# A Site on Rod G Protein α Subunit That Mediates Effector Activation

Helen M. Rarick, Nikolai O. Artemyev, Heidi E. Hamm*

The heterotrimeric guanine nucleotide binding proteins (G proteins) are activated by sensory or hormone receptors. In turn, the G proteins activate effector proteins such as adenylyl cyclase, cyclic guanosine 3′,5′-monophosphate phosphodiesterase (cGMP PDE), phospholipase C, and potassium and calcium ion channels by mechanisms that are poorly understood. A site on the α subunit of the G protein transducin ($\alpha_t$) has been identified that interacts with and activates cGMP phosphodiesterase, the effector enzyme in rod photoreceptors. A 22–amino acid peptide, corresponding to residues 293 to 314 from the COOH-terminal region of $\alpha_t$, fully mimicked $\alpha_t$ and potently activated PDE. This region is adjacent to the receptor activation domain; thus, the α subunit of this G protein has a site for interaction with both its effector and receptor that maps near the COOH-terminus.

In vertebrate rod cells, the G protein, transducin ($G_t$), couples the photoreceptor rhodopsin to the effector cGMP PDE. The α subunits of G proteins in the guanosine diphosphate (GDP)–bound form bind to both the βγ subunit complex and their cognate activated receptor (*1*). Binding to the activated receptor stimulates replacement of GDP with guanosine triphosphate (GTP) at the guanine nucleotide binding site of the α subunits, which results in a conformational change in α (*2*). The α subunits with GTP bound (α-GTP) have decreased affinity for the receptor and for the βγ subunit (*1*) and can stimulate effectors (*3*). This alteration of protein to protein interactions indicates that the conformation of α-GTP must reveal specific amino acid residues that take part in effector activation; however, it is uncertain which residues on the α subunit interact with and activate the effector. Analysis of chimeras of the α subunits of $G_i$ ($\alpha_i$) and $G_s$ ($\alpha_s$) showed that the smallest linear stretch of $\alpha_s$ sufficient to stimulate adenylyl cyclase was a 120-residue segment near the COOH-terminus (residues 235 to 356) (*4*, *5*). We have determined that a 22–amino acid peptide from within the COOH-terminal region of $\alpha_t$ is able to activate cGMP PDE.

On the rod outer segment (ROS) disk membrane, $G_t$ in the GTP-bound form activates its effector, cGMP PDE, which cleaves cGMP to 5′ GMP and results in plasma membrane hyperpolarization (*6*). Bovine PDE consists of two catalytic subunits, Pα (88 kD) and Pβ (84 kD) and two identical inhibitory subunits, Pγ (11 kD) (*7*, *8*). The α subunit of transducin in the GTP-bound form activates PDE by removing inhibitory constraints imposed by the γ subunits (*6*). Bovine $\alpha_t$, bound to the nonhydrolyzable GTP analog guanosine-5′-O-(3-thiotriphosphate (GTP-γ-S), forms a complex with Pγ (*9*). PDE can also be activated by limited trypsin proteolysis, which cleaves Pγ (*8*). In addition, $\alpha_t$ with GDP bound ($\alpha_t$-GDP) inhibits $\alpha_t$–GTP-γ-S–stimulated PDE and trypsinized PDE (tPDE) (*10*). These results indicate that $\alpha_t$ contains sites of binding for Pγ, Pαβ, or both.

We synthesized various peptides that correspond to specific regions of $\alpha_t$ to find sites of interaction with PDE (Fig. 1) (*11*). Synthetic peptides can either block the interaction between two proteins or mimic the effect of one protein on another. Because $\alpha_t$ has a different conformation depending on whether GDP or GTP is bound, the $\alpha_t$ peptides could mimic either $\alpha_t$-GDP or $\alpha_t$–GTP-γ-S. Thus, we examined whether synthetic $\alpha_t$ peptides could mimic $\alpha_t$–GTP-γ-S and directly activate basal PDE, block stimulation of PDE activity by $\alpha_t$–GTP-γ-S, or mimic $\alpha_t$-GDP and inhibit active PDE.

