In Vivo Coupling of Growth Factor II/Mannose 6-Phosphate Receptor to Heteromeric G Proteins

DISTINCT ROLES OF CYTOPLASMIC DOMAINS AND SIGNAL SEQUESTRATION BY THE RECEPTOR*

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We examined the signaling function of the IGF-II/mannose 6-phosphate receptor (IGF-IIR) by transfecting IGF-IIR cDNAs into COS cells, where adenyl cyclase (AC) was inhibited by transfection of constitutively activated Ga1 cDNA (Ga1Q205L). In cells transfected with IGF-IIR cDNA, IGF-II decreased cAMP accumulation promoted by cholera toxin or forskolin. This effect of IGF-II was not observed in untransfected cells or in cells transfected with IGF-IIRs lacking Arg2410-Lys2423. Thus, IGF-IIR, through its cytoplasmic domain, mediates the Ga1-linked action of IGF-II in living cells. We also found that IGF-IIR truncated with C-terminal 28 residues after Ser2424 caused Gbg2-dependent response of AC in response to IGF-II by activating Gi1. Comparison with the Ga1-dominant response of AC by intact IGF-IIR suggests that the C-terminal 28-residue region inactivates Gbg2. This study not only provides further evidence that IGF-IIR has IGF-II-dependent signaling function to interact with heteromeric G proteins with distinct roles by different cytoplasmic domains, it also suggests that IGF-IIR can separate and sequestrate the Ga1 and Gbg2 signals following Gi1 activation.

Insulin-like growth factor II (IGF-II)1 promotes growth, mainly in fetal development. In cultured cells, it exerts mitogenic and metabolic stimulation by binding to cell surface receptors. IGF-IIR is a high-affinity receptor for IGF-II (1–3). It is also a receptor for M6P (3). However, these two distinct ligands bind to different sites in IGF-IIR, which has been indicated by competition experiments and by the fact that only mammalian IGF-IIR can bind IGF-II (4). For several reasons (for review, see Ref. 4), it remains unclear whether the IGF-IIR executes signaling functions in response to IGF-II. Nonetheless, there are multiple lines of independent evidence that IGF-IIR has signaling function activated by IGF-II. In multiple cultured-cell systems, IGF-II evokes cellular responses, most likely through IGF-IIR (3, 5–15). We and another group independently showed that IGF-II stimulation of IGF-IIR promotes calcium influx through Gi1, a member of the heteromeric G protein family, in Balb/c3T3 or CHO cells (12–15). In reconstituted vesicles, purified IGF-IIR directly couples to Gi1 in response to IGF-II (16–18), and human IGF-IIR has a cytoplasmic 14-residue region at Arg2410-Lys2423, which can directly activate Gi1 (18, 19). In cell-free systems, this region most likely functions as the effector domain of IGF-IIR for Gi1 coupling (18–20). Although failure of IGF-IIR coupling to G proteins in cell-free systems was once reported (21), a subsequent paper (15), with two of the same authors, suggested the Gi1 coupling function of IGF-IIR, based on the observation that IGF-II stimulates Ca2+ influx via a pertussis toxin-sensitive G protein in a manner resistant to tyrosine kinase inhibitors.

Intensive studies of the molecular signaling function of IGF-IIR have so far been conducted only on cell-free experimental systems, which have serious limitations. The present study was conducted to establish a more physiological system, where one can investigate the signaling functions of IGF-IIR. Here we report that IGF-II links recombinant IGF-IIR to the Ga1 system in living cells. Furthermore, to the extreme C terminus of IGF-IIR, we assigned a novel function of inactivating Gbg2, which is another component of heteromeric G proteins. This study not only offers further evidence for the interaction of IGF-IIR with heteromeric G proteins, it provides a novel insight into the differential regulation of G protein subunit signals by receptors.

**EXPERIMENTAL PROCEDURES

Ga1 and gi2 (Ga1Q205L) cDNAs were provided by Dr. H. R. Bourne. Wild-type Ga1 cDNA, human IGF-IIR cDNA, D2410–2423 cDNA, and the construction method of IGF-IIR mutants were described previously (20, 22). Oligonucleotide-directed mutagenesis was done to construct ΔCT41 and ΔCT28 according to the Kunkel method (23). Oligonucleotides used were GAGCGTGAGGACGATTGATGAAGGGTGGGGCTG for ΔCT41 and ΔCT28 according to the Kunkel method (23). Oligonucleotides used were GAGCGTGAGGACGATTGATGAAGGGTGGGGCTG- GTC for ΔCT41, and GCGAGGAAGAGAATTGATGATCCAGCTT- GCAACAG for ΔCT28.

