Binding of the β2 Adrenergic Receptor to N-Ethylmaleimide-sensitive Factor Regulates Receptor Recycling

Received for publication, June 29, 2001, and in revised form, September 18, 2001
Published, JBC Papers in Press, September 27, 2001, DOI 10.1074/jbc.M106087200

Mei Cong‡, Stephen J. Perry‡, Liaoyuan A. Hu‡, Phyllis I. Hanson§, Audrey Claing‡, and Robert J. Lefkowitz¶

From the Howard Hughes Medical Institute, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710 and the Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

Following agonist stimulation, most G protein-coupled receptors become desensitized and are internalized, either to be degraded or recycled back to the cell surface. What determines the fate of a specific receptor type after it is internalized is poorly understood. Here we show that the rapidly recycling β2 adrenergic receptor (β2AR) binds via a determinant including the last three amino acids in its carboxy-terminal tail to the membrane fusion regulatory protein, N-ethylmaleimide-sensitive factor (NSF). This is documented by in vitro overlay assays and by cellular communoprecipitations. Receptors bearing mutations in any of the last three residues fail to interact with NSF. After stimulation with the agonist isoproterenol, a green fluorescent protein fusion of NSF colocalizes with the wild type β2AR but not with a tail-mutated β2AR. The β2AR-NSF interaction is required for efficient internalization of the receptors and for their recycling to the cell surface. Mutations in the β2AR tail that ablate NSF binding reduce the efficiency of receptor internalization upon agonist stimulation. Upon subsequent treatment of cells with the antagonist propranolol, wild type receptors return to the cell surface, while tail-mutated receptors remain sequestered. Thus, the direct binding of the β2AR to NSF demonstrates how, after internalization, the fate of a receptor is dependent on a specific interaction with a component of the cellular membrane-trafficking machinery.

G protein-coupled receptors (GPCRs) are a family of integral plasma membrane proteins that transduce signals into cells from diverse extracellular ligands. An almost universal feature of these receptors is their ability to be desensitized in response to prolonged exposure to ligand (1). This is achieved mainly by the action of GPCR kinases that only phosphorylate agonist-occupied receptor molecules, allowing β-arrestin molecules to then bind and physically interdict further coupling of the receptor to heterotrimeric G proteins (2).

Receptor desensitization is usually accompanied by the rapid internalization of the receptor molecules. This serves two main purposes. Firstly, it allows a cell to resesthetize its responsiveness to a ligand by trafficking the receptors through a series of intracellular vesicular compartments in which they are returned to a naïve state (i.e. dephosphorylated) before recycling them back to the cell surface (3–6). Secondly, by trafficking the receptors to alternative vesicular structures in which they are degraded, a cell can permanently reduce the receptor density in the plasma membrane and thus diminish its responsiveness to subsequent exposures to the ligand (7–9).

The proportion of a GPCR that is targeted for recycling or degradation varies greatly between receptor types, as do the rates with which these two processes occur. Some GPCRs, including the β2 adrenergic receptor (β2AR), are rapidly recycled back to the cell surface within minutes of being internalized (3, 10–12). Other receptors are detained within the cell for much longer periods of time before they are recycled (13–17), while still others are mostly degraded (18, 19). The postendocytic fate of some receptors appears to be determined by the mechanism by which they are internalized (20, 21). For others it is an intrinsic property of the receptor molecule itself. This is demonstrated most clearly in situations where two types of receptors internalizing by the same mechanism subsequently suffer different postendocytic fates. For example, in HEK293 cells both the β2AR and δ opioid receptor are internalized through clathrin-coated pits, but while almost all the β2AR is then rapidly recycled, the δ opioid receptor is mainly targeted to lysosomes for degradation (18). Furthermore, studies utilizing receptor chimeras between the β2AR and vasopressin V2 receptor (V2R) (16) and between the substance P receptor and the thrombin PAR1 receptor (22) have shown that interchanging the carboxyl termini of receptors is sufficient to bestow the recycling or degradative sorting properties of one receptor onto the other. From such studies it has been hypothesized that there exist domains within the carboxyl termini of receptors that act as determinants of receptor fate. These domains are presumably amino acid sequences that act as specific binding sites for protein factors involved in sorting, trafficking, and recycling or degrading the receptor. One such domain, a serine cluster in the tail of the V2 receptor, has implicated the β-arrestins as members of this group of factors (15–17). Upon agonist binding, phosphorylation of the V2R within the cluster promotes the formation of a stable complex between the recep-
tor and β-arrestin. The complex is subsequently internalized and targeted to a recycling pathway in which the receptor is detained within endosomal compartments for long periods of time before being returned to the cell surface. Furthermore, removal of such a serine cluster from, or its addition to, the tail of a receptor is sufficient to bestow on it fast or slow recycling kinetics.

A number of other proteins have been identified that interact with the tails of specific receptors (23–25). While in some cases such proteins have been shown to alter the rate of receptor internalization, their effects on receptor fate are largely unknown. One exception is the Na+/H+ exchanger regulatory factor (NHERF), which binds to the tails of a small number of GPCRs including the β2AR (26–28). Disruption of the interaction between the β2AR and NHERF is accomplished by a shift in the postendocytic sorting of the receptor from being rapidly recycled to instead being mostly targeted for degradation (28). It is likely that NHERF represents just one member of a large group of factors that influence the fate of specific sets of GPCRs. Identification of these factors is an important step in furthering our understanding of the complex mechanisms that regulate receptor fate. Here we describe the identification of such a fate-determining factor for the β2AR, the N-ethylmaleimide-sensitive factor (NSF), and describe its ability to regulate the sorting of the internalized receptor.