We found that one peptide, corresponding to residues 293 to 314 (peptide 293–314), and a truncated analog of this peptide, corresponding to residues 300 to 314 (peptide 300–314), activated basal PDE, whereas all other peptides had no effect (*12*). The maximal activity of PDE stimulated by the first peptide was ~40% of the activity of tPDE (Fig. 2); the activation constant ($K_a$) was ~8.3 μM (Fig. 2). The second peptide stimulated PDE less ($K_a$ ~ 40 μM). The presence of membranes allows optimal activation of PDE by $\alpha_t$–GTP-γ-S (*6*). However, the effect of either peptide on PDE was not altered by the presence of urea-washed ROS membranes (which were stripped of peripheral proteins) (*13*).

In our experiments, the $K_a$ for $\alpha_t$–GTP-γ-S stimulation of PDE in solution was 1 to 2 μM. Phosphodiesterase was activated by $\alpha_t$–GTP-γ-S to a maximum of ~40% of the activity of tPDE (*13*). Because the effects of peptide 293–314 and $\alpha_t$–GTP-γ-S on PDE activation were similar and all other peptides tested, including those with similar charge characteristics, did not activate PDE, the effects of this peptide on PDE appear to be significant. We tested whether peptide 293–314 could activate tPDE that had been inhibited by exogenous Pγ. A

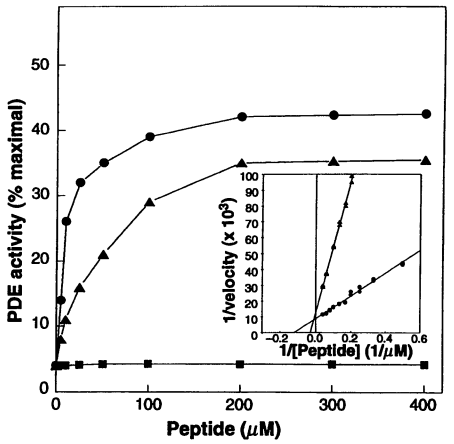


**Fig. 2.** Effects of $\alpha_t$ peptides on basal PDE activity. Peptides at the indicated concentrations were added to PDE in isotonic buffer (10 nM); cGMP (4 mM) was then added. PDE activity was measured by the proton release assay (*12*) and expressed as a percentage of maximal activity of PDE (10 nM) after limited trypsin proteolysis (tPDE activity) (100% = 19.4 μmol of cGMP hydrolyzed per second per milligram of PDE. ●, Peptide 293–314; ▲, 300–314; ■, all other peptides. The inset shows a double reciprocal plot of the data; velocity is equal to micromoles of cGMP hydrolyzed per second per milligram of PDE.

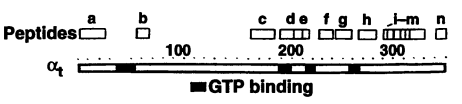


**Fig. 1.** Localization of the synthetic $\alpha_t$ peptides in relation to a linear map of $\alpha_t$. The following $\alpha_t$ peptides were synthesized: (a) residues 1 to 23 (MGAGASAEEKHSRELEKKLKEDA, (b) residues 53 to 65 (HQDGYSLEECLEF), (c) residues 162 to 181 (GYVPTEQDVLRSRVKTTGII), (d) residues 189 to 210 (DLNFRMFDVGGQRSERKKWIHC), (e) residues 201 to 215 (RSERKKWIHCFEGVT), (f) residues 224 to 239 (SAYDMVLVEDDEVNRM), (g) residues 242 to 259 (SLHLFNSICNHRYFATTS), (h) residues 265 to 280 (NKKDVFSEKIKKAHLS), (i) residues 288 to 310 (QPNTYEDAGNYIKVQFLELNMRR), (j) residues 293 to 314 (EDAGNYIKVQFLELNMRRDVKE), (k) residues 300 to 314 (KVQFLELNMRRDVKE), (l) residues 305 to 329 (ELNMRRDVKEIYSHMTCATDTQNVK), (m) residues 311 to 329 (DVKEIYSHMTCATDTQNVK), and (n) residues 340 to 350 (IKENLKDCGLF) (*11*). Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Department of Physiology and Biophysics, University of Illinois College of Medicine at Chicago, Box 6998, Chicago, IL 60680.

*To whom correspondence should be addressed.

purified fusion protein of Pγ (fPγ) (*14*) nearly completely inhibited the activity of tPDE (Fig. 3A). Addition of peptide 293–314 (20 μM) (Fig. 3A) or $\alpha_t$–GTP-γ-S (8 μM) (*13*) in the presence of fPγ restored approximately 40% of the activity of tPDE.