COS cells were grown in DMEM plus 10% calf serum and streptomycin/penicillin. For stable expression of Ga1, COS cells were transfected by the calcium phosphate method using 10 μg of Ga1 cDNA and 0.3 μg of pBabe/Puro, a puromycin resistance gene. Cells were then selected with 3 μg/ml puromycin and tested for immunoblot analysis with AS57. The COS cell line used here expresses Ga1 at an approximately half the level of endogenous Ga1.

Plasmids were transfected by the lipofection method as described (22). Intracellular accumulation of cAMP was measured as described.
(24). A day before transfection, 5 × 10^4 cells were seeded on a 12-well plate. Unless otherwise specified, cDNAs encoding IGF-IIRs or G_{i2}/gip2 (0.5 μg/ml each) were transfected with 1 μl/ml LipofectAMINE (LifeTechnologies, Inc.) and incubated for 24 h in a serum-free culture. After washing cells with fresh media, cells were labeled with 3 μCi of [3H]jadenine for another 23 h 30 min. It should be emphasized that cells were then washed rigorously with solution containing M6P, as follows. DMEM-Hepes (DMEM containing 25 mM Hepes/NaOH, pH 7.4) containing 10 mM M6P was added to cells after discarding media. Cells were then incubated for 15 min at room temperature and washed four times with DMEM-Hepes. These procedures, which dissociate M6P and M6P-containing proteins from IGF-IIR, ensured reproducibility for inhibition of AC by IGF-IIR stimulation. This was reasonable because M6P binding to IGF-IIR impaired the action of IGF-II to inhibit AC in cells transfected with IGF-IIR cDNA (see "Results"). Cells were then treated with 2.5 μg/ml CTX (Calbiochem) and 1 mM isobutylmethylxanthine with or without IGF-II (or IGF-I) in DMEM-Hepes at 37°C for 30 min. Reactions were terminated by aspiration and the immediate addition of 5% ice-cold trichloroacetic acid (1 ml/well). Acid-soluble nucleotides were separated on two-step ion-exchange columns as described (24), and specific accumulation of cAMP is expressed as (cAMP/ADP ~ ATP) × 10^3.

For binding assay of IGF-IIR, cells (3 × 10^5/dish) were transfected with 10 μg of IGF-IIR cDNAs and 20 μl of LipofectAMINE in 5 ml of DMEM plus streptomycin/penicillin. Twenty-four hours after transfection, the medium was renewed to DMEM plus 10% calf serum and streptomycin/penicillin. By scraping cells 48 h after transfection, membranes were prepared and IGF-II binding assay was performed as described (20). Specific binding was calculated by subtracting nonspecific binding, the binding in the presence of 100 nM IGF-II. All other materials were obtained from commercial sources. Data were analyzed with Student's t test.

**RESULTS AND DISCUSSION**

We initially examined whether our COS cells were appropriate to see the effects of G_{i2}. Indeed, COS cells have not frequently been used to examine the effects of G_{i} or G_{i2}-coupled receptors, although Bell and co-workers (25) have described the G_{i2}-coupled effect of somatostatin receptors using COS cells. For this reason, we tested the effect of transfection of wild-type G_{i2} or constitutively activated G_{i2} mutant gip2 cDNA on AC activity. As shown in Fig. 1A, transfection of gip2 resulted in dose-dependent inhibition of CTX-stimulated cAMP accumulation, whereas that of wild-type G_{i2} had no effect. Therefore, our COS cells seemed to be suitable for examining the G_{i2}-coupling function of receptors with transient transfection of cDNAs.

Intact IGF-IIR cDNA was transfected into these COS cells, which were treated with IGF-IIR before cell lysis. Parental COS cells expressed 3.6 fmol/μg of endogenous IGF-IIR binding sites having the K_d of 0.90 nM. With cDNA transfection, these cells expressed recombinant IGF-IIRs with comparable affinities by severalfold of the endogenous binding level (Fig. 1B, B_max and K_d were 7.3 fmol/μg and 1.3 nM in intact IGF-IIR, 9.5 fmol/μg and 1.4 nM in ΔCT41 transfection, 7.1 fmol/μg and 0.85 nM in ΔCT28 transfection, and 6.0 fmol/μg and 0.88 nM in Δ2410–2423 transfection, respectively). The endogenous IGF-IIR binding site appears to be virtually IGF-IIR, because only a ~110-kDa protein was cross-linked with radioactive IGF-IIR in an IGF-IIR-inhibitable manner in parental COS cell membranes under the same condition as in the IGF-IIR binding assay (data not shown). This assessment is not only consistent with the report of Steele-Perrin et al. (26) that IGF-IIR exhibits considerably high affinity for IGF-II, but is also strongly supported by the report of Oshima et al. (27) showing that endogenous IGF-IIR is scarce in COS cells.

