Feedback Regulation of β-arrestin1 Function by Extracellular Signal-regulated Kinases*

(Received for publication, March 19, 1999)
Fang-Tsyr Lin, William E. Miller, Louis M. Luttrell, and Robert J. Lefkowitz‡

From the Howard Hughes Medical Institute, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

The functions of β-arrestin1 to facilitate clathrin-mediated endocytosis of the β2-adrenergic receptor and to promote agonist-induced activation of extracellular signal-regulated kinases (ERK) are regulated by its phosphorylation/dephosphorylation at Ser-412. Cytoplasmic β-arrestin1 is almost stoichiometrically phosphorylated at Ser-412. Diphosphorylation of β-arrestin1 at the plasma membrane is required for targeting a signaling complex that includes the agonist-occupied receptors to the clathrin-coated pits. Here we demonstrate that β-arrestin1 phosphorylation and function are modulated by an ERK-dependent negative feedback mechanism. ERK1 and ERK2 phosphorylate β-arrestin1 at Ser-412 in vitro. Inhibition of ERK activity by a dominant-negative MEK1 mutant significantly attenuates β-arrestin1 phosphorylation, thereby increasing the concentration of dephosphorylated β-arrestin1. Under such conditions, β-arrestin1-mediated β2-adrenergic receptor internalization is enhanced as is its ability to bind clathrin. In contrast, if ERK-mediated phosphorylation is increased by transfection of a constitutively active MEK1 mutant, receptor internalization is inhibited. Our results suggest that dephosphorylated β-arrestin1 mediates endocytosis-dependent ERK activation. Following activation, ERKs phosphorylate β-arrestin1, thereby exerting an inhibitory feedback control of its function.

The life cycle of G protein-coupled receptors (GPCRs)1 includes receptor activation, desensitization, sequestration, and either resensitization (recycling) or degradation (1). β-Arrestins were initially discovered as molecules that bind to agonist-occupied receptors following receptor phosphorylation by G protein-coupled receptor kinases, thereby interdicting signal transduction to G proteins and causing receptor desensitization (2, 3). More recently, however, β-arrestins have been shown to be involved in the internalization and signaling of GPCRs (4). For example, they serve as clathrin adaptors, which help to target agonist-occupied GPCRs to clathrin-coated pits for internalization (5). This function is regulated by phosphorylation/dephosphorylation of β-arrestin1 at a carboxyl-terminal serine, Ser-412 (6). Cytosolic β-arrestin1 is constitutively phosphorylated by heretofore unidentified kinase(s) and is rapidly dephosphorylated when it is recruited to the plasma membrane in response to agonist stimulation. Dephosphorylation of β-arrestin1 at the plasma membrane is not required for receptor binding and receptor desensitization but is required for its clathrin binding and receptor internalization. The S412A mutant of β-arrestin1, which mimics the dephosphorylated form, has been shown to be more active than wild-type β-arrestin1 in promoting clathrin-mediated endocytosis of the β2-adrenergic receptor. In contrast, the S412D mutant, which simulates the phosphorylated form of β-arrestin1, acts as a dominant-negative inhibitor of receptor endocytosis (6). Moreover, in addition to regulating the internalization of classical GPCRs, such as the β2-adrenergic receptor, β-arrestin1 has been shown to bind to the tyrosine kinase insulin-like growth factor I receptor and mediate its endocytosis in an analogous fashion (7).

Recently, several studies have shown that clathrin-mediated internalization is required for mitogenic signaling by various GPCRs and tyrosine kinase growth factor receptors (7–12). Thus, inhibition of clathrin-mediated internalization reduces agonist-induced activation of ERK1 and 2. The Ras-dependent activation of ERKs by GPCRs also requires c-Src (13, 14). Very recently, it has been shown that β-arrestins serve to recruit the activated c-Src to the agonist-occupied β2-adrenergic receptors as well as to target this signaling complex to the clathrin-coated pits for internalization and activation of the ERK cascade (15). Like clathrin targeting, the recruitment and activation of c-Src kinase is modulated by phosphorylation/dephosphorylation of β-arrestin1 (15). The S412D β-arrestin1 mutant, defective in both binding to Src and targeting the receptors to clathrin-coated pits, acts as a dominant-negative inhibitor of agonist-induced ERK activation. In contrast, the S412A β-arrestin1 mutant, which binds to Src as well as the wild-type β-arrestin1, is active in promoting agonist-induced ERK phosphorylation.