The degree of activation of PDE depends reciprocally on the concentrations of $\alpha_t$–GTP-γ-S and exogenous Pγ (*15*). The effects of fPγ on activation of PDE by peptide 293–314 (Fig. 3B) were similar to the effects of Pγ on stimulation of PDE by $\alpha_t$–GTP-γ-S (*15*). The fPγ protein shifted the dose-response curve to the right and changed its shape from hyperbolic to sigmoidal.

The $\alpha_t$–GTP-γ-S complex activates PDE by promoting dissociation of Pγ from its inhibitory site on the catalytic subunits (*15*). To determine whether peptide 293–314 activates PDE by a similar mechanism, we used gel filtration high-performance liquid chromatography (HPLC) to examine the effect of this peptide on the binding of $^{125}$I-labeled fPγ to tPDE (*16*); two other $\alpha_t$ peptides, corresponding to residues 53 to 65 (peptide 53–65) and residues 265 to 280 (peptide 265–280), that did not activate PDE were used as controls. $^{125}$I-fPγ added to tPDE comigrated with tPDE (Fig. 4). When $^{125}$I-fPγ was incubated with tPDE in the presence of peptide 293–314 (50 μM), the amount of $^{125}$I-fPγ bound to tPDE after gel filtration was decreased. Peptide 293–314 caused a dose-dependent decrease in the amount of $^{125}$I-fPγ bound to tPDE (Fig. 4). The concentration of the peptide (10 μM) near its $K_a$ (8.3 μM) reduced the amount of $^{125}$I-fPγ bound to tPDE by about 54%. Peptides 53–65 and 265–280 (each at 50 μM) had no effect. These results suggest that peptide 293–314 activates PDE by lowering the affinity of Pγ for the catalytic subunits.

Each $\alpha_t$ peptide was also tested for effects on $\alpha_t$–GTP-γ-S–stimulated PDE and tPDE activity (*17*). Peptide 53–65 maximally inhibited $\alpha_t$–GTP-γ-S–stimulated PDE activity by 60% with an $IC_{50}$ (concentration required to inhibit the activity of PDE by 50%) equal to 60 μM and inhibited tPDE by 75% with an $IC_{50}$ equal to 80 μM. Another peptide, corresponding to residues 201 to 215 (peptide 201–215), inhibited only tPDE activity by 60% with an $IC_{50}$ equal to 100 μM. All the other peptides had no effect. These data suggest that regions corresponding to residues 53 to 65 and 201 to 215 on $\alpha_t$ may interact with the catalytic subunits of PDE.

The $\alpha_t$ peptide 293–314 (as well a truncated analog of this peptide) directly activated PDE. Peptide 293–314 activated PDE with a $K_a$ value (8.3 μM) (Fig. 2) that was within one order of magnitude of that for $\alpha_t$–GTP-γ-S ($K_a$ = ~1 to 2 μM). In addition, peptide 293–314 decreased the binding of $^{125}$I-fPγ to tPDE. With $K_a$ in the micromolar range, this peptide weakened interaction between the PDE γ subunit or subunits and PDEαβ, an interaction with an affinity in the picomolar range (*15*). Because the peptide was added after the addition of $^{125}$I-fPγ, the decreased binding of $^{125}$I-fPγ to tPDE was probably a result of an increase in the rate of dissociation of $^{125}$I-fPγ from tPDE. This suggests that the peptide causes a conformational change in the PDE holoenzyme complex that decreases the affinity of fPγ for tPDE.

Peptide 293–314 appears to mimic $\alpha_t$ in the GTP-γ-S–bound form and activates PDE by reversing the inhibitory effect of Pγ. This implies that this region on $\alpha_t$ is a site for PDE binding and is involved in PDE activation. The smallest known region on $\alpha_s$ capable of activating adenylyl cyclase lies between residues 235 to 356 (*4, 5*), which corresponds to residues 208 to 314 of $\alpha_t$ (if $\alpha_s$ and $\alpha_t$ amino acid sequences are aligned by their conserved GTP binding domains). Peptide 293–314 lies within this region. Thus, this region may be an essential part of the effector activation domain for many G proteins.