In the cells transfected with either gene, 2.5 μg/ml CTX constantly increased AC activity by ~5-fold over the basal level. In cells transfected with intact IGF-IIR cDNA, IGF-II significantly impaired the CTX-stimulated AC activity in a dose-dependent manner (Fig. 1C). IGF-II also inhibited forsko-
FIG. 2. Effect of IGF-IIR and cytoplasmic mutants on AC activity in COS cells. COS cells were transfected with recombinant IGF-IIR cDNAs or pECE vector (each 0.5 μg/ml). Cells were then stimulated by 2.5 μg/ml CTX in the presence or absence of 10 nm IGF-II, and AC activity was measured. As a control, the effect of 10 nm IGF-I was examined in the IGF-IIR-transfected COS cells. Each value represents the mean ± S.E. of single determinants done with three independent transfections. AC activity is indicated as a percentage of CTX-stimulated activity in mock-transfected COS cells, which was 22.7 ± 2.6. Each value represents the mean ± S.E. of four independent experiments. Note that IGF-II augmented CTX-stimulated AC activity by ~200% in parental COS cells that received ΔCT28 transfection under the same condition. E, effect of PTX on IGF-II/ΔCT28-induced augmentation of CTX-stimulated AC activity. COS cells were transfected with ΔCT28 for 24 h and treated with 10 ng/ml PTX for another 24 h. During the last 30 min, cells were stimulated by 2.5 μg/ml CTX with or without 10 nm IGF-II. AC activity is indicated as a percentage of CTX-stimulated activity in mock-transfected COS/Gαt, cells, which was 20.7 ± 2.8.

TABLE I
Effects of PTX and M6P on the IGF-II action in IGF-IIR-transfected COS cells

| Treatment          | No IGF-II | 10 nm IGF-II |
|--------------------|-----------|-------------|
| No treatment       | 2.1 ± 0.1 | 5.7 ± 0.1   |
| + CTX              | 9.9 ± 1.0 | 8.2 ± 0.8   |
| + CTX + PTX        | 7.4 ± 0.6 | 8.2 ± 0.8   |
| + M6P              |           | 8.2 ± 0.8   |

has been implicated in its Gbg coupling function (18–20). To examine whether this is the case in living cells, we constructed mutant 1GF-IIRs lacking the C-terminal 41 residues after Arg2410 (ΔCT41) or the 28 residues after Ser2424 (ΔCT28) and lacking Arg2410–Lys2423 (Δ2410–2423). Despite remarkable expression of ΔCT41 (Fig. 1B), IGF-II failed to inhibit CTX-stimulated AC activity in cells transfected with this mutant (Fig. 2), indicating an essential role of the C-terminal 41 residues for AC suppression. We unexpectedly found a novel AC-linked function of ΔCT28. In ΔCT28-transfected COS cells, CTX augmented AC activity to the same level as in COS cells transfected with other IGF-IIRs; however, only in the ΔCT28-transfected cells, did IGF-II further potentiate CTX-stimulated AC activity (Fig. 2). This effect of IGF-II depended on the amount of ΔCT28 cDNA used for transfection (Fig. 3A). In the same ΔCT28-transfected cells, IGF-II did not affect AC activity without CTX (not shown). These suggest that AC potentiation by ΔCT28 is mediated by the Gbg subunit of heteromeric G proteins (28, 29).

To confirm the Gbg mediation, we examined the effect of Gαt on the function of ΔCT28 (Fig. 3B). In COS cells overexpressing Gαt (COS/Gαt), CTX-stimulated AC activity was measured. Each value represents the mean ± S.E. of four independent experiments. AC activity is indicated as percentage of the CTX-stimulated activity in Δ2410–2423-transfected COS cells, which was 20.7 ± 2.8.

