Volume 260, number 2, 313–317 FEB 08039 January 1990

The γ subunit of brain G-proteins is methyl esterified at a C-terminal cysteine

Bernard K.-K. Fung, Harvey K. Yamane, Irene M. Ota† and Steven Clarke†

Jules Stein Eye Institute and †Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024, USA

Received 18 October 1989; revised version received 1 December 1989

The γ polypeptide of brain G-proteins is carboxyl methylated when the purified $\beta\gamma$ subunit complex is reconstituted with S-adenosyl-[^{3}H-methyl]-L-methionine and a methyltransferase present in detergent-stripped brain membranes. By chromatographic analysis of the ^{3}H-amino acid generated by exhaustive proteolysis and performic acid oxidation of the ^{3}H-methylated $\beta\gamma$ complex, we show that this modification occurs on the α-carboxyl group of a C-terminal cysteine residue. Our result suggests that brain G-proteins may undergo multiple covalent modification steps, including proteolytic removal of the three terminal amino acids from the predicted common C-terminal Cys-Xaa-Xaa-Xaa sequence, and the methyl esterification of the resulting terminal cysteine residue. This modification is likely to be associated with lipidation at the sulfhydryl group of the same cysteine, which would explain the tight membrane binding property of the brain $\beta\gamma$ complex.

G-Protein; Posttranslational modification; Carboxyl methylation; Lipidation

1. INTRODUCTION

G-proteins play a key role in transducing a diverse array of extracellular signals into intracellular responses (reviewed in [1]). Members of this family of regulatory proteins are heterotrimers composed of α, β and γ subunits. To date, over twelve unique GTP-binding α subunits have been discovered. They all appear to mediate signal coupling between a receptor and an effector enzyme [1]. In contrast, only two distinct forms of the β [2–4] and four types of the γ subunits have been identified [5–8]. In mammalian systems, the β and γ subunits form a tightly associated $\beta\gamma$ complex which interacts with the α subunit to facilitate binding to receptors [9,10] and to attenuate adenylate cyclase activity [11,12]. Recent evidence further suggests that the $\beta\gamma$ complex itself may directly activate phospholipase A_2 [13,14] and regulate pheromone-mediated mating in yeast [6].

In this paper, we present evidence showing that at least one γ subunit of bovine brain G-proteins is methyl esterified at a C-terminal cysteine residue. This process may involve a common C-terminal Cys-Xaa-Xaa-Xaa motif that provides a general recognition sequence for proteolytic removal of the three terminal residues, followed by lipidation and methylation of the resulting C-terminal cysteine [15]. We postulate that carboxyl methylation and a putative lipidation event may play important roles in the membrane localization and functioning of the $\beta\gamma$ complex.

Correspondence address: B.K.-K. Fung, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024, USA

Abbreviations: AdoMet, *S*-adenosyl-L-methionine; CHAPS, 3-((3-cholamidopropyl)-dimethyl-ammonio)-1-propanesulfonate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; G_α and $G_{\beta\gamma}$, α and $\beta\gamma$ subunit of brain G-proteins; MOPS, 3-(*N*-morpholino)propanesulfonic acid; T_α and $T_{\beta\gamma}$, α and $\beta\gamma$ subunit of transducin

2. MATERIALS AND METHODS

2.1. *Preparation of ^{3}H-methylated $G_{\beta\gamma}$*

Bovine brain $G_{\beta\gamma}$ was partially purified by successive chromatographies on DEAE-Sephacel, Bio-Gel P200 and hydroxylapatite according to a modified procedure [16] of Sternweis and Robishaw [17]. Final purification of $G_{\beta\gamma}$ was achieved on a Mono-Q HR5/5 column eluted with a gradient of 0–300 mM NaCl in Mono-Q buffer (20 mM Tris, pH 8.0, 1 mM EDTA, 5 mM $MgCl_2$, 0.5% CHAPS). Fractions containing $G_{\beta\gamma}$ were pooled, concentrated to over 20 mg/ml, and stored frozen at −20°C.

