Modulation of GDP Release from Transducin by the Conserved Glu$^{134}$–Arg$^{135}$ Sequence in Rhodopsin*

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A superfamily of seven-transmembrane helix receptors catalyzes GDP release from heterotrimeric guanine nucleotide-binding proteins (G proteins) to initiate the intracellular signaling cascade. The photoreceptor rhodopsin is a prototypical member of the superfamily that activates the retinal G protein transducin (Gt). The cytoplasmic domain of rhodopsin binds and activates Gt, but residues that stimulate GDP release from Gt have not been identified until now. We show here that the abnormal signal transduction phenotypes of several different mutations affecting the highly conserved Glu$^{134}$–Arg$^{135}$ charge pair result from alteration of the GDP release step in the Gt activation cascade. We propose that Glu$^{134}$ and Arg$^{135}$ constitute the site that directly provides the signal from rhodopsin to activate GDP release from Gt. Because the Glu/Asp–Arg sequence occurs at a topologically identical location in most of the seven-transmembrane helix receptors, we propose that these residues constitute a switch for signal transfer.

Heterotrimeric (αβγ) G proteins function as signal transducers coupling seven transmembrane helical receptors for neurotransmitters and sensory stimuli to many intracellular effector enzymes or ion channels. GDP release is the rate-limiting step in switching a G protein conformation from the inactive GDP-bound state to the active GTP-bound state. Activated receptors catalyze guanine-nucleotide exchange, predominantly by lowering the barrier for GDP release (1–5). Receptor-coupled GDP-GTP exchange and subunit dissociation represent a paradigm for signal transduction by all G protein-coupled receptors (GPCRs) (1–5).

Visual transduction in rod cells is a prototypical example of a G protein-coupled signaling system. In rod cells, the cytoplasmic surface of nonactivated rhodopsin is peripherally associated with transducin, in the GDP-bound state (3). Signal transduction is initiated by the photon-induced isomerization of the photoreceptor chromophore 11-cis-retinal to all-trans-retinal (Fig. 1). Structural changes in rhodopsin lead to an active intermediate, metarhodopsin II (M II or R$^*$). The R$^*$ stabilizes interaction with transducin. R$^*$ also catalyzes the process by which transducin is switched from the GDP-bound state to a GTP-bound state and G$^\gamma$-GTP and G$^\beta\gamma$ subunits dissociate from R$^*$. Evidence from studies using peptide competition (6) and mutational (7–12), biochemical (13), and antibody competition (14) have implicated residues in three cytoplasmic loops as critical for Gt interaction. The mechanism of individual steps, binding transducin in the GDP-bound state, GDP release, GTP uptake, and dissociation of the α and βγ-subunits, catalyzed by the activated rhodopsin is still unclear. This is because interactions of mutant rhodopsin with transducin have mainly been inferred from loss of the mutants’ ability to catalyze the GDP/GTP exchange in transducin. We have employed assays that measure transducin-independent metarhodopsin II stabilization and [$\gamma$-32P]GDP release from transducin to characterize abnormal transducin-coupling to rhodopsin mutants. Our data suggest that Glu$^{134}$–Arg$^{135}$ in rhodopsin constitutes a “switch” for Gt activation (Fig. 1).

**EXPERIMENTAL PROCEDURES**

Materials—N-Dodecyl-β-o-maltoside (DM) (Anatrace, Cleveland, OH) and digitonin (Calbiochem) were purchased. Radionuclides were from DuPont NEN, nitrocellulose BA8885 filters were from Schleicher & Schuell, nucleotides were from Boehringer Mannheim, frozen bovine retina were from Lawson Co. (Omaha, NE), and 11-cis-retinal was supplied by Dr. R. Crouch.

Preparation of Rhodopsin Mutant Pigments—Procedures for the construction of mutants and expression of opsins have been described earlier (15–17). The concentration of expressed opsin in COS cell membrane was estimated by PhosphorImager analysis of immunoblots calibrated against purified bovine rhodopsin standards. Opsin was expressed in COS cells by transient transfection of the corresponding gene. Harvested cells were incubated with 40 μM 11-cis-retinal in the dark for 60 min to regenerate the rhodopsin state. The pigments were solubilized in DM and purified by an immunoadfinity procedure (15–17). Purified rhodopsins in 10 mM HEPES buffer, pH 7.2, containing 100 mM NaCl and 0.1% DM were used for recording a dark spectrum at 5°C. The pigment concentration was calculated from its absorbance at 500 nm based on ε$^{500}$ = 42.700 M$^{-1}$ cm$^{-1}$.