FIG. 3. IGF-II-dependent augmentation of AC stimulation by IGF-IIR/ΔCT28. A, effect of ΔCT28 cDNA on CTX-stimulated AC activity in COS cells. COS cells were transfected with increasing doses of ΔCT28 cDNA and stimulated by 2.5 μg/ml CTX with or without 10 nm IGF-II. Basal AC activity in mock-transfected COS cells was 2.0 ± 0.1. Each value represents the mean ± S.E. of single determinants done with four independent transfections. *, p < 0.02; **, p < 0.05 versus no IGF-II. B, effect of ΔCT28 on CTX-induced AC stimulation in COS cells overexpressing Gαt (COS/Gαt). COS/Gαt cells were transfected with ΔCT28 cDNA (or vector), and stimulated by 2.5 μg/ml CTX with or without 10 nm IGF-II. AC activity is indicated as a percentage of CTX-stimulated activity in mock-transfected COS/Gαt, cells, which was 22.7 ± 2.6. Each value represents the mean ± S.E. of four independent experiments. Note that IGF-II augmented CTX-stimulated AC activity by ~200% in parental COS cells that received ΔCT28 transfection under the same condition. C, effect of PTX on IGF-II/ΔCT28-induced augmentation of CTX-stimulated AC activity. COS cells were transfected with ΔCT28 for 24 h and treated with 10 ng/ml PTX for another 24 h. During the last 30 min, cells were stimulated by 2.5 μg/ml CTX with or without 10 nm IGF-II. AC activity is indicated as a percentage of CTX-stimulated activity in ΔCT28-transfected COS cells. Each value represents the mean ± S.E. of four independent experiments. D, effect of IGF-IIR/Δ2410–2423 on AC activity in COS cells. COS cells were transfected with Δ2410–2423 cDNA and then stimulated by 2.5 μg/ml CTX in the presence or absence of 10 nm IGF-II, and AC activity was measured. Each value represents the mean ± S.E. of four independent experiments. AC activity is indicated as percentage of the CTX-stimulated activity in Δ2410–2423-transfected COS cells, which was 20.7 ± 2.8.
4 h treatment of 10 ng/ml PTX blocked the stimulatory effect of 10 nM IGF-II in COS cells transfected with ΔCT28, while the CTX response was not changed by PTX. These data indicate that the action of ΔCT28 on Gβγ is through Gαi, again suggesting that the Arg2410-Lys2423 region is interactive with Gβγ.

We confirmed the inability of Δ2410–2423 to affect AC (Fig. 3D). In COS cells transfected with Δ2410–2423, IGF-II could neither inhibit nor augment AC activity, despite the expression of this mutant comparable to that of intact IGF-IIR (Fig. 1B). Δ2410–2423 is mutant IGF-IIR that lacks Arg2410-Lys2423 but retains the extreme C-terminal 28 residues that ΔCT28 lacks. Therefore, this finally demonstrates that the domain that is essential for the interaction with Gαi is not the extreme C terminus but the Arg2410-Lys2423 region.

We have herein established a whole-cell system in which IGF-II-triggered signaling function of IGF-IIR can be examined. Using this system, multiple lines of evidence show that recombinant human IGF-IIR activates Gαi and suppresses AC in response to IGF-II. IGF-IIR transfection was required to observe the effect of IGF-II on AC, consistent with the scariness of endogenous IGF-IIR in COS cells. IGF-I could not reproduce the effect of IGF-II. In addition, M6P treatment of transfected COS cells blocked the effect of IGF-II, reproducing our in vitro data (16). It is thus emphasized that insufficient removal of lysosomal enzymes from IGF-IIR precludes this receptor from responding to IGF-II. Furthermore, the Arg2410-Lys2423 region is shown here to be essential for AC suppression by IGF-IIR, as predicted by our in vitro study (18–20).

In this study, an IGF-IIR mutant has pointed to a novel function of the receptor C terminus. ΔCT28 enhanced cAMP production in response to IGF-II. Multiple lines of evidence indicate that this response was mediated by Gβγ, the source of which was the activated Gαi. In contrast, intact IGF-IIR activated Gαi and mainly generated the signal of Gβγ in response to the same stimulation. These indicate that the C-terminal Ser2424-Ile2451 region of IGF-IIR can inactivate Gβγ. This inactivation suggests direct or indirect interaction of this C-terminal region with Gβγ. In further support of this idea, the Ser2424-Ile2451 region is homologous to a part of the PH domains (31) of multiple proteins including β-adrenergic receptor kinase (Fig. 4), which are known to bind Gβγ (32). It has also been shown that the isolated PH domain of β-adrenergic receptor kinase inactivates the action of Gβγ (33). These findings suggest that this region of IGF-IIR inactivates Gβγ through interaction. Because of technical difficulty, we were not able to examine the effects of the isolated regional peptides on AC augmentation by ΔCT28 observed in this whole-cell system.