Bovine brain membranes were prepared and extracted with TED buffer (20 mM Tris, pH 8.0, 1 mM EDTA, 1 mM DTT) containing 1% cholate as described by Sternweis and Robishaw [17]. Following centrifugation at 50000 × *g* for 1 h, the detergent-stripped membranes were washed once with TED buffer containing 0.1 M NaCl, and then resuspended in the same buffer. This membrane preparation was used as a source of methyltransferase in carboxyl methylating $G_{\beta\gamma}$.

Methylation of $G_{\beta\gamma}$ was routinely performed by rapidly mixing 20 μl of 20 mg/ml $G_{\beta\gamma}$ with 780 μl of methylation buffer (50 mM Hepes, pH 7.0, 100 mM NaCl, 5 mM $MgCl_2$, 0.1 mM DTT) containing 2 mg of cholate-stripped brain membranes and 0.6 nmol of [^{3}H]AdoMet (79.6 Ci/mmol). After 3 h of incubation at 37°C, the membranes were washed 3 times with 3 ml of buffer A (10 mM MOPS, pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT) to remove all residual [^{3}H]AdoMet. To extract the ^{3}H-methylated $G_{\beta\gamma}$, the membranes were incubated in 0.8 ml of 10 mM sodium cholate in buffer A for 15 min, followed by centrifugation in a Beckman airfuge at 120000 × *g* for 3 min. This extract, which contained approximately 50–70% of the total ^{3}H-methylated $G_{\beta\gamma}$ associated with the

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

membranes, was used for immunoprecipitation and amino acid analysis.

2.2. *Total enzymatic digestion of ^{3}H-methylated $G_{\beta\gamma}$*

^{3}H-methylated $G_{\beta\gamma}$ extract (8 μg protein) was digested with 50 μg of diphenyl carbamyl chloride-treated bovine pancreatic trypsin (Sigma) in 100 mM 2-bis(2-hydroxyethyl)-amino-2-(hydroxylmethyl)-1,3-propanediol buffer, pH 6.0, at 37°C in a total volume of 1.0 ml. After 6 h, an additional 50 μg of trypsin was added and the incubation continued for 12 h. The reaction was then quenched by the addition of 300 μl of 10% (w/v) trifluoroacetic acid. The sample was lyophilized and digested with 65 μg of porcine kidney microsomal leucine aminopeptidase (Sigma, type IV-S, 0.6 unit) and 65 μg of prolidase (Sigma, 5.2 unit) in 150 mM 2-bis(2-hydroxylethyl)-amino-2-(hydroxylmethyl)-1,3-propanediol buffer, pH 6.0 containing 30 mM $CaCl_2$ and 30 mM $MnCl_2$ in a total volume of 1 ml. The digestion was continued for 240 min at 40°C and then quenched with 150 μl of 10% (w/v) trifluoroacetic acid. This material was lyophilized and oxidized with 1.9 ml of performic acid for 23 h at 4°C as described by Ota and Clarke [18].

2.3. *Analytical methods*

SDS-PAGE analyses were performed either with the Laemmli buffer system [19] or the tricine buffer system [20]. The latter system allows the separation of proteins in the range from 1 to 20 kDa. Radioactivity was determined either by excision of appropriate gel regions followed by digestion in 20% hydrogen peroxide at 65°C overnight and liquid scintillation counting, or by autoradiography using Kodak XAR-5 film following equilibration in the fluorographic reagent Amplify (Amersham). $G_{\beta\gamma}$ concentrations were measured by Coomassie blue binding using γ-globulin in Mono-Q buffer as a standard [21]. Membrane protein concentrations were determined by measuring the absorbance at 280 nm of membranes solubilized in 1% SDS. One mg/ml of membrane proteins is arbitrarily defined as 1 OD unit at 280 nm. Analysis of methyl ester linkages was performed by the vapor-phase equilibration assay [22].

3. RESULTS

The $\beta\gamma$ subunits of G proteins were extracted from bovine brain membranes with sodium cholate and purified by successive chromatographies on DEAE-Sephacel, Bio-Gel P200, hydroxylapatite, and Mono-Q anion exchange columns. SDS-PAGE analysis of the purified proteins showed a doublet corresponding to 35000 and 36000 Da and a protein band of 8000 Da, consistent with the electrophoretic pattern of brain $G_{\beta\gamma}$ preparations reported previously [23]. Based on the Coomassie blue staining intensity, these three bands accounted for over 96% of the total protein (left panel of fig.1, lane 1). In addition to the evidence provided by SDS-PAGE analysis, affinity-purified antibodies directed against the $T_{\beta\gamma}$ subunit of transducin [24] were found to cross-react with both the 35 and 36 kDa bands, but not with the 8 kDa band (data not shown). These results provide conclusive evidence for the two forms of β subunit in our purified $G_{\beta\gamma}$ preparation [23] and confirm the immunochemical difference between the γ subunits of brain G-proteins and transducin [24–26].