Other regions of α subunits may participate in G protein binding to effectors. In $G_s$, residues $Trp^{263}$, $Leu^{268}$, and $Arg^{269}$ are critical in the determination of the affinity of $\alpha_s$ for adenylyl cyclase (*18*). Our data suggest that other regions on $\alpha_t$ may also interact with the effector PDE. The correspondence between the studies on the $\alpha_i$-$\alpha_s$ chimeras and our finding that peptide 293–314 activates PDE suggest that similar sites on G protein α subunits are involved in effector activation even though there does not seem to be strong conservation of amino acid sequences among G protein–regulated effector enzymes and channels.

*Note added in proof*: One of the $\alpha_s$ regions required for activation of adenylyl cyclase (residues 349 to 356) corresponds to residues 307 to 314 on $\alpha_t$ (*19*).

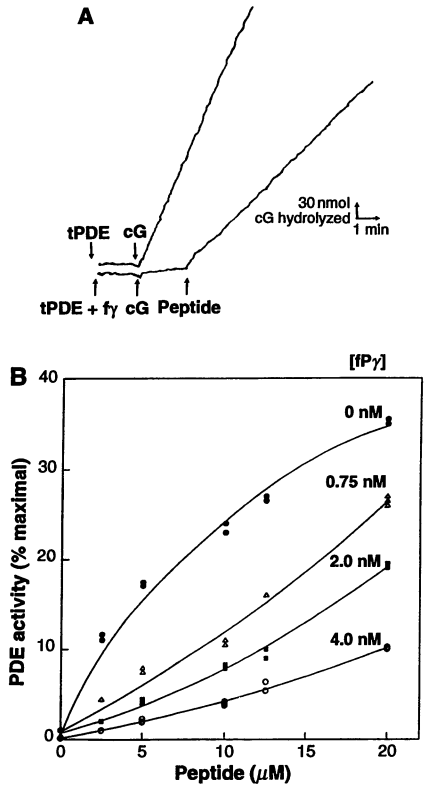


**Fig. 3.** Reciprocal relations between peptide 293–314 and fPγ. (**A**) Activation of fPγ-inhibited tPDE by peptide 293–314. Upper trace: cGMP (4 mM) (cG) was added to tPDE (2.5 nM) in isotonic buffer. Lower trace, tPDE (2.5 nM) and fPγ (5 nM) (fγ) were mixed in the reaction vial and then cGMP (4 mM) was added. After 2 min, peptide 293–314 (20 μM) was added. PDE activity was measured by the proton release assay. (**B**) Reciprocal effects of peptide 293–314 and fPγ on PDE activity. PDE activity was measured with the inorganic phosphate release method (*12*). The fPγ protein was added at the indicated concentrations to PDE (0.7 nM), and then various concentrations of peptide 293–314 were added. After 3 min of incubation, cGMP (1 mM) was added. PDE activity is expressed as a percentage of tPDE activity.

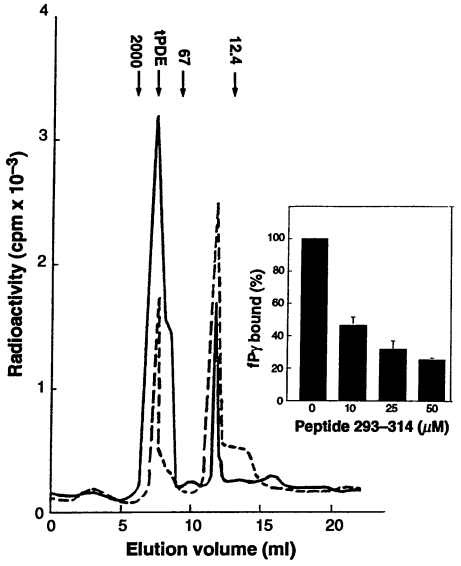


**Fig. 4.** Peptide 293–314 decreased binding of $^{125}$I-fPγ to tPDE. The $^{125}$I-fPγ (40 pmol) was preincubated with tPDE (40 pmol) alone or in the presence of 10 μM, 25 μM, or 50 μM peptide 293–314 and eluted with Buffer B at a flow rate of 0.7 ml/min. Typical radioactivity profiles after HPLC gel filtration of tPDE preincubated with $^{125}$I-fPγ alone (solid line) or in the presence of peptide 293–314 (50 μM) (dashed line). Inset: relative amounts of $^{125}$I-fPγ bound to tPDE in the presence of increasing concentrations of peptide 293–314 (100% equals the amount of $^{125}$I-fPγ bound to tPDE in the absence of peptide). Molecular weight standards: blue dextran (2000 kD), bovine serum albumin (67 kD), and cytochrome c (12.4 kD).

