitation at 500 psi for 30 min) and centrifugation (100,000 × g for 30 min), PCaK was purified chromatographically using DEAE-Sephacel, hydroxyapatite, Phenyl-Superose HR10/10, and Mono Q HR5/5 (23) and assayed as described by Yasuda et al. (24). The yield was 1 mg from a 1-liter culture, the specific activity was 800 units/mg, and the protein was unaffected by sonication. After storage in liquid nitrogen, PCaK, α, and β were purified by the same method and were kindly supplied by Dr. William D. Singer (UT Southwestern Medical Center) and Dr. Shige Ohno (Yokohama City University).

Phosphorylation of G<sub>12</sub> Subunits by PKC—Candidate PKC substrates (in buffer containing 0.7% CHAPS) were incubated at 30°C for 30 min with PKC (Calbiochem) or purified recombinant PCaK (in 100 μl of 25 mM Tris-HCl (pH 7.5), 5 mM MgCl<sub>2</sub>, 125 mM CaCl<sub>2</sub>, 1 mM DTT, 10 μM γ<sup>32</sup>PATP (5000 cpm/pmol), and 10 μg/ml phosphorylase-diolein (Sigma). The final concentration of CHAPS was less than 0.07%, PKC (1 milliunit/mg of G<sub>12</sub>) was added to start the reaction. When induced, G<sub>12</sub> was first incubated with G<sub>a</sub> subunits for 10 min on ice. Reactions were stopped by addition of 500 μl of 1% SDS, 2 mM ATP, and 500 μl of 30% trichloroacetic acid. The mixtures were filtered to collect precipitated protein (BAB5 filters; Schleicher & Schuell), and the filters were washed (12 ml of 5% trichloroacetic acid) and counted. The stoichiometry of phosphorylation was approximately 0.5 for α<sub>12</sub> and 1.5 for α<sub>13</sub>; protein concentrations were determined by staining with Amido Black (25).

Trypsin Protection—Phosphorylated α<sub>12</sub> was incubated with GDP (100 μM) or GDP + AMP for 10 min at 0°C prior to treatment with TPCK-treated trypsin (20% of the mass of α<sub>12</sub>) for 20 min at 30°C. After addition of an equal volume of 2 × sample buffer, the products were analyzed by SDS-PAGE, followed by autoradiography or Western blotting with α<sub>12</sub> antisemur J169 (5).

Inhibition of GTPγS Binding to α by βγ-G, and αβ subunits were mixed at 0°C and incubated for 10 min in 50 mM NaHepes (pH 8.0), 50 mM βγ-G, and αβ subunits (0.5 mM) for α<sub>12</sub> or 0.5 mM EDTA. 1 mM DTT, 0.3% CHAPS, and 0.1% C<sub>e</sub>-E<sub>o</sub>. (final volume 25 μl). GTPγS binding solution was added (25 μl of 50 mM NaHepes (pH 8.0), 1 mM EDTA, 1 mM DTT, and 10 μM [γ<sup>32</sup>PS]GTPγS (8000 cpm/pmol)), and the mixture was incubated at 30°C for 60 min. After addition of 2 ml of ice-cold 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 20 mM MgCl<sub>2</sub>, bound GTPγS was quantified by filtration as described (26).

CD Gelatination of α<sub>12</sub> and βγ-G<sub>12</sub> (250 pmo1) was incubated on ice for 10 min with or without 750 pmo1 of βγ-G<sub>12</sub> in 180 μl of 50 mM NaHepes (pH 8.0), 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 3 mM MgCl<sub>2</sub>, and 0.7% CHAPS prior to application to a Superdex 200 HR 10/30 gel filtration column (Pharmacia) equilibrated with 50 mM NaHepes (pH 8.0), 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 3 mM MgCl<sub>2</sub>, and 1% octyl-β-gluco side (Calbiochem). Fractions of 0.4 ml were collected at a flow rate of 0.3 ml/min and were analyzed by SDS-PAGE and silver staining.

Expression, Labeling, and Immunoprecipitation of α<sub>12</sub>—NIH 3T3 cells that stably express α<sub>12</sub> (NIH 3T3-G12) were obtained by transfection with plasmids pCMVα<sub>12</sub> and pSV2Neo and selection in medium containing 600 μg/ml G418 (Life Technologies, Inc.). A mixture of G418-resistant colonies was collected 20 days after transfection. Expression of α<sub>12</sub> was confirmed by immunoblotting of cell membrane extracts with antisemur J169.