The Stability of the R$^*$ State—The stability of light-activated rhodopsin was estimated by following the hydrolysis of retinal-protein linkage by the method of Morton and Pitt (18). Pigments purified as above in 0.1% DM were used for recording a dark spectrum. The sample was then exposed to light for 20 s using a 150-W Fiber-Lite fitted with a 490 nm cut-off filter, and the sample was allowed to decay in the dark at 23°C. At the specified time, an aliquot of the sample was placed in 10 mM HCl and kept on ice. Spectra were recorded 20 min later. The time taken for conversion of the 440 nm peak (represents acid-denatured Schiff base linkage between all-trans-retinal and apoprotein opsin) to a 380 nm peak (represents free all-trans-retinal released from Schiff base hydrolysis) is a measure of the deactivation of M II (18).

The Nucleotide Exchange Assay—The ability to catalytically activate transducin was assayed by [$\gamma$-32P]GTP-γS (1130 Ci/mmol) binding assay as described earlier (9).

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1 The abbreviations used are: G protein, guanine nucleotide-binding protein; DM, n-dodecyl-β-o-maltoside; GTPγS, guanosine 5′-O(3-thiotriphosphate); M I, metarhodopsin I (λ$^{\text{max}}$ = 480 nm); M II, metarhodopsin II (λ$^{\text{max}}$ ≈ 380 nm); R$, light-activated rhodopsin (M II); Gt, transducin; G$^{\alpha},$ the α-subunit of transducin; G$^{\beta\gamma},$ the βγ subunit of transducin; GPCR, G protein-coupled receptor.

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RESULTS AND DISCUSSION

Phenotypic Properties of Glu134-Arg135 Mutants

Generation of Recombinant Pigments—To understand the functional significance of the highly conserved Asp/Glu-Arg sequence in GPCRs located in the cytoplasmic portion of the third transmembrane segment (Fig. 2), we evaluated its role in rhodopsin-transducin interaction. We mutated the Glu134, Arg135 sequence of rhodopsin to Gln134-Gln135. In addition, five single mutants were generated at position 134 and two at 135 (Fig. 2). None of these mutations, which would be expected to change the environment around the substituting amino acids, affected the level of expression or the glycosylation pattern described for opsin expressed in COS cells. A 500-nm absorbing chromophore was yielded by all mutant opsins upon incubation with 11-cis-retinal, detergent solubilization, and immunooaffinity purification (Table I). Illumination of each pigment yielded a spectral species with a λmax value of 380 nm, a characteristic feature attributed to the formation of the catalytically active M II state. The Schiff base-cleavage (see “Experimental Procedures”) in the light-activated pigments (M II) for each mutant followed a time course identical to that determined for native M II pigment (t½ = 15 min; data not shown). Thus, regeneration of opsin to the rhodopsin state (λmax 500 nm), light-dependent conversion to the M II state (λmax 380 nm), and the stabilities of these intermediate states of all the mutants were indistinguishable from those of the wild type. This indicates that the native rhodopsin-like structure is achieved by each of the mutants and the wild type opsin expressed in COS cells.

Stimulation of GTP·S-Binding Activity of Transducin—The transducin-activating ability of different mutants assayed in the purified COS cell membranes varied significantly (Table I). The double mutant E134Q-R135Q and the single mutants R135G and R135Q did not activate the GTP·S-binding activity of Gt upon light activation. The transducin-activating phenotype of the R135Q mutant assayed in COS membrane that we report here is significantly different from that reported by Cohen et al. (9). Therefore, we compared transducin activation by the double mutant E134Q-R135Q and the single mutants R135G and R135Q under different conditions reported by various investigators earlier (Table II). The results suggest that the phenotypes of the E134Q, R135Q and the R135G and R135Q mutants are identical under various transducin assay conditions. Therefore, preservation of Arg135 is critical for stimulation of transducin independent of the environments provided. Thus, in patients carrying the R135G mutation, the mutant rhodopsin in vivo is expected to be defective in activating the light-transduction cascade and thus may be a potential mechanism for the pathogenesis of autosomal dominant retinitis pigmentosa. Previously, the R135Q, R135L, and R135W mutants expressed in COS cells and affinity purified were found to form spectroscopically normal pigments that did not activate guanine nucleotide exchange by transducin (7, 8, 22–24).