**EXPERIMENTAL PROCEDURES**

Mammalian expression constructs for FLAG-β2AR, FLAG-β1AR, and HA-NHERF are described previously (23, 27, 29, 30). Mammalian expression constructs for HA-V2R, HA-V2δ2, and HA-β2V2 were kind gifts from Dr. Marc Caron (16). Bacterial expression constructs for the four point mutations introducing alanines at Asp-410, Ser-411, Ser-412, and Ser-413 (the entire carboxyl terminus) of the β2AR (26–28). Purified NSF protein and the Four Point Mutations FLAG-NSF were kind gifts from Dr. James Rothman. The anti-HA 12CA5 antibody-agarose conjugate and the Four Point Mutations FLAG-NSF-c-Myc were kind gifts from Dr. Marc Caron (16). Bacterial expression constructs for the sorting of the internalized receptor.

**RESULTS**

To identify potential binding partners for the tail of the β2AR, a yeast two-hybrid screen of a rat brain cDNA library was performed using as bait the GAL4 binding domain fused to residues 328–413 (the entire carboxyl terminus) of the β2AR. The library contains three clones encoding the rat NSF (22, 33) that were isolated, which allow growth on selective media containing 2 µg/ml of yeast coexpressing both fusion proteins (data not shown). The interaction is detectable in vitro using an overlay assay where recombinant NSF binds to a GST fusion protein of the β2AR tail but not to a GST fusion of the β1 adrenergic receptor (β1AR) tail or to GST alone (Fig. 1A). Furthermore, NSF coex-
Fig. 1. NSF interacts with the tail of the β2AR. A, 4 μg of GST, GST-β1ARct (carboxyl-terminal tail) fusion, and GST-β2ARct fusion were separated by PAGE and transferred to a nitrocellulose filter (equal loading was assessed by Coomassie staining an identical gel, upper panel). The filter was incubated overnight in 100 nM purified NSF protein and washed, and a Western blot was performed using an anti-NSF monoclonal antibody (IB: NSF, bottom panel). Shown are representative blots of three experiments. B, in COS7 cells, His6-NSF-c-Myc was overexpressed alone or with FL-β1AR or FL-β2AR. After treatment with a cell-permeable cross-linker, receptors were immunoprecipitated (IP: FL-ARs) with M2 anti-FLAG affinity beads, and the amount of NSF in the immune complex was detected with an anti-hexahistidine antiserum (IB: His6-NSF). Results are representative of three experiments.

Fig. 2. Mapping of the NSF binding site in the β2AR. A, a tail truncation mutant of the β2AR fail to bind NSF. In COS7 cells, His6-NSF-c-Myc was overexpressed alone or with wild type FL-β2AR (wt: residues 1–413), FL-β2ART360 (residues 1–385) or FL-β2ART360 (residues 1–360). After treating the cells with a cell-permeable cross-linker, receptors were immunoprecipitated (IP: FL-ARs) using M2 anti-FLAG affinity beads, and proteins resolved by PAGE. The amount of NSF in the immune complex (upper panel) and cell lysate (lower panel) was detected with an anti-hexahistidine antiserum (IB: His6-NSF). Results are representative of three experiments. B, the β2AR carboxy terminus confers NSF binding on the V2R. In COS7 cells, HA-tagged V2 receptor (HA-V2R), V2β2 receptor chimeras were overexpressed together with His6-NSF-c-Myc. Cells were treated with a cell-permeable cross-linker, receptors were immunoprecipitated (IP: HA-ARs) using 12CA5 anti-HA affinity beads, and proteins resolved by PAGE. The amount of NSF in the immune complex (upper panel) and cell lysate (lower panel) was detected with an anti-hexahistidine antiserum (IB: His6-NSF). Results are representative of three experiments. C, schematic diagram showing the GST-β2AR tail fusion proteins used in overlay assays to determine the region to which NSF binds. D, overlay assays identify the distal region of the β2AR as the NSF binding domain. 4 μg of the GST-fusion proteins represented in C were separated by PAGE and transferred to a nitrocellulose filter (loading of proteins was assessed by Coomassie staining an identical gel, upper panel). The filter was incubated overnight in 100 nM purified NSF protein and washed, and a Western blot was performed using an anti-NSF monoclonal antibody (NSF overlay, IB: NSF, bottom panel). Results are representative of three experiments.
and proteins resolved by PAGE. The amount of NHERF in the immune complex (IB: HA-NHERF) was detected with an anti-hexahistidine antiserum (IB: His<sub>6</sub>-NSF). Results are representative of three experiments. C, in COS<sup>7</sup> cells, HA-NHERF was overexpressed alone or with wild type FL-β<sub>2</sub>AR, FL-β<sub>2</sub>AR-410A, FL-β<sub>2</sub>AR-411A, FL-β<sub>2</sub>AR-412A, or FL-β<sub>2</sub>AR-413A. After treating the cells with a cell-permeable cross-linker, receptors were immunoprecipitated (IP: FL-β<sub>2</sub>ARs) using M2 anti