For labeling with either [35S]methionine or [32P]Pi, cells were incubated with methionine phosphate-free Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) for 2 h, followed by incubation with methionine-free medium containing [35S]methionine (50 μCi/ml; 3 h) or [32P]Pi (0.5 mM; 2 h). Cells were washed twice with 20 mM NaHepes (pH 7.5) and 150 mM NaCl, harvested, suspended in 500 μl of hypotonic buffer (20 mM NaHepes (pH 7.5), 1 mM EDTA, 1 mM DTT), frozen, and thawed three times, and centrifuged at 125,000 × g at 4°C for 20 min to prepare cytosolic and crude membrane fractions. Naf (5 mM) and β-glycerophosphate (10 mM) were included in the lysis buffer for cells labeled with [32P]Pi. Membrane extracts were prepared with 500 μl of 20 mM NaHepes (pH 7.5), 150 mM NaCl, 1% sodium cholate, 1% Triton X-100, and 0.5% SDS (RIPA buffer) prior to centrifugation at 125,000 × g for 20 min.

For immunoprecipitation, 25 μl of membrane extract was incubated with 2.5 μl of 10% fixed Staphylococcus aureus (Pansorbín; Calbiochem) on ice for 30 min. After centrifugation at 15,000 × g for 5 min, supernatants were incubated overnight at 4°C with 7.5 μg of anti-α<sub>12</sub>IgG or control rabbit IgG. Pansorbín (5 μl; 10%) was added for an additional 30 min prior to collection of immunoprecipitates by centrifugation and suspension in 100 μl of RIPA buffer. The suspension was layered over 1 ml of RIPA buffer containing 20% sucrose (w/v) and centrifuged at 15,000 × g for 5 min. Pellets were extracted with SDS-PAGE sample buffer, heated (90°C; 3 min), and subjected to SDS-PAGE followed by autoradiography. Gels containing [35S]methionine-labeled proteins were treated with ENHANCE (DuPont NEN). The yield was 1 mg from a 1-liter culture, the specific activity was 800 units/mg, and the protein was unaffected by sonication. After storage in liquid nitrogen, PCaK, α, and β were purified by the same method and were kindly supplied by Dr. William D. Singer (UT Southwestern Medical Center) and Dr. Shige Ohno (Yokohama City University).

**RESULTS**

Phosphorylation of α<sub>12</sub> and α<sub>13</sub> by PKC—The results of Dhanasekaran et al. (10), described above, prompted examination of possible phosphorylation of α<sub>12</sub> by PKC. Of the nine G protein α subunits tested, only α<sub>12</sub> and α<sub>13</sub> were phosphorylated in vitro by PKC (Fig. 1A); the effect on α<sub>13</sub> was anticipated based on the work of Lounsbury et al. (17). The related α subunit, α<sub>12</sub>, is not a substrate for PKC, also consistent with Dhanasekaran et al. (10). Phosphorylation of α<sub>12</sub> was dependent on Ca<sup>2+</sup> and phosphorylase-dependent, characteristic of PKC (Fig. 1B). Of the several types of PKC tested (PKCa, δ, ε, and θ), all showed the same pattern, phosphorylating only α<sub>12</sub> and α<sub>13</sub> (data not shown).

We examined NIH 3T3 cells that had been stably transfected with an expression plasmid encoding α<sub>12</sub> to test phosphorylation of the protein in vivo. Immunoblotting of membranes from these cells (NIH 3T3-G12) demonstrates significant expression of α<sub>12</sub> (Fig. 2A); we could not detect the protein in these cells prior to transfection (using antisemur J169). This antisemur could be used to immunoprecipitate α<sub>12</sub> from a membrane extract of [35S]methionine-labeled NIH 3T3-G12 cells (Fig. 2B), and phosphorylated α<sub>12</sub> was immunoprecipitated from cells.
labeled with [32P]P, after exposure to PMA (Fig. 2C). Thus, α12 appears to be phosphorylated in vivo after PKC is activated by phorbol esters.