## REFERENCES AND NOTES


1. L. Stryer and H. R. Bourne, *Annu. Rev. Cell. Biol.* **2**, 391 (1986); A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987); A. M. Spiegel, *Vitam.*

*Horm.* (*N.Y.*) **44**, 47 (1988).
2. T. Higashijima *et al.*, *J. Biol. Chem.* **262**, 752 (1987); B. K.-K. Fung and C. R. Nash, *ibid.* **258**, 10503 (1983); R. T. Miller, S. B. Masters, K. A. Sullivan, B. Beiderman, H. R. Bourne, *Nature* **334**, 712 (1988).
3. D. C. May, E. M. Ross, A. C. Gilman, M. D. Smigel, *J. Biol. Chem.* **260**, 15829 (1985); B. K.-K. Fung, J. B. Hurley, L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 152 (1981); S. J. Taylor, H. Z. Chae, S. G. Rhee, J. H. Exton, *Nature* **350**, 516 (1991).
4. S. B. Masters *et al.*, *Science* **241**, 448 (1988).
5. C. W. Woon, S. Soparkar, L. Heasley, G. L. Johnson, *J. Biol. Chem.* **264**, 5687 (1989); S. Osawa, N. Dhanaseharan, C. W. Woon, G. L. Johnson, *Cell* **63**, 697 (1990).
6. L. Stryer, *Annu. Rev. Neurosci.* **9**, 87 (1986); M. Chabre and P. Deterre, *Eur. J. Biochem.* **179**, 225 (1989).
7. J. B. Hurley and L. Stryer, *J. Biol. Chem.* **257**, 11094 (1982).
8. P. Deterre, J. Bigay, F. Forquet, M. Robert, M. Chabre, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2424 (1988).
9. P. Deterre *et al.*, *Proteins* **1**, 188 (1986).
10. S. Kroll, W. J. Phillips, R. A. Cerione, *J. Biol. Chem.* **264**, 4490 (1989).
11. Some of the synthetic $\alpha_t$ peptides were chosen for the following reasons. (i) Several peptides were made within the COOH-terminal region of $\alpha_t$ that corresponded to a putative effector domain on the $\alpha_i$-$\alpha_s$ chimeras (*4, 5*). (ii) Peptide 162–181 corresponds to a proposed effector contact region in Ras p21 [I. Sigal, J. B. Gibbs, J. S. D'Alonzo, E. M. Scolnick, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4725 (1986)]. (iii) Peptide 53–65 is from a region on $\alpha_t$ that has least sequence identity to other $\alpha$ chains, contains an identical sequence with P$\alpha$ ($\alpha_t$: residues 59 to 63 and P$\alpha$: residues 120 to 124) [D. J. Takemoto and J. S. Cunnick, *Cell. Signal.* **2**, 99 (1990)], and corresponds to a putative effector binding region in bacterial elongation factor EF-Tu [R. A. Laursen, J. J. L'Italien, S. Nagarkatti, D. L. Miller, *J. Biol. Chem.* **256**, 8102 (1981)]. (iv) Two peptides (189–210, 201–215) are contained in a region on $\alpha_t$ analogous to a region on $\alpha_s$ important in the GTP-induced conformational change. (v) Peptides were made corresponding to regions on $\alpha_t$ predicted to be on the surface of the protein. The peptides were synthesized with an acetyl group at the $NH_2$-terminus and an amide group at the COOH-terminus by the solid-state Merrifield method on an automated synthesizer (Applied Biosystems). Crude peptides were purified by reversed-phase HPLC on a preparative Aquapore Octyl (C-8) column (25 × 1) (Applied Biosystems) with a 0 to 60% gradient of acetonitrile in 0.1% trifluoroacetic acid. Only those peptides with a single peak that corresponded to the predicted molecular weight on a fast atom bombardment mass spectrogram and a single peak shown by analytical HPLC were used. All peptides were filtered through a 0.22-μm Nylon filter (Micron Separations). Peptides 242–259 and 288–310 were insoluble in the PDE assay conditions (*12*) and were not tested further.