The purified $G_{\beta\gamma}$ was tested as a methyl acceptor by incubation with [^{3}H]AdoMet and detergent-stripped brain membranes as a source of protein methyltransferase. The products of the reaction were

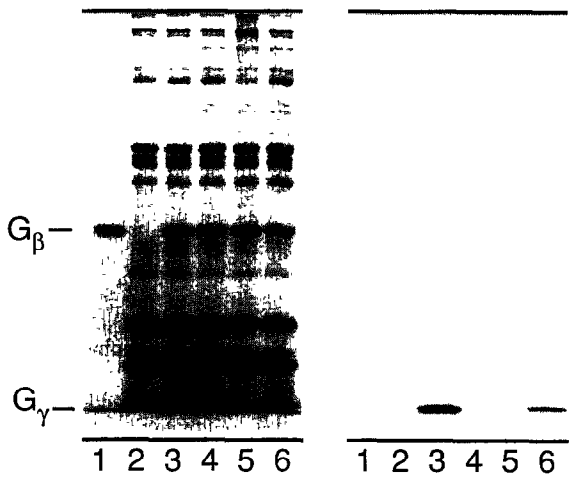


Fig.1. Methylation of $G_{\beta\gamma}$ by brain membranes. Purified brain $G_{\beta\gamma}$ was methylated as described in section 2, except 0.5 μM of [^{3}H]AdoMet was used. Each lane contains 5 μg of $G_{\beta\gamma}$ and 25 μg of stripped brain membranes where indicated, and substituted with the appropriate buffer where omitted. Following incubation at 37°C for 3 h, samples were quenched with electrophoresis sample buffer and subjected to SDS-PAGE. Left panel, Coomassie blue-stained gel. Right panel, corresponding autoradiogram. Lane 1, $G_{\beta\gamma}$ only; lane 2, stripped brain membranes only; lane 3, $G_{\beta\gamma}$ + stripped brain membranes; lane 4, $G_{\beta\gamma}$ + stripped brain membranes, in presence of 100 μM *S*-adenosylhomocysteine; lane 5, $G_{\beta\gamma}$ + stripped brain membranes denatured by heating for 15 min at 100°C; lane 6, $G_{\beta\gamma}$ denatured by heating for 15 min at 100°C + stripped brain membranes.

then analyzed by SDS-PAGE at pH 8.6. Under this alkaline condition, the less stable aspartyl α- and β-methyl esters are hydrolyzed, but carboxyl terminal α-methyl esters remain stable [18,27]. As shown in lane 3 of fig.1, radioactivity was detected in a protein band co-migrating with G_γ at the 8 kDa position. Over 95% of the radioactivity associated with this gel band was base-labile and recovered as [^{3}H]methanol when analyzed by the vapor phase equilibration assay [22], indicating that the 8 kDa protein is carboxyl methylated. Methylation was not observed if brain membranes (lane 1) or $G_{\beta\gamma}$ (lane 2) were omitted from the reaction mixture. Incorporation of radioactivity was almost completely blocked in the presence of an excess of the reaction product, *S*-adenosylhomocysteine (lane 4), demonstrating that the methylation observed is the result of a competitive enzymatic reaction. Methylation was also absent if the membranes were denatured by heating at 100°C for 15 min (lane 5), and was greater than 80% inhibited when $G_{\beta\gamma}$ was similarly heat-denatured before incubation with stripped brain membranes and [^{3}H]AdoMet (lane 6). These results indicate that carboxyl methylation of the 8 kDa polypeptide is mediated by a methyltransferase present in the stripped brain membranes.