The time course and stoichiometry of phosphorylation of α12 in vitro are shown in Fig. 3A. Since the substrate is over 90% pure (based on silver staining; Fig. 1A) and other phosphorylated proteins do not appear in the reaction mixtures (Fig. 1B), we estimated stoichiometry by filtration. When 7 pmol of α12 was included in the assay, the maximal incorporation of phosphate was about 3 pmol. Since the stoichiometry of binding of GTP·S to the α12 used here was about 50% (based on the protein assay), we believe that 1 mol of phosphate is incorporated per mol of α12. (α12 is not phosphorylated when denatured; data not shown.) Of interest, α12 was phosphorylated very poorly after incubation with a 2-fold excess of β1γ2 (Fig. 3A); the reaction is almost completely suppressed when α12 and β1γ2 are present at equimolar concentrations (Fig. 3B). Similar results were obtained with α2 (Fig. 3C). Nonpreylated βγ subunit complexes have reduced affinity for at least certain Gα subunits (22); appropriately, the βγ complex comprised of β1 and the nonpreylated Cy568 → Ser12 γ2 mutant was a less potent inhibitor of α12 phosphorylation (Fig. 3B). Since β1γ2 did not inhibit the activity of PKC when a specific substrate peptide from myelin basic protein (MBP a12) was utilized (data not shown), we conclude that α2 and α2 are not substrates for PKC when associated with βγ in the G protein heterotrimer.

Both the GDP-bound and the GTP·S-bound forms of α12 and α2 are phosphorylated almost equally well by PKC (Fig. 4A); there was no significant difference in the time course of phosphorylation of both forms of both proteins (data not shown). Lounsbury et al. (17) reported that the GDP-bound form of α2 was phosphorylated more efficiently than the GTP·S-activated species. The discrepancy may be explained by the fact that the Gα subunits used in this work were purified from S93 cells and thus myristoylated at their amino termini; the protein used by Lounsbury et al. (17) was synthesized in Escherichia coli and was not so modified. Myristoylation of the amino terminus may alter the conformation of this domain, which is the site of phosphorylation (see below).

Since βγ inhibits the phosphorylation of α12 and α2, we assessed the dependence of this effect on βγ concentration (using both GDP- and GTP·S-bound forms of α2 at the lowest possible concentrations (0.5 nM)) in an attempt to estimate the affinity of βγ for the protein (Fig. 4B). Efforts to measure these affinities have been thwarted in the past by the very high affinity of α-GDP for βγ and resultant difficulty in detection of an effect of βγ on α at appropriately low concentrations. However, phosphorylation of α by PKC offers a very sensitive signal. The concentrations of β1γ2 required to inhibit (by 50%) phosphorylation of α12-GDP and α2-GTP·S were 0.5 and 50 nM, respectively. Since the effect of β1γ2 on α12-GDP was still close to stoichiometric, there exists at least a 100-fold difference in apparent affinity of β1γ2 for α12-GDP and α2-GTP·S.

The Site of Phosphorylation of α12—Phosphorylated α12 was digested with trypsin either in the presence of GDP or GTP + AMF. Activation of α12 by AMF protects the bulk of the protein from digestion, and a 40-kDa fragment accumulates (Fig. 5). This fragment is recognized by antiserum J169, which was generated using a peptide corresponding to the carboxyl terminus of α12 (Fig. 5, lane 2). However, this fragment is no longer phosphorylated (Fig. 5, lane 5). Thus, phosphorylated α12 can still be activated by AMF, and phosphorylation by PKC occurs near the amino terminus. Similar results were obtained with α2, in which Ser16 and Ser27 both appear to be phosphorylated by PKC (17).

Characterization of Phosphorylated α12 and α2— α12 and α2 were phosphorylated and repurified as described under “Experimental Procedures.” The stoichiometry of phosphorylation of α12 was approximately 0.5 based on total protein concentration (presumed stoichiometry approximately 1), while that for α2 was 1–1.5; it is possible that α2 is phosphorylated at more than one site (17). Phosphorylation did not change the time course of GTP·S binding (and thus of GDP dissociation) for either α12 or α2 (Fig. 6).

The rate of binding of GTP·S to nonphosphorylated α12 or α2 is inhibited by βγ (Fig. 7). This reflects the well-known capacity...
of γ to stabilize the GDP-bound form of G protein α subunits. However, the rate of GTPγS binding to phosphorylated α12 (Fig. 7A) or phosphorylated α2 (Fig. 7B) is not inhibited substantially by a 10-fold molar excess of β1γz, and the modest effects seen could reflect the presence of small amounts of nonphosphorylated protein in the preparations. The effect of γ on GTPγS binding to mock-treated proteins (PKC in the absence of ATP) was the same as that on the nonphosphorylated proteins (data not shown).