12. Bovine ROS preparation, PDE extraction from ROS membranes with hypotonic buffer and purification, and trypsinization of PDE were carried out as described [N. O. Artemyev and H. E. Hamm, *Biochem. J.* **283**, 273 (1992)]. Because there was no difference between the functional effects observed with PDE extract from ROS membranes (80% pure) and purified PDE (95% pure), PDE extract was used. PDE activity was measured by two different methods. (i) The rate of proton release was measured with a pH microelectrode (Microelectrodes) as described [P. A. Liebman and A. T. Evanczuk, *Methods Enzymol.* **81**, 532 (1982)]. In each assay, various components were added to a small vial that contained Buffer A [10 mM Hepes (pH 8.0), 100 mM KCl, 2 mM $MgCl_2$, and 1 mM dithiothreitol (DTT)] in a final volume of 200 μl. The hydrolysis of cGMP was measured and then quantified by titrating the suspension with known amounts of NaOH. (ii) Release of inorganic phosphate was measured with purified 5′ nucleotidase (Sigma) in microtiter plates (Dynatech) as described [P. G. Gillespie and J. A. Beavo, *Mol. Pharmacol.* **36**, 773 (1990)].
13. H. M. Rarick and N. O. Artemyev, unpublished results.
14. A fusion protein that contained the γ subunit of PDE (fPγ) was expressed in *Escherichia coli* and purified as described [R. L. Brown and L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4922 (1989)]. The fusion protein, consisting of 38 residues of the bacteriophage λ CII repressor, a seven-residue joining sequence, and the 87-residue Pγ subunit was as effective as native Pγ in inhibiting tPDE and $\alpha_t$–GTP-γ-S–activated PDE. Pγ has one tyrosine at residue 84. The fusion protein (1 mg) was iodinated with Iodogen (Pierce) and desalted twice on a P-6 desalting gel (Bio-Rad) to remove free $^{125}I$. The specific activity was approximately 3.9 Ci/mmol. Purity of $^{125}I$-fPγ was determined by polyacrylamide gel electrophoresis and autoradiography.
15. T. G. Wensel and L. Stryer, *Struct. Funct. Genet.* **1**, 90 (1986); *Biochemistry* **29**, 2155 (1990).
16. $^{125}I$-fPγ (40 pmol) was preincubated with an equimolar amount of tPDE for 5 min at room temperature alone or in the presence of the indicated concentration of peptide in 100 μl of Buffer B [20 mM Hepes (pH 8.0), 1 mM $MgCl_2$, 100 mM NaCl, and 1 mM DTT] and injected into an Ultraspherogel 3000SW (30 × 0.75) HPLC gel filtration column (Beckman) equilibrated with the same buffer. Proteins were eluted from the column with a flow rate of 0.7 ml/min, and radioactivity was measured with an M170 flow radioisotope detector (Beckman). The amount of $^{125}I$-fPγ bound to tPDE was estimated by integration of the radioactive peak that corresponded to tPDE.
17. The $\alpha_t$–GTP-γ-S was purified as described [M. R. Mazzoni, J. A. Malinski, H. E. Hamm, *J. Biol. Chem.* **266**, 14072 (1991)]. Increasing concentrations of each peptide (1 μM to 5 mM) were added with $\alpha_t$–GTP-γ-S (1 μM) either to a vial that contained PDE (10 nM) or to a vial that contained tPDE (10 nM). The reactions were initiated by addition of cGMP (4 mM), and PDE activity was measured by the proton release assay.
18. H. Itoh and A. G. Gilman, *J. Biol. Chem.* **266**, 16226 (1991).
19. C. Berlot and H. R. Bourne, *Cell* **68**, 911 (1992).
20. Supported by a National Eye Institute grant (EY 06062) to H.E.H. H.M.R. was supported by NIH training grant T32 HL 07692-02. We thank L. Stryer and R. L. Brown for the fPγ expression construct pLcIIFXSG, N. Skiba for expressing fPγ in *E. coli*, the Protein Sequencing/Synthesis Laboratory at the University of Illinois for peptide synthesis, and T. Puryear, L. Hinz, and T. Garcia for technical assistance.

11 February 1992; accepted 3 April 1992