Over 50% of the membrane-bound *^{3}H-methylated $G_{\beta\gamma}$* can be re-extracted from the stripped brain membranes of the reconstituted system with a low concen-

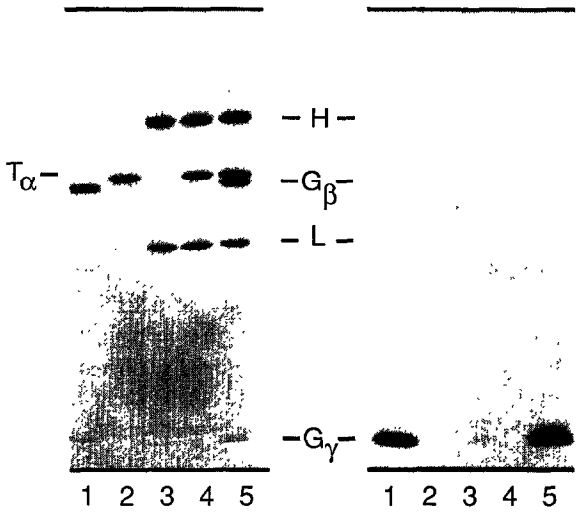
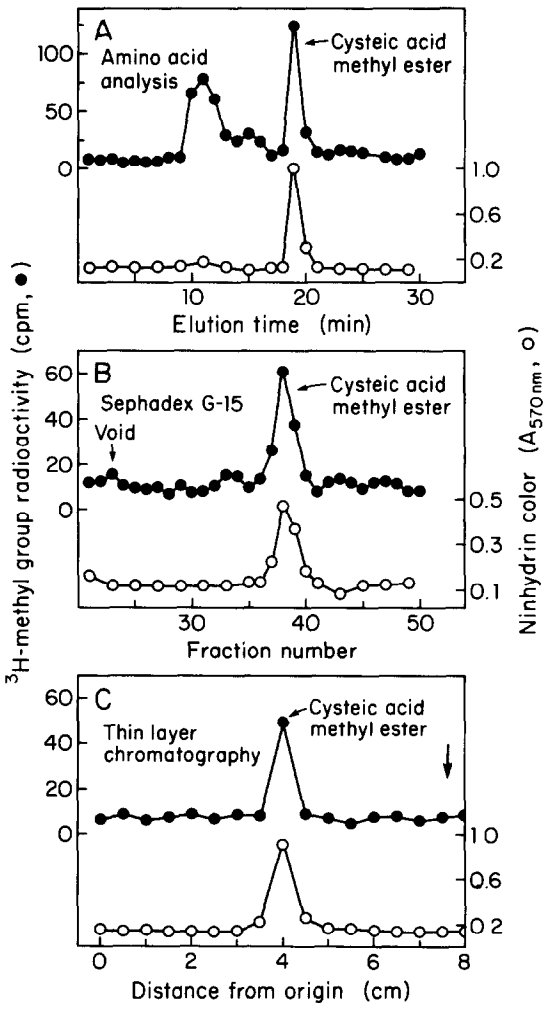


Fig.2. Confirmation of the identity of ^{3}H-methylated G_γ by co-immunoprecipitation of $G_{\beta\gamma}$ with T_α. Left panel, Coomassie blue-stained gel of 2 μg of cholate-extracted ^{3}H-methylated $G_{\beta\gamma}$ standard (lane 1), 2.5 μg of purified T_α standard (lane 2), and proteins extracted from the TF16-pansorbin immunoprecipitates of ^{3}H-methylated $G_{\beta\gamma}$ only (lane 3), T_α only (lane 4), and a mixture of ^{3}H-methylated $G_{\beta\gamma}$ and T_α (lane 5). Right panel, corresponding autoradiogram. Immunoprecipitation was performed as described [28] using 6 μg of mAb TF16, 4.5 μg of ^{3}H-methylated $G_{\beta\gamma}$ extract and 5.5 μg of T_α where indicated.