We also examined the interaction of α12 and βγ by gel filtration. The peaks of both phosphorylated and nonphosphorylated α12 were in fractions 38–40 (Fig. 8), corresponding to molecular weights of about 45,000. Addition of β1γz to nonphosphorylated α12 shifted the peak of α about three fractions (35–37), while the migration of phosphorylated α12 was unchanged after incubation with β1γz. The results shown in Figs. 7 and 8 indicate that phosphorylation of α12 and α2 interfere with their capacity.
to form oligomers with the G protein $\beta\gamma$ subunit complex.

Finally, we examined the effect of phosphorylation of $\alpha_2$ on its ability to inhibit the activity of type V adenyl cyclase (5), since this is the only assay available for interaction of $\alpha_2$ or $\alpha_{12}$ with an effector (Fig. 9). Okadaic acid (1 $\mu$M) was included in the assay to inhibit phosphatases that might be present in the Sf9 cell membranes utilized as the source of adenyl cyclase. Phosphorylation of $\alpha_2$ had little or no effect on its inhibitory interactions with adenyl cyclase.

**DISCUSSION**

We have demonstrated that $\alpha_{12}$ is phosphorylated by PKC both in vitro and in vivo; the homologous subfamily member $\alpha_{13}$ is not a substrate. Among the large number of G protein $\alpha$ subunits tested, the only other efficient substrate for phosphorylation by various isoforms of PKC was $\alpha_x$. The stoichiometry of phosphorylation of $\alpha_{12}$ was equal to that for GDP-$\gamma$S binding and is thus assumed to be 1.

Phosphorylation of $\alpha_{12}$ occurs within the amino-terminal domain that is removed by trypsin selectively from activated G protein $\alpha$ subunits (Table I). Examination of corresponding sites of proteolysis in other G$\alpha$ subunits indicates that trypsin probably removes the first 49 or 50 residues from $\alpha_{12}$. There are three serine residues (2, 9, and 38) and one threonine (7) within the relevant sequence. Although Ser9 and Ser38 are both can-

The indicated concentrations of $\alpha_2$ were mixed with 20 $\mu$g of membranes from Sf9 cells expressing type V adenyl cyclase in the presence of 50 nM GTP-$\gamma$S-$\alpha_1$. Adenylyl cyclase activity was assayed as described under “Experimental Procedures.” $\alpha_1$ subunits were nonphosphorylated $\alpha_2$-GDP ($\bullet$), nonphosphorylated $\alpha_2$-GTP-$\gamma$S ($\circ$), phosphorylated $\alpha_2$-GDP ($\nabla$), and phosphorylated $\alpha_2$-GTP-$\gamma$S ($\bigcirc$). The concentrations of GTP-$\gamma$S-activated $\alpha_1$ subunits were estimated from $[^{35}S]$GTP-$\gamma$S binding. Data shown are the average of duplicate determinations from a single experiment that is representative of three such experiments.

![Figure 9. Inhibition of type V adenyl cyclase by phosphorylated $\alpha_2$.](image)

**Table I**

| $\alpha_{12}$ | MSGVVRTLSRLP|AEGAR|GAGAAR(D|RARD|DALLAERRR|VRR |
| $\alpha_{13}$ | MADFLP-SRSV|LSCFP |----------|GCV|LNGAEQ|QK|E|D|C|LS|KTYVRK |
| $\alpha_2$ | MCRQSSE|KEAR|R|R|DH|LR|BQ|RQK |
| $\alpha_{11}$ | MGC|TL|SAED|K|AVERS|K|D|NL|RED|GK |
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REFERENCES