In contrast, the light-activated activity of E134S, E134I, and E134Q mutant rhodopsins was 40–44% enhanced in comparison with wild type (see Table I). The opsin forms of the E134S, E134I, and E134Q mutants are able to activate transducin in the absence of 11-cis-retinal, whereas the wild type and R135G/Q mutant opsins are inactive (Table I). Reconstitution with 11-cis-retinal suppressed the constitutive activity of E134S, E134I, and E134Q mutants in the dark (Table I). The E134L and E134F mutant opsins, however, did not activate transducin basally. It is worth noting that these two side chains, although hydrophobic like the Ile side chain, do not transducin cycle. A similar assay was reported earlier (12). The [α-32P]GDP-labeled (3000 Ci/mmol) Gt used for this assay was prepared by a modification of the method described earlier (19). Excess GTP was removed by Amicon filtration. Conversion of [α-32P]GDP to [α-32P]GTP by the intrinsic GTPase activity of Gt was estimated by extraction of the nucleotide with 0.1 M HCl followed by TLC. An assay mixture containing 50 nm purified rhodopsin forms (after 1 min of illumination with 490 nm filtered light) or opsin forms in the COS membranes was mixed with [α-32P]GDP-labeled transducin (200 nM) in a total volume of 100 μl at 23 °C. The assay conditions were the same as those described for the nucleotide exchange assay. Two different controls, [α-32P]GDP-labeled transducin alone and [α-32P]GDP-labeled transducin mixed with untransfected COS membrane, were used to estimate use of [α-32P]GDP from transducin during the assay in the absence of stimulation by opsin or rhodopsin. The wild type and mutants were assayed in parallel with same batch of [α-32P]GDP-labeled transducin for 20 min. 3 × 10 μl aliquots were removed and vacuum filtered through BAS5 nitrocellulose filter discs. The filters were washed with cold buffer rapidly and air dried, and the bound radioactivity was quantitated. The average value of three measurements for [control] − [light-activated wild type rhodopsin] was taken as 100%. The standard error was ± 5%. The mean [alpha-32P]GDP release values for mutants in experiments carried out in parallel were normalized to the wild type value.

The αt(340–350) Mutants of E134S and E134Q E134Q and E134S mutant rhodopsins were synthesized based on a method previously detailed (20). Wild type and mutant rhodopsins were prepared in 1% digitonin as described earlier (16). Varying concentrations of the αt(340–350) peptide (Ac-IKENLqDCGLF) was taken as 100%. The standard error was of three measurements for [control]. The purified rhodopsin forms (after 1 min of illumination with 490 nm cut-off filter, and the sample was allowed to equilibrate in the dark at 5°C for 20 min. The amount of M II formed was calculated from the differences in peak areas as a function of peptide concentration. An αt(340–350) analogue (Ac-IKENLqDCGLF) peptide was used as a negative control due to its inability to compete with transducin in the [35S]GTP·S binding assay. This peptide was found to be inactive in earlier studies as well (23).
contain branching at the β-carbon atom. The rhodopsin forms of these mutants were similar to the wild type. The negative charge of the residue at position 134 of opsin was previously suggested to be crucial for producing opsin’s basally inactive state (8–12). The observations that E134S, E134I, and E134Q mutations cause constitutive activation and E134L and E134F mutations do not cause constitutive activation of opsin suggest that the mechanism of basal activation of opsin is more complex than discharging the negative Glu134 residue. We propose that a combination of charge and side chain-packing interactions of Glu134 in the wild type opsin controls the inactive state of opsin. The most significant outcome, however, is the finding that the gain-of-function caused by the E134Q mutation is completely suppressed when this mutation is combined with the loss-of-function mutation, R135Q. The fact that both opsin
and rhodopsin forms of the E134Q, R135Q mutant are defective indicates that Arg135 plays a crucial role both in opsin and rhodopsin.