Among known AC subtypes, no single AC that responds to both Gαi (inhibitory) and Gβγ (stimulatory) has been specified. However, among AC types I–VI, our COS cells express type VI, which is inhibited by Gαi, and type IV, which is stimulated by Gβγ (data not shown). It is thus reasonable to assume that the whole response of AC to Gαi in intact COS cells is the sum of the respective effects of Gαi and Gβγ on these AC subtypes, thus allowing the total AC activity to respond to both G-protein subunits.

In summary, this study shows that IGF-IIR, in living cells, activates Gαi and affects AC through differential actions of multiple cytoplasmic domains of its own. In our cells, it activates Gαi through Arg2410-Lys2423 and inactivates Gβγ through Ser2424-Ile2451, resulting in the predominant action of Gαi. Therefore, the distinct roles played by multiple domains of IGF-IIR separate and sequester the Gαi and Gβγ signals following Gαi activation. This is potentially a very interesting mechanism that allows a receptor to differentially activate Gαi and Gβγ and selectively turn on each subunit-specific pathway. With this novel mechanism, there is no longer necessity that a receptor must always turn on both subunit pathways by activating one heteromeric G protein complex. It is thus important to investigate whether a similar mechanism is possessed by other receptors.

It is also conceivable that this novel property of IGF-IIR may contribute to its unique signaling function in vivo. Multiple effects of Gαi depend on Gαi-released Gβγ (34). Thus, IGF-IIR-induced Gαi activation may lack some of the Gβγ outputs induced by conventional receptors. It is also conceivable that the Gβγ inactivating effect of the C terminus of this receptor may be affected by the amount of free Gβγ inside the cell. There may be an intracellular free Gβγ pool with different sizes in different cells (35). Excess Gβγ may thus occupy the C terminus and attenuate its inhibitory effect. In accord with this idea, IGF-II binding to IGF-IIR can potentiate AC stimulation in human fibroblasts (36) but not in COS cells (this report) and can stimulate PI turnover in renal cells (5) but not in Balb/c3T3 (12) or CHO cells (15). Alternatively, the Gβγ-linked function of IGF-II might be involved in its trafficking function as an M6P receptor, as one of the established functions of Gβγ is translocation of target proteins (37). This is, however, less likely, because the residues essential for IGF-IIR trafficking have been mainly localized near the N terminus of the cytoplasmic domain, particularly before Arg2410 (38). This possibility is further lowered by the fact that the cation-dependent M6P receptor, another trafficking receptor for M6P, has no cytoplasmic regions homologous to the PH-like domain in the extreme C terminus of IGF-IIR. In conclusion, this study demonstrates the coupling of IGF-II with heteromeric G proteins in native cell environments. While calcium influx is one of its most likely outputs (12–15), it is important to determine which cellular function is executed by the demonstrated IGF-IIR interaction with the G proteins.