1. Hepler, J. R., and Gilman, A. G. (1992) Trends Biochem. Sci. 17, 383–387
2. Kaziro, Y., Itoh, H., Kozasa, T., Nakafuku, M., and Satch, T. (1991) Annu. Rev. Biochem. 60, 349–400
3. Neer, E. J. (1995) Cell 80, 249–257
4. Strathmann, M. P., and Simon, M. I. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 5582–5586
5. Kozasa, T., and Gilman, A. G. (1995) J. Biol. Chem. 270, 1734–1741
6. Singer, W. D., Miller, R. T., and Sternweis, P. C. (1994) J. Biol. Chem. 269, 19796–19802
7. Chan, A. M. L., Fleming, T. P., McGovern, E. S., Chedid, M., Miki, T., and Aaronson, S. A. (1993) Mol. Cell. Biol. 13, 762–768
8. Jiang, H. P., Wu, D. Q., and Simon, M. I. (1993) FEBS Lett. 330, 319–322
9. Kozasa, T., and Gilman, A. G. (1995) J. Biol. Chem. 270, 1734–1741
10. Dhanasekaran, N., Varaprasad, M. V. S., Wadsworth, S. J., Dermott, J. M., and van Rossum, G. (1994) J. Biol. Chem. 269, 11802–11806
11. Vojnov-Yasukawa, T. A., Face, A. M., and Bourne, H. R. (1994) Oncogene 9, 2509–2515
12. Kawabe, J., Iwami, G., Ebina, T., Ohno, S., Katada, T., Ueda, Y., Homcy, C. J., and Ishikawa, Y. (1994) J. Biol. Chem. 269, 16554–16558
13. Jacobowitz, O., and Iyengar, R. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 10630–10634
14. Orellana, S. A., Solski, P. A., and Brown, J. H. (1985) J. Biol. Chem. 260, 5236–5239
15. Lynch, C. J., Charest, R., Bocckino, S. B., Exton, J. H., and Blackmore, P. F. (1985) J. Biol. Chem. 260, 2844–2851
16. Takano, K., Stanfield, P. R., Nakajima, S., and Nakajima, Y. (1995) Neuron 14, 999–1008
17. Lonsbury, K. M., Schlegel, B., Poncz, M., Brass, L. F., and Manning, D. R. (1993) J. Biol. Chem. 268, 3494–3498
18. Katada, T., Gilman, A. G., Watanabe, Y., Bauer, S., and Jakobs, K. H. (1985) Eur. J. Biochem. 151, 431–437
19. Bushfield, M., Murphy, G. J., Lavan, B. E., Parker, P. J., Hruby, V. J., Milligan, G., and Housley, M. D. (1996) Biochem. J. 268, 449–457
20. Daniel-Issakani, S., Spiegel, A. M., and Strulov, B. (1989) J. Biol. Chem. 264, 20240–20247
21. Fields, T. A., and Casey, P. J. (1995) J. Biol. Chem. 270, 23119–23125
22. Iniguez-Lluhi, J. A., Simon, M. I., Robishaw, J. D., and Gilman, A. G. (1992) J. Biol. Chem. 267, 23409–23417
23. Fujise, A., Mizuno, I., Ueda, Y., Osada, S., Hirai, S., Takayanagai, A., Shimizu, N., Owada, M. K., Nakajima, H., and Ohno, S. (1994) J. Biol. Chem. 269, 31642–31648
24. Yasuda, I., Kishimoto, A., Tanaka, S., Tominaga, M., Sakurai, A., and Nishizuka, Y. (1990) Biochem. Biophys. Res. Commun. 166, 1220–1227
25. Schaffert, W., and Weissmann, C. (1973) Anal. Biochem. 56, 502–514
26. Northup, J. K., Smigel, M. D., and Gilman, A. G. (1982) J. Biol. Chem. 257, 11416–11423
27. Laemmli, U. K. (1970) Nature 227, 680–685
28. Wray, W., Bourlikas, T., Wray, V. P., and Hancock, R. (1981) Anal. Biochem. 118, 197–203
29. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
30. Pearson, R. B., and Kemp, B. E. (1991) Methods Enzymol. 200, 62–81
31. Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G., and Sprang, S. R. (1994) Science 265, 1405–1412
32. Mixon, M. B., Lee, E., Coleman, D. E., Berghuis, A. M., Gilman, A. G., and Sprang, S. R. (1995) Science 270, 954–960
33. Wall, M. A., Coleman, D. E., Lee, E., Iniguez-Lluhi, J. A., Posner, B. A., Gilman, A. G., and Sprang, S. R. (1998) Cell 83, 1047–1058
34. Barford, D., and Johnson, L. N. (1989) Nature 340, 609–616