Localization of Defect in Coupling of Mutant Rhodopsins to Transducin Activation

The formation of rhodopsin and M II states in the Glu134 and Arg135 residue-mutants indicates that these residues must be crucial for coupling to the G protein rather than overall structure of rhodopsin. Studies on (Asp/Glu)-Arg sequence mutants so far have not localized the defect to one of the several steps in the Gi activation scheme presented in Fig. 1. For example, the inability of the R135G mutation could result from a defect at any one of the steps involved in linking the catalytically active R* state to uptake of GTPγS by the R*Gt-empty complex (Fig. 1). These individual steps were investigated as follows.

Stabilization of R* State of the R135G Mutant—A peptide derived from transducin was used to examine whether the R135G mutant harbors a defect in generating a site for transducin binding or in stabilization of the active state. The stability of the M II state in both wild-type and R135G rhodopsins (t1/2 = 15 min) was identical, as indicated by decay of the Schiff base monitored by the acid-trapping experiment shown in Fig. 3A. Because the mutation did not interfere with light activation properties, we investigated Gi binding properties of its M II intermediate. Conventionally, Gi binding is spectroscopically monitored by stabilization of the M II intermediate. As indicated in Fig. 1, this involves binding of Gi-GDP to R* followed by release of GDP to produce a R*Gt-empty complex. Hence, using Gi-GDP will not distinguish between the two steps that are known to be involved in the stabilization of the M II intermediate. Hamm et al. (20) and Dratz et al. (21) demonstrated that stabilization of M II can be measured by direct interaction of the α1(340–350) peptide, which corresponds to the carboxy-terminal sequence of Gt. Because M II stabilization by α1(340–350) could potentially distinguish the defect in the Gi binding step from the defect in the GDP release step, we used it as a surrogate assay for testing the mutants defective in Gi activation (Fig. 3B). In this assay, illumination for 20 s, the peptide-free sample produced a small amount of M II (380 nm) and predominantly a M I (480 nm) intermediate. In the presence of 0.1–1 mM peptide, a transition from the 480 to the 380 nm form was observed as a function of peptide concentration (Fig. 3, B and C). Equilibrium distribution of M I and M II was obtained at around 500 μM (S.E. ± 18%, n = 3) peptide concentration for the wild type as well as the defective R135G mutant. An analogue of the α1(340–350) peptide with a critical lysine (corresponding to Lys345 of Gt-GTP) modified to glutamine did not promote the transition from M I to M II at 1 mM concentration (Fig. 3C). This analogue peptide was earlier found to be inactive in a similar assay carried out in disc membrane (20, 21).

Does the α1(340–350) peptide binding ability of the mutant truly represent its ability to bind G? A larger binding surface is involved in the interaction between G and M II than between the α1(340–350) peptide and M II. Evidence suggests that the Gi binding site is in an inactive conformation in the R state (6–10). Upon light activation, a conformational change is responsible for relaxation of the binding constraint. Because binding of the peptide is not affected, the R135G mutation does not abolish the conformational change, suggesting that in the wild type Arg135 is involved neither in the conformational tran-
sition nor in binding the $\alpha_{i}(340–350)$ peptide directly. Therefore, it should be possible to characterize a rhodopsin mutant in which the peptide fails to bind, even though interaction with $G_{i}$ is relatively normal.

**Defect of the R135G Mutant in GDP Release from G** —Measurement of GDP release from $G_{i}$ in the absence of GTP was employed to examine the ability of mutants to catalyze the rate-limiting step in the transducin cycle. Photoexcited rhodopsin catalyzes stoichiometric GDP release from [α-32P]GDP labeled $G_{i}$ (Fig. 4A). The GDP release induced by the light-activated R135G mutant rhodopsin was comparable with that by wild type opsin ($\pm 15\%$; Fig. 4B). This value varied between 10 and 15% with different batches of transducin preparations. The same result was obtained in the presence of a 10-fold molar excess of R135G mutant rhodopsin.

**Constitutive Stimulation of GDP release by the Glu134 Mutants** —The opsins forms of mutant E134Q, E134S, and E134I were active in the GTPγS binding assay (Table I). Binding affinity of $G_{i}$ and uptake of the [β55S]GTPγS by Rα-Gi-empty state are not affected in the E134Q mutant as shown earlier (9–12). This is also true for the E134I and E134S mutants.2 E134Q and E134I opsins stimulated nearly 50% GDP release from $G_{i}$ without reconstitution with 11-cis-retinal (Fig. 4B). The light-activated rhodopsin forms of E134Q/I mutants were 20–30% more active in stimulating GDP release.