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REFERENCES

1. Morgan, D. O., Edman, J. C., Standring, D. N., Freed, V. A., Smith, M. C., Roth, R. A., and Rutter, W. J. (1987) Nature 329, 301–307.
2. MacDonald, R. G., Pfeffer, S. R., Coussens, L., Tepper, M. A., Brocklebank, C. M., Møler, J. E., Anderson, J.-K., Chen, E., Czech, M. P., and Ullrich, A. (1988) Science 239, 1134–1136.
3. Roth, R. A. (1988) Science 239, 1269–1271.
4. Kornfeld, S. (1992) Annu. Rev. Biochem. 61, 307–330.
5. Rogers, S. A., and Hamerman, M. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4037–4041.
6. Rogers, S. A., and Hamerman, M. R. (1989) J. Biol. Chem. 264, 7962–7969.
7. Mathieu, M., Rochefort, H., Barenton, B., Preboks, C., and Vignon, F. (1990) Mol. Endocrinol. 4, 1327–1335.
8. Minisci, C. P., Kohn, E. S., Grubb, J. H., Sly, W. S., Oh, Y., Müller, H., Rosenfeld, R. G., and Helman, L. J. (1992) J. Biol. Chem. 267, 9000–9004.
9. Hari, J., Pierce, S. B., Morgan, D. O., Iiri, T., Ogata, E., and Rosenfeld, R. G. (1987) Annu. Rev. Biochem. 56, 3367–3371.
10. Mohan, S., Linkhart, T., Rosenfeld, R. G., and Baylink, D. J. (1989) J. Cell. Physiol. 140, 169–176.
11. Kojima, I., Nishimoto, I., Iiri, T., Ogata, E., and Rosenfeld, R. G. (1988) Biochem. Biophys. Res. Commun. 154, 9–19.
12. Nishimoto, I., Hata, Y., Ogata, E., and Kojima, I. (1987) J. Biol. Chem. 262, 12120–12126.
13. Matsunaga, H., Nishimoto, I., Kojima, I., Yamashita, N., Kurokawa, K., and Ogata, E. (1988) Am. J. Physiol. 255, C442–C446.
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14. Okamoto, T., Asano, T., Harada, S., Ogata, E., and Nishimoto, I. (1991) J. Biol. Chem. 266, 1085–1091
15. Pfeifer, A., Nürnberg, B., Kamen, S., Uhde, M., Schultz, G., Ruth, P., and Hofmann, F. (1995) J. Biol. Chem. 270, 9052–9059
16. Murayama, Y., Okamoto, T., Ogata, E., Asano, T., Iiri, T., Katada, T., Ui, M., Grubb, J. H., Sly, W. S., and Nishimoto, I. (1990) J. Biol. Chem. 265, 17456–17462
17. Nishimoto, I., Murayama, Y., Katada, T., Ui, M., and Ogata, E. (1989) J. Biol. Chem. 264, 14029–14038
18. Okamoto, T., Katada, T., Murayama, Y., Ui, M., Ogata, E., and Nishimoto, I. (1990) Cell 62, 709–717
19. Okamoto, T., and Nishimoto, I. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 8020–8023
20. Takahashi, K., Murayama, Y., Okamoto, T., Yokota, T., Ikezu, T., Takahashi, S., Giambarella, U., Ogata, E., and Nishimoto, I. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 11772–11776
21. Körner, C., Nürnberg, B., Uhde, M., and Braulke, T. (1995) J. Biol. Chem. 270, 287–295
22. Ikezu, T., Okamoto, T., Murayama, Y., Okamoto, T., Homma, Y., Ogata, E., and Nishimoto, I. (1994) J. Biol. Chem. 269, 31955–31961
23. Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 488–492
24. Wong, Y. H., Federman, A., Pace, A. M., Zachary, I., Evans, T., Pouyssegur, and Bourne, H. R. (1991) Nature 351, 63–65
25. Yasuda, K., Rens-Doriano, S., Breder, C. D., Law, S. F., Saper, C. B., Reisine, T., and Bell, G. I. (1992) J. Biol. Chem. 267, 20422–20428
26. Steele-Perkins, G., Turner, J., Edman, J. C., Hari, J., Pierce, S. B., Stover, C., Rutter, W. J., and Roth, R. A. (1988) J. Biol. Chem. 263, 11486–11492
27. Oshima, A., Nolan, C. M., Kyle, J. W., Grubb, J. H., and Sly, W. S. (1988) J. Biol. Chem. 263, 2553–2562
28. Tang, W.-J., and Gliman, A. G. (1991) Science 254, 1500–1503
29. Federman, A. D., Conklin, B. R., Schrader, K. A., Reed, R. R., and Bourne, H. R. (1992) Nature 356, 159–161
30. Birnbaumer, L. (1992) Cell 71, 1069–1072
31. Mayer, B. J., Ren, R., Clark, K. L., and Baltimore, D. (1993) Cell 72, 629–630
32. Touhara, K., Inglese, J., Pitcher, J. A., Shaw, G., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 10217–10220
33. Koch, W. J., Hawes, B. E., Inglese, J., Luttrel, L. M., and Lefkowitz, R. J. (1994) J. Biol. Chem. 269, 6193–6197
34. Reuveny, E., Stesinger, P. A., Inglese, J., Morales, J. M., Iliguez-Llusi, J. A., Lefkowitz, R. J., Bourne, H. R., Jan, Y. N., and Jan, L. Y. (1994) Nature 370, 143–146
35. Neer, E. J., and Clapham, D. E. (1990) in G Proteins (Iyengar, R., and Birnbaumer, L., eds) pp. 41–61, Academic Press, San Diego
36. Danne, H., Bouterfa, H., and Braulke, T. (1994) Mol. Cell. Endocrinol. 99, R25–R29
37. Inglese, J., Koch, W. J., Caron, M. G., and Lefkowitz, R. J. (1992) Nature 359, 147–150
38. Lobel, P., Fujimoto, K., Ye, R. D., Griffiths, G., and Kornfeld, S. (1989) Cell 57, 787–796
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