Fig.3. Chromatographic identification of cysteic acid ^{3}H-methyl ester. (A) Amino acid analysis of the enzymatic digests of $G_{\beta\gamma}$. ^{3}H-methylated $G_{\beta\gamma}$ was converted to its amino acid constituents as described in section 2. The oxidized digest was lyophilized, resuspended in pH 2.2 sodium citrate sample buffer (Pierce) and mixed with 4 μmol of L-cysteic acid methyl ester standard. This mixture was fractionated by amino acid analysis on a column of Beckman AA-15 sulfonated polystyrene resin (0.9 × 54 cm) at 56°C in a pH 3.25 sodium citrate buffer (0.2 M in sodium ion). One minute fractions (1.1 ml) were collected and assayed for total radioactivity (●—●) by counting 50 μl of each fraction in 5 ml of scintillation fluor. The elution position of the cysteic acid methyl ester standard was determined by manual ninhydrin analysis of 20-μl aliquots of column fractions (○—○). A peak of radioactivity was found to co-elute with the cysteic acid methyl ester standard at 19 min. Radioactivity was absent at 60 and 90 min where aspartate β-methyl ester and glutamate γ-methyl ester, respectively, were expected to be eluted from the column. The identity of the radioactive peak at 9–11 min has not been established. (B) Sephadex G-15 gel filtration chromatography of the cysteic acid methyl ester peak. Fraction 19 from the amino acid analysis column was lyophilized and redissolved in 200 μl of 10 mM synthetic cysteic acid methyl ester. This mixture was applied to a 1.5 × 85 cm column of Sephadex G-15 equilibrated with 0.1 M acetic acid. Fractions (2.2 ml) were collected and analyzed for radioactivity (●—●) and ninhydrin-reactive material (○—○) as described above. (C) Thin-layer chromatography of cysteic acid ^{3}H-methyl ester. Fraction 38 of the Sephadex G-15 column above was lyophilized and dissolved in a minimal volume of water. An aliquot was applied to a cellulose sheet and chromatographed at room temperature in a solvent of *n*-propanol/water (7:3, v/v). Strips (0.5 cm) were cut and analyzed for radiolabel in methyl ester linkages (●—●) and for ninhydrin reactivity (○—○) as described [18]. The position of the solvent front is marked with an arrow.

tration of sodium cholate with few protein contaminants (fig.2, lane 1). When this preparation was incubated with the purified T_α subunit of transducin (lane 2) and the mixture immunoprecipitated with a monoclonal antibody TF16 directed against T_α [28], a heteromeric complex consisting of T_α, both the 35 kDa and 36 kDa forms of G_β, and G_γ were observed in the immunoprecipitate (lane 5). $G_{\beta\gamma}$ was absent if T_α was not included in the incubation mixture (lane 3). These results indicate that $G_{\beta\gamma}$ and T_α form a tightly associated complex that can be immunoprecipitated with monoclonal antibody TF16. When the autoradiogram of this gel was examined, a ^{3}H-methylated protein band at the 8 kDa position was found only in the preparation containing added T_α (lane 5, left panel). Based on the ability of the ^{3}H-methylated protein to form a complex with T_α, we conclude that the carboxyl methylated 8 kDa protein is the γ polypeptide of brain G-proteins. From the amount of radioactivity associated with the G_γ band of the SDS-PAGE gel and the specific activity of the [^{3}H]AdoMet, we calculated that 1–2 mol% of the added $G_{\beta\gamma}$ were



methylated after 3 h of incubation. This low level of labeling can be explained if most of the purified $G_{\beta\gamma}$ has already been methylated in vivo.

In order to identify the methyl esterified amino acid, a preparation of cholate-extracted ^{3}H-methylated $G_{\beta\gamma}$ was enzymatically converted to its amino acid constituents by the combined actions of trypsin, leucine aminopeptidase, and prolidase [18]. The proteolysis was followed by performic acid oxidation, which quantitatively cleaves thiol ester-linked lipids and may partially cleave thiol ether-linked lipids [18]. Under these conditions, ^{3}H-methylated C-terminal cysteine residues will be released as free cysteic acid ^{3}H-methyl ester. This oxidized digest was fractionated on an amino acid analysis column. As shown in fig.3A, a major peak of radioactivity, accounting for 6.2% of that present as methyl esters in the starting material, was found to elute in the position of the cysteic acid methyl ester standard. The 6.2% yield represents a minimum estimate of the content of C-terminal cysteine methyl esters because of spontaneous hydrolysis of the methyl ester during isolation, esterolytic activity of the proteolytic enzymes, and potential incomplete cleavage of the polypeptide or the thioether-linked lipids on the cysteine residue [18]. When the radioactive material eluting at 19 min was fractionated by size on a Sephadex G-15 gel filtration column, all of the radiolabel was found to co-elute with the synthetic standard of cysteic acid methyl ester (fig.3B). To further confirm the identification of cysteic acid ^{3}H-methyl ester, fractions 37–39 were taken from the Sephadex G-15 gel filtration column and analyzed by cellulose thin layer chromatography using either *n*-propanol/water (fig.3C) or *n*-butanol/acetic acid/water (data not shown). In both chromatography systems, the radioactive materials from each fraction co-eluted with the cysteic acid methyl ester standard. These results show that methylation of G_γ occurs at the α-carboxyl group of a C-terminal cysteine residue.