**The R135Q Mutation Is Dominant over the E134Q Mutation** —The properties of the light-activated E134Q-R135Q mutant rhodopsin are similar to those of the R135G single mutant rhodopsin in the GTPγS binding, GDP release and the $\alpha_{i}(340–350)$ peptide-dependent M II-stabilization assays (see Figs. 3 and 4 and Tables I and II). Unlike the E134Q mutant, the opsin form of the E134Q-R135Q mutant was not constitutively active in transducin activation as measured by [32P]GDP release and [β55S]GTPγS binding assays. We examined whether the $\alpha_{i}(340–350)$ peptide leads to the formation of an M II-like state from the rhodopsin forms of these two mutants without light activation. The rhodopsin forms of E134Q and E134Q,R135Q mutants incubated with 500 mM $\alpha_{i}(340–350)$ peptide for 1–15 h in the dark did not cause a transition to a 380 nm absorbing spectral form (data not shown). This observation is consistent with the inhibition of the constitutive activity of E134Q opsin when 11-cis-retinal binds. A mutant rhodopsin with charge reversal (Arg135,Glu134 instead of Glu134,Arg135) was previously reported to be defective in $G_{i}$-mediated stabilization of the mutant M II and therefore may be unable to promote the release of GDP and initiate the transducin cycle (7). It is not known whether this charge reversal preserved the ability to bind the $\alpha_{i}(340–350)$ peptide. In this regard also, our data demonstrate that the role of Arg135 is important for rhodopsin coupling to transducin.

These results indicate that Glu134 is responsible for constraining the $G_{i}$-activating ability of the apoprotein opsin and rhodopsin in the dark state. Specific inability of the R135G mutant to catalyze release of GDP demonstrates that the role of the Arg135 side chain in wild type rhodopsin is to stimulate the release of GDP from the R*,$G_{i}$,GDP complex. Because the phenotype of R135Q overrides the E134Q mutant phenotype in the double mutant (E134Q,R135Q), it can be argued that the role of Arg135 is pivotal for the gain-of-function phenotype of E134Q, E134I, and E134Q mutants. We speculate that Arg135 likely makes contact with Gα,GDP to trigger GDP release and to stabilize the R*,$G_{i}$,empty state. Until GDP is released, the critical residues of $G_{i}$ required for stabilization of the M II state might be inaccessible.

Recent structural evidence indicates that the Glu134,Arg135 charged pair is located within the helical region bordering the third transmembrane domain and that activation may proceed through a loss of tertiary constraints involving critical residues in this region (25). Uptake of a proton by Glu134 during rhodopsin activation has been shown previously (11). Our study suggests that the properties of the side chain in addition to negative charge play a very important role in release of the constraint postulated above. The finding that preservation of the Arg135 side chain is crucial in the E134Q,R135Q mutant suggests that the release of Glu134 from the constrained state enables the Arg135 side chain to engage the GDP release switch of transducin. Although transducin is capable of weak association with rhodopsin in the dark (3), a stable interaction between them must require GDP release (Fig. 1). Arg135 is the only residue in the cytoplasmic domain of rhodopsin that completely abolishes $G_{i}$ activation with subtle alteration of side chain structure (8). Interaction of $G_{i}$ with Arg135 therefore appears to be the primary trigger for concerted GDP release and stabilization of the M II and $G_{i}$-empty complex. This implies that Glu134,Arg135 in rhodopsin function together to constitute an important regulatory switch for transfer of an activating signal from the receptor to the $G_{i}$ protein to activate the rate-limiting GDP release step that is fundamental to the in vivo role of light-activated rhodopsin.

An (Asp/Glu)-Arg-Tyr sequence is present near the putative cytoplasmic border of third transmembrane segment in the majority of GPCRs. Conservative substitutions are tolerated at the Asp/Glu and Tyr positions, but Arg is invariant (5, 26). Mutagenesis at the Asp-Arg sequence produces receptors with moderate to severe defects in eliciting effector response. The mechanistic basis for these defects has not been clearly elucidated, but those phenotypes are consistent with the paradigm described in this report for the rhodopsin-transducin interac-

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2 S. Acharya and S. Karnik, unpublished observations.
The receptor-G protein binding and dissociation scheme outlined for the Glu\textsuperscript{134}-Arg\textsuperscript{135} mutants of rhodopsin in Fig. 1 may be applicable to most of these mutant receptors as well. We conclude, therefore, that the (Asp/Glu)-Arg charged pair is most likely a canonical sequence for activating GDP release in the family of GPCRs.