ERK activity appears to be tightly regulated by an activation/inactivation cycle. GPCR-mediated activation of ERKs involves the sequential involvement of components of a Ras activation complex, including c-Src, Shc, Grb2, Gab1, and Sos1, followed by activation of Raf-1 kinase and MEK1 (13, 14). It has been shown that the inactivation of this cascade is associated with the induction of mitogen-activated protein kinase phosphatase (MKP-1) by agonist stimulation (16). Previous studies also suggest that it may involve the negative feedback phosphorylation of upstream activators, including Sos1, Raf-1 kinase, and MEK1, by the activated ERK (17–21). Recently ERK has been reported to phosphorylate IRS-1 and reduce its function, thereby inhibiting further insulin signaling (22). These findings underscore the requirement for stringent control of cellular ERK activity by feedback regulatory mechanisms. Here we demonstrate a novel form of feedback regulation controlling GPCR-mediated activation of ERKs. Once stimulated, the ERKs phosphorylate β-arrestin1 at Ser-412, thereby reduc-

* This work was supported by the Howard Hughes Medical Institute and by Grant HL16037 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Investigator of the Howard Hughes Medical Institute. To whom correspondence should be addressed: Howard Hughes Medical Inst., Dept. of Medicine and Biochemistry, Duke University Medical Center, Box 3821, Durham, NC 27710. E-mail: lefk001@mc.duke.edu.

1 The abbreviations used are: GPCR, G protein-coupled receptor; ERK, extracellular signal-regulated kinase(s); PAGE, polyacrylamide gel electrophoresis.
FLAG-tagged HEK 293 cells were transiently transfected with the plasmid encoding β-arrestin1 purified from E. coli were phosphorylated in vitro by ERK1, ERK2, or GSK-3 in the presence of γ-32P[ATP as described under “Experimental Procedures.” The phosphoproteins were fractionated by SDS-PAGE. The gel was dried and developed by autoradiography.

The FLAG-tagged MEK1(K97A) plasmid into HEK 293 cells. Two days after transfection, pores. The phospho-

**EXPERIMENTAL PROCEDURES**

**Expression of β-Arrestin1 in Escherichia coli and Phosphorylation in Vitro—** A 1.26-kilobase KpnI/HinIII fragment encoding (S412D)β-arrestin1-His6, was removed from pBS/S412D/βarr1-His6, and subcloned into pKK223–3 vector (Amersham Pharmacia Biotech). After transfection of pKK/βarr1-His6 or pKK/S412D/βarr1-His6, plasmid into E. coli, expression of the proteins was induced by isopropyl-1-thio-

**Two-dimensional Tryptic Phosphopeptide Mapping**—Phospho-

**Metabolic Labeling**—The His-tagged β-arrestin1 expression vector was transfected alone or with the dominant-negative MEK1(K97A) plasmid (25) into HEK 293 cells. Cells were labeled with [32P]orthophosphate for 1 h and then harvested for β-arrestin1 purification as described (6). Co-immunoprecipitation and Immunoblotting—The FLAG-tagged β-arrestin1 expression vector was transfected alone or with the MEK1(K97A) plasmid into HEK 293 cells. Two days after transfection, cells were harvested and lysed for co-immunoprecipitation as described (6). The FLAG-tagged β-arrestin1 (15) was immunoprecipitated with a polyclonal antibody directed against the FLAG epitope (Santa Cruz Inc.). After SDS-PAGE, the immunoblot was probed with a monoclonal antibody specific to clathrin heavy chain (Transduction Laboratories) and was visualized by enhanced chemiluminescence assay (ECL, Amersham Pharmacia Biotech). The expression levels of phospho-ERKs, total cellular ERKs, and MEK1 mutants (K97A and S218D/S222D) (25, 26) in whole cell extracts were determined by probing the immunoblots separately with the antibodies specific to phospho-ERK (Promega), cellular ERK2 (Transduction Laboratories), or MEK1 (Transduction Laboratories).