4. DISCUSSION

In this study we present the first evidence that at least one γ subunit of bovine brain G-proteins is methylated at the α-carboxyl group of a C-terminal cysteine residue. Other proteins shown to be similarly methyl esterified at a C-terminal cysteine residue include the ras proteins [15,29–31], several fungal mating factor peptides (cf. [32]), the α subunit of retinal cGMP phosphodiesterase [33], and at least one polypeptide of bovine rod outer segments in the molecular mass range of between 23000 and 29000 Da [18]. In each of these methylated proteins for which the cDNA sequence has been determined, the deduced amino acid sequence predicts a cysteine residue at the fourth position from the C-terminus. We have previously proposed that methylation of the α-carboxyl group of this cysteine residue in these proteins occurs following the post-translational removal of three amino acid residues from the C-terminus [15]. The known C-terminal sequence of Cys-Ala-Ile-Leu deduced from the cDNA sequence of a bovine brain G-protein γ subunit [7] and our observation of methyl esterification of bovine brain G_γ at a terminal cysteine residue further support this model.

In addition to being modified by methyl esterification, several members of this group of proteins may be associated with membranes through a lipophilic moiety covalently attached to the sulfhydryl group of the C-terminal cysteine residue [15,30]. Consistent with the possible lipidation of G_γ is the observation that the brain $G_{\beta\gamma}$ subunit complex alone can bind tightly to phospholipid vesicles [34], as well as to the stripped brain membranes as described here. In ras proteins, the C-terminal cysteine residue appears to be polyisoprenylated in a biosynthetic step that occurs prior to the attachment of a palmitoyl group to a nearby cysteine residue [30]. Since chemical analysis of $G_{\beta\gamma}$ failed to reveal the presence of fatty acids [35], we speculate that the C-terminal methylated cysteine of the γ subunit may also be modified by polyisoprenylation.

What might be the role of the post-translational processing of the γ subunit in signal transduction? A priori, the methylation and possible lipidation of the γ subunit might serve to direct the G-protein to the membrane, where it can be properly oriented with its receptor and effector enzyme in preparation for a cycle of signal transduction [9–12]. Alternatively, this modification may be essential for converting the γ subunit to an active form, which can then act on a putative effector to generate a cellular response [6,13,14]. Which, if either, of these pathways occurs remains to be elucidated, but the existence of large families of signal transducing proteins with a Cys-Xaa-Xaa-Xaa motif [15] raises the possibility that carboxyl methylation of the C-terminal cysteine may play an important role in cellular regulation.

Acknowledgements: This work was supported by the Jules and Doris Stein Research to Prevent Blindness Professorship from Research to Prevent Blindness, Inc. and a grant from the National Institutes of Health (EY05895) to B.K.-K.F., and grants from the National Institutes of Health (GM-26020) and the National Science Foundation (DMB 8602102) to S.C.

REFERENCES

[1] Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615–649.
[2] Sugimoto, K., Nukada, T., Tanabe, T., Takahashi, H., Noda, M., Mianmino, N., Kangawa, K., Matsuo, H., Hirose, T., Inayama, S. and Numa, S. (1986) FEBS Lett. 191, 235–240.
[3] Fong, H.K.W., Amatruda, T.T., iii, Birren, B.W. and Simon, M.I. (1987) Proc. Natl. Acad. Sci. USA 84, 3792–3794.
[4] Gao, B., Gilman, A.G. and Robishaw, J. (1987) Proc. Natl. Acad. Sci. USA 84, 6122–6125.