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REFERENCES

1. Gilman, A. G. (1984) Cell 36, 577–579
2. Bourne, H. R., Sanders, D. A., and McCormick, F. (1990) Nature 348, 125–132
3. Stryer, L. (1986) Annu. Rev. Neurosci. 9, 87–119
4. Conklin, B. R., and Bourne, H. R. (1993) Cell 73, 631–641
5. Dohlman, H. G., Thorner, J., Caron, M. G., and Lefkowitz, R. J. (1991) Annu. Rev. Biochem. 60, 653–688
6. König, B., Arendt, A., McDowell, J. H., Kahlert, M., Hargrave, P. A., and Hofmann, K. P. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6878–6882
7. Franke, R. R., Konig, B., Sakmar, T. P., Khorana, H. G., and Hofmann, K. P. (1990) Science 250, 123–125
8. Franke, R. R., Sakmar, T. P., Graham, R. M., and Khorana, H. G. (1992) J. Biol. Chem. 267, 14767–14774
9. Cohen, G. B., Yang, T., Robinson, P. R., and Oprian, D. D. (1993) Biochemistry 32, 6111–6115
10. Fahmy, K., and Sakmar, T. P. (1993) Biochemistry 32, 7229–7236
11. Arnin, S., Fahmy, K., Hofmann, K. P., and Sakmar, T. P. (1994) J. Biol. Chem. 269, 23879–23881
12. Ernst, O. P., Hofmann, K. P., and Sakmar, T. P. (1995) J. Biol. Chem. 270, 10580–10586
13. Kuhn, H., and Hargrave, P. A. (1981) Biochemistry 20, 2410–2417
14. Weiss, E. R., Hadeck, J. R., Johnson G. L., and Malbon C. C. (1987) J. Biol. Chem. 262, 4319–4323
15. Kar Shiv, S., Sakmar, T. P., Chen, H. B., and Khorana, H. G. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8459–8463
16. Oprian, D. D., Molday, R. S., Kaufman, R. J., and Khorana, H. G. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8874–8878
17. Ferretti, L., Kar Shiv, S., Khorana, H. G., Nassil, M., and Oprian, D. D. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 599–603
18. Morton R. A., and Pitt, G. A. J. (1955) Biochem. J. 59, 128–134
19. Fung, B. K., Hurley, J. B., and Stryer, L. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 152–156
20. Hamm, H. E., Deretic, D., Arendt, A., Hargrave, P. A., Koenig, B., and Hofmann, K. P. (1986) Science 241, 832–835
21. Dratz, E. A., Furstenau, J. E., Lambert, C. G., Thireault, D. L., Rarick, H., Schepers, T., Pakhlevaniants, S., and Hamm, H. E. (1993) Nature 363, 276–281
22. Kau Shal, S., and Khorana, H. G. (1994) Biochemistry 33, 6121–6128
23. Sung, C.-H., Davenport, C. M., and Nathans, J. (1993) J. Biol. Chem. 268, 26645–26649
24. Min, R. C., Zvyaga, T. A., Cypress, A. M., and Sakmar, T. P. (1993) J. Biol. Chem. 268, 9400–9404
25. Farahbakhsh, Z. T., Ridge K. D., Khorana, H. G., and Hubbell, W. L. (1995) Biochemistry 34, 8812–8819
26. Baldwin, J. M. (1995) EMBO J. 12, 1693–1703
27. O'Dowd, B. F., Hnatowich, M., Regan, J. W., Leader, W. M., Caron, M. G., and Lefkowitz R. J. (1988) J. Biol. Chem. 263, 15985–15992
28. Fraser, C. M., Wang, C.-D., Robinson, D. A., Gocayne, J. D., and Venter J. C. (1989) Mol. Pharmacol. 36, 840–847
29. Wang, C.-D., Buck, M. A., and Fraser, C. M. (1991) Mol. Pharmacol. 40, 168–179
30. Zhu, S. Z., Wang, S. Z., Hu, J., and el-Fakahany, E. E. (1994) Mol. Pharmacol. 45, 517–523