**Agonist-promoted Sequestration of the β3-Adrenergic Receptors—** HEK 293 cells were transiently transfected with the plasmid encoding FLAG-tagged β3-adrenergic receptors with or without the expression vectors of β-arrestin and a MEK1 mutant. Two days after transfection, cells were incubated with 10 μM (-)-isoproterenol in 0.1 M ascorbic acid for 30 min before harvesting. The agonist-promoted sequestration of β3-adrenergic receptors was determined by immunofluorescence flow cytometry as described previously (27).

**RESULTS AND DISCUSSION**

ERKs Phosphorylate β-Arrestin1 at Ser-412 in Vitro—Previously we have shown that cytosolic β-arrestin1 is highly phosphorylated and is dephosphorylated only when it is recruited to the plasma membrane in response to agonist stimulation (6). The major phosphorylation site is located at the carboxyl-ter-

**FIG. 1.** Phosphorylation of β-arrestin1 by ERK1, ERK2, and GSK-3 in vitro. 20 pmol of His-tagged wild-type (WT) or S412D β-arrestin1 purified from E. coli were phosphorylated in vitro by ERK1, ERK2, or GSK-3 in the presence of γ-32P[ATP as described under “Experimental Procedures.” The phosphoproteins were fractionated by SDS-PAGE. The gel was dried and developed by autoradiography.

**FIG. 2.** Two-dimensional tryptic phosphopeptide mapping of cellular phospho-β-arrestin1 and ERK2-phosphorylated β-arrestin1. A, 32P-labeled β-arrestin1 (βarr1) was purified from HEK 293 cells overexpressing His-tagged β-arrestin1, resolved by SDS-PAGE, and transferred to Immobilon polyvinylidene difluoride membranes. The 32P-labeled β-arrestin1 was cut out, digested with trypsin, resolved in two dimensions by electrophoresis and chromatography, and detected by autoradiography. × is the origin of sample loading. The arrows indicate the major partially digested phosphopeptides a1 and a2 and the minor phosphopeptide b. B, tryptic phosphopeptides as described above were purified by reverse-phase high pressure liquid chromatography. Two major phosphopeptides identified as amino acids 401–418 and 398–418 (6) were mixed and subjected to two-dimensional phosphopeptide mapping analysis. C and D, ERK-phosphorylated wild-type (C) and S412D (D) β-arrestin1 as described in Fig. 1 were digested with trypsin and subjected to two-dimensional phosphopeptide mapping analysis as described.

minal Ser-412, which accounts for 90% of β-arrestin1 phosphorylation. To identify the candidate kinase(s) that phosphorylate β-arrestin1 at Ser-412, we tested the ability of several kinases to phosphorylate β-arrestin1 in vitro. Because Ser-412 is followed by a proline residue, a consensus phosphorylation sequence recognized by members of the mitogen-activated protein kinase family as well as by glycogen synthase kinase-3 (GSK-3), we speculated that these kinases might be potential candidates for mediating Ser-412 phosphorylation. Therefore, equal amounts of wild-type and S412D β-arrestin1 purified from E. coli were subjected to phosphorylation by ERK1, ERK2, or GSK-3 in vitro. As shown in Fig. 1, wild-type β-arrestin1 was highly phosphorylated by either ERK1 or ERK2. The stoichiometry was ~0.8 mol of P/mol of protein. Mutation of Ser-412 to Asp markedly reduced ERK-mediated β-arrestin1 phosphorylation. Both wild-type and S412D β-arrestin1 were equally but weakly phosphorylated by GSK-3, indicating that GSK-3 is not the kinase responsible for Ser-412 phosphorylation.

Next, we compared the two-dimensional tryptic phosphopeptide map of cellular β-arrestin1 with those of wild-type and S412D β-arrestin1 phosphorylated by ERK2 in vitro. As shown
we employed a dominant-negative MEK1(K97A) inhibitor (25) to cellular ERKs. Overexpression of MEK1(K97A) was detected by an anti-reprobed with an ERK2 antibody to ensure equal expression of total a specific anti-phospho-ERK antibody. This blot was then stripped and from cellular phospho-in vitro

b

mutant in HEK 293 cells significantly reduced ERK phosphorylation. Overexpression of the MEK1(K97A) phosphorylation. indicating that these kinases are not responsible for Ser-412 reduce not shown). Moreover, mutation of Ser-412 to Asp did not the high stoichiometry of cellular phosphorylation of β-arrestin1 in cells, transiently expressing His-tagged β-arrestin1 alone or with MEK1(K97A), were split onto two plates each. Upper panel, one plate was metabolically labeled with [32P]orthophosphate for 1 h. β-Arrestin1 was purified, resolved by SDS-PAGE, and transferred to nitrocellulose membranes. After autoradiography, this membrane was subjected to Western blot analysis using an antibody specific to β-arrestin1. Lower panel, equal amounts of whole cell lysates from the other plate were fractionated by SDS-PAGE and subjected to Western blot analysis using a specific anti-phospho-ERK antibody. This blot was then stripped and reprobed with an ERK2 antibody to ensure equal expression of total cellular ERKs. Overexpression of MEK1(K97A) was detected by an antibody specific to MEK1.

in Fig. 2A, the two-dimensional phosphopeptide mapping of cellular β-arrestin1 purified from HEK 293 cells indicates that it contains three phosphopeptides: a1, a2, and b. The major phosphopeptides, a1 and a2, are partial digestion products (amino acids 401–418 and 398–418) containing Ser-412 (Fig. 2B) as confirmed by amino acid sequencing (6). The two-dimen-

sional phosphopeptide map of β-arrestin1 phosphorylated by ERK2 in vitro (Fig. 2C) is identical with the pattern derived from cellular phospho-β-arrestin1. This was further confirmed by the identical map derived from a mixture of equal amounts of cellular phospho-β-arrestin1 and ERK2-phosphorylated β-arrestin1 (data not shown). These two phosphopeptides, a1 and a2, were missing in the map of S412D β-arrestin1 phospho-

by ERK2 (Fig. 2D). Taken together, our results indicate that ERK is capable of phosphorylating Ser-412 of β-arrestin1. Although β-arrestin1 could be phosphorylated by protein kinases A and C, GRK2, and casein kinase I and II in vitro, in no case did the two-dimensional tryptic phosphopeptide maps match those of β-arrestin1 purified from cells (data not shown). Moreover, mutation of Ser-412 to Asp did not reduce in vitro phosphorylation of β-arrestin1 by these kinases, indicating that these kinases are not responsible for Ser-412 phosphorylation.

Inhibition of β-Arrestin1 Phosphorylation in HEK 293 Cells by a Dominant-negative MEK1 Inhibitor—To investigate whether ERK1 and ERK2 mediate β-arrestin1 phosphorylation in cells, we employed a dominant-negative MEK1(K97A) inhibitor (25) to determine whether inhibition of ERK activity might affect β-arrestin1 phosphorylation. Overexpression of the MEK1(K97A) mutant in HEK 293 cells significantly reduced ERK phosphorylation (Fig. 3, lower panel). This was associated with −70% reduction of β-arrestin1 phosphorylation (Fig. 3, upper panel). Increasing the level of activated ERKs with a constitutively active S218D/S222D mutant of MEK1 (26) did not significantly elevate β-arrestin1 phosphorylation (data not shown), consistent with the high stoichiometry of cellular phosphorylation of β-arrestin1 at Ser-412 (0.85 mol P/mol protein) (6). These results demonstrate that inhibition of ERK activation blocks β-arrestin1 phosphorylation in HEK 293 cells, thus further implicating ERKs as the kinases responsible for phosphorylating β-arrestin1 in cells.

Regulation of β-Arrestin1 Function by Constitutively Active and Dominant-negative Mutants of MEK1—Previously we have shown that dephosphorylation of β-arrestin1 at the plasma membrane is required for clathrin binding and agonist-induced internalization of the β2-adrenergic receptor (6). Thus,
Feedback Modulation of β-Arrestin1 Function by ERK

![Diagram](image)

**Fig. 5. A model for the negative feedback regulation of β-arrestin1 function by ERK-mediated phosphorylation.** Cytosolic β-arrestin1 is predominantly phosphorylated at Ser-412. It is dephosphorylated when recruited to the plasma membrane in response to agonist stimulation. Dephosphorylated β-arrestin1 binds to Src and also targets the agonist-occupied, GRK-phosphorylated GPCR to the clathrin-coated pits for internalization. ERK is activated subsequent to the receptor internalization event. Afterward, the activated ERK phosphorylates β-arrestin1 at Ser-412, reduces its ability to bind Src (15) and clathrin (6), and thereby attenuates ERK signaling. Ultimately the receptors are dephosphorylated and recycled back to the plasma membrane for resensitization. A, agonist; GRK2, G protein-coupled receptor kinase 2; (+), stimulatory effect; (−), inhibitory effect.

it would be expected that increasing the level of dephosphorylated β-arrestin1 in cells by inhibiting ERK activation with the dominant-negative MEK1(K97A) mutant would augment its clathrin binding ability and function in receptor internalization. As shown in Fig. 4A, the dominant-negative MEK1(K97A) mutant significantly enhances the co-immunoprecipitation of wild-type β-arrestin1 with clathrin heavy chain. The S412A mutant of β-arrestin1, which mimics the dephosphorylated form of β-arrestin1, also robustly co-immunoprecipitated clathrin (Fig. 4A). We did not observe any effect of the constitutively active S218D/S222D mutant of MEK1 on clathrin binding of β-arrestin1 (data not shown), presumably because cellular β-arrestin1 is already so highly phosphorylated that we could not detect its binding with clathrin.

We further investigated the effect of dominant-negative K97A and constitutively active S218D/S222D mutants of MEK1 on β-arrestin1-mediated sequestration of the β2-adrenergic receptors. In the presence of the MEK1(K97A) mutant, receptor sequestration was increased in control HEK 293 cells. It was further promoted by overexpressing β-arrestin1 (Fig. 4B), presumably because the level of active, dephosphorylated β-arrestin1 is highly increased by MEK1(K97A) mutant (as it is by S412A β-arrestin1 (6)). In contrast, the constitutively active S218D/S222D mutant of MEK1 slightly reduced receptor sequestration in control cells. This reduction was even more dramatic in cells overexpressing β-arrestin1 where receptor sequestration was now predominantly mediated by transfected β-arrestin1 (in contrast to control cells where both endogenous β-arrestin1 and 2 participate). In such cells, levels of the phosphorylated β-arrestin1 are increased to such high levels by the constitutively active MEK1 mutant that phospho-β-arrestin1 now acts essentially as a dominant-negative inhibitor of receptor internalization (as does S412D β-arrestin1 (6)).

To determine whether the effect of MEK mutants was specifically due to altered β-arrestin1 function, we also tested their effects on receptor sequestration mediated by β-arrestin2. Interestingly, this enhancement was not significantly affected by either MEK1 mutant in cells overexpressing β-arrestin2 (Fig. 4B). This result suggests that ERKs can modulate the function of β-arrestin1 but not β-arrestin2. Although β-arrestin2 is also a phosphoprotein in cells (data not shown), it has no site corresponding to Ser-412 of β-arrestin1. This suggests that ERKs are not the kinases that phosphorylate β-arrestin2 in cells.

A Model for Negative Feedback Regulation of β-Arrestin1 Function by ERK-Mediated Phosphorylation—Fig. 5 provides a model for the feedback regulation of β-arrestin1 function by ERK-mediated phosphorylation of Ser-412. Cytosolic β-arrestin1, which is predominately phosphorylated at Ser-412 (6), is recruited to the plasma membrane upon agonist stimulation. Membrane-bound β-arrestin1 is dephosphorylated by as yet unknown phosphatases. Although dephosphorylation of β-arrestin1 is not required for its receptor binding, it is required for several of its other functions including Src recruitment (15) and clathrin binding (6). These events in turn are necessary for GPCR-mediated activation of the Ras-dependent ERK pathway (13). Once activated, the ERKs are able to phosphorylate β-arrestin1 at Ser-412, thereby reducing these functions and, in a feedback regulatory fashion, reducing further ERK signaling.

Acknowledgments—The model shown in this paper was kindly provided by Dr. Stuart Maudsley. The expression vectors of MEK1(K97A) and MEK1(S218D/S222D) mutants were generous gifts from Dr. Edwin G. Krebs and Dr. Raymond L. Erikson, respectively. We thank Drs. Yehia Daaka, Julie A. Pitcher, and Randy Hall for helpful discussions. We also thank Donna Addison and Mary Holben for excellent secretarial assistance.

REFERENCES

1. Lefkowitz, R. J. (1998) J. Biol. Chem. 273, 18677–18680
2. Lohse, M. J., Benovic, J. L., Codina, J., Caron, M. G., and Lefkowitz, R. J. (1990) Science 248, 1547–1550
3. Attromodal, H., Arriza, J. L., Aski, C., Dawson, T. M., Codina, J., Kwatra, M. M., Snyder, S. H., Caron, M. G., and Lefkowitz, R. J. (1992) J. Biol. Chem. 267, 17882–17890
4. Ferguson, S. S. G., Downey, W. E. III, Colapietra, A.-M., Barak, L. S., Menard, L., and Caron, M. G. (1996) Science 271, 363–366
5. Goodman, O. B., Jr., Krupnick, J. G., Santini, F., Gurevich, V. V., Penn, R. B., Bagnon, A. W., Keen, J. H., and Benovic, J. L. (1996) Nature 383, 447–450
6. Lin, F.-T., Krueger, K. K., Kendall, H. E., Daaka, Y., Fredericks, Z. L., Pitcher, J. A., and Lefkowitz, R. J. (1997) J. Biol. Chem. 272, 31051–31057
7. Lin, F.-T., Daaka, Y., and Lefkowitz, R. J. (1998) J. Biol. Chem. 273, 31640–31643
8. Vieira, A. V., Lamaze, C., and Schmid, S. L. (1996) Nature 274, 2086–2089
9. Ribeiro, A., Pierichalla, B. A., Ciurlaro, C. L., and Ginty, D. D. (1997) Science 274, 1097–1100
10. Chow, J. C., Condorelli, G., and Smith, R. J. (1998) J. Biol. Chem. 273, 4672–4680
11. Luttrell, L. M., Daaka, Y., della Rocca, G. J., and Lefkowitz, R. J. (1997) J. Biol. Chem. 272, 31648–31656
12. Daaka, Y., Luttrell, L. M., Ahn, S., della Rocca, G. J., Ferguson, S. S. G., Caron M. G., and Lefkowitz, R. J. (1998) J. Biol. Chem. 273, 665–688
13. van Biesen, T., Luttrell, L. M., Hawes, B. E., and Lefkowitz, R. J. (1996) Endocr. Rev. 17, 698–714
14. Gutkind, J. S. (1998) J. Biol. Chem. 273, 1839–1842
15. Luttrell, L. M., Ferguson, S. S., Daaka, Y., Miller, W. E., Maudsley, S., della Rocca, G. J., Lin, F.-T., Kawakatsu, H., Owada, K., Luttrell, D. K., Caron, M. G., and Lefkowitz, R. J. (1999) J. Biol. Chem. 274, 655–661
16. Charles, C. H., Sun, H., Iau, L. F., and Tonks, N. K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5292–5296
17. Pitarke, E., and McCormick F. (1996) J. Biol. Chem. 271, 5871–5877
18. Formchi, M., Chari, S., Dunn, M. J., and Sorokin, A. (1997) EMBO J. 16, 4639–4651
19. Lee, R., Cobb, M. H., and Blackshear, P. J. (1992) J. Biol. Chem. 267, 1088–1092
20. Ueki, K., Matsuda, S., Tobe, K., Gotoh, Y., Tamemoto, H., Yachi, M., Akuma, Y., Yaxaki, Y., Nishida, E., and Kadowaki, T. (1994) J. Biol. Chem. 269, 15756–15761
21. Brunet, A., Pages, G., and Pouyssegur, J. (1994) FERS Let. 346, 299–303
22. De Fea, K., and Roth, R. A. (1997) J. Biol. Chem. 272, 31400–31406
23. Luo, K., Hurley, T. R., and Setton, B. M. (1991) Methods Enzymol. 201, 149–152
24. Huganir, R. L., Miles, K., and Greengard, P. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 6968–6972
25. Coggeshall, R., Seger, R., Reszka, A. A., Munar, E. S., Eldar-Finkelman, H., Dobrowolska, G., Jensen, A. M., Campbell J. S., Fischer, E. H., and Krebs, E. G. (1994) J. Biol. Chem. 269, 25699–25709
26. Huang, W., Kessler D. S., and Erikson, R. L. (1995) Mol. Biol. Cell 6, 237–245
27. Barak, L. S., Tiber, M., Freedman, N. J., Kwatra, M. M., Lefkowitz, R. J., and Caron, M. G. (1994) J. Biol. Chem. 269, 2796–2799