[5] Hurley, J.B., Fong, H.K.W., Teplow, D.B., Dreyer, W.J. and Simon, M.I. (1984) Proc. Natl. Acad. Sci. USA 81, 6948–6952.
[6] Whiteway, M., Hougan, L., Dignard, D., Thomas, D.Y., Bell, L., Saari, G.C., Grant, F.J., O'Hara, P. and Mackay, V.L. (1989) Cell 56, 467–477.
[7] Gautam, N., Baetscher, M., Aebersold, R. and Simon, M.I. (1989) Science 244, 971–974.
[8] Robishaw, J.D., Kalman, V.K., Moomaw, C.R. and Slaughter, C.A. (1989) J. Biol. Chem. 264, 15758–15761.
[9] Fung, B.K.-K. (1983) J. Biol. Chem. 258, 10495–10502.
[10] Florio, V.A. and Sternweis, P.C. (1985) J. Biol. Chem. 260, 3477–3483.
[11] Katada, T., Bokoch, G.M., Smigel, M.D., Ui, M. and Gilman, A.G. (1984) J. Biol. Chem. 259, 3586–3595.
[12] Cerione, R.A., Staniszewski, C., Gierschik, P., Codina, J., Somers, R.L., Birnbaumer, L., Spiegel, A.M., Caron, M.G. and Lefkowitz, R.J. (1986) J. Biol. Chem. 261, 9514–9520.
[13] Jelsema, C. and Axelrod, J. (1987) Proc. Natl. Acad. Sci. USA 84, 3623–3627.
[14] Kim, D., Lewis, D.L., Graziadei, L., Neer, E.J., Bar-Sagi, D. and Clapham, D.E. (1989) Nature 337, 557–560.
[15] Clarke, S., Vogel, J.P., Deschenes, R.J. and Stock, J. (1988) Proc. Natl. Acad. Sci. USA 85, 4643–4647.
[16] Yamane, H.K. and Fung, B.K.-K. (1989) J. Biol. Chem. 264, 20100–20105.
[17] Sternweis, P.C. and Robishaw, J.D. (1984) J. Biol. Chem. 259, 13806–13813.
[18] Ota, I.M. and Clarke, S. (1989) J. Biol. Chem. 264, 12879–12884.
[19] Laemmli, U.K. (1970) Nature 227, 680–685.
[20] Schägger, H. and Von Jagow, G. (1987) Anal. Biochem. 166, 368–379.
[21] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[22] Murray, E.D., jr and Clarke, S. (1986) J. Biol. Chem. 261, 306–312.
[23] Evans, T., Fawzi, A., Fraser, E.D., Brown, M.L. and Northup, J.K. (1987) J. Biol. Chem. 262, 176–181.
[24] Fung, B.K.-K. (1986) Prog. Retinal Res. 6, 151–177.
[25] Huff, R.M., Axton, J.M. and Neer, E.J. (1985) J. Biol. Chem. 260, 10864–10871.
[26] Roof, D.J., Applebury, M.L. and Sternweis, P.C. (1985) J. Biol. Chem. 260, 16242–16249.
[27] Clarke, S. (1985) Annu. Rev. Biochem. 54, 479–506.
[28] Navon, S.E. and Fung, B.K.-K. (1987) J. Biol. Chem. 262, 15746–15751.
[29] Gutierrez, L., Magee, A.I., Marshall, C.J. and Hancock, J.F. (1989) EMBO J. 8, 1093–1098.
[30] Hancock, J.F., Magee, A.I., Childs, J.E. and Marshall, C.J. (1989) Cell 57, 1167–1177.
[31] Deschenes, R.J., Stimmel, J.B., Clarke, S., Stock, J. and Broach, J.R. (1989) J. Biol. Chem. 264, 11865–11873.
[32] Anderegg, R.J., Betz, R., Carr, S.A., Crabb, J.W. and Duntze, W. (1988) J. Biol. Chem. 263, 18236–18240.
[33] Ong, O.C., Ota, I.M., Clarke, S. and Fung, B.K.-K. (1989) Proc. Natl. Acad. Sci. USA 86, 9238–9242.
[34] Sternweis, P.C. (1986) J. Biol. Chem. 261, 631–637.
[35] Buss, J.E., Mumby, S.E., Casey, P.J., Gilman, A.G. and Sefton, B.M. (1987) Proc. Natl. Acad. Sci. USA 84, 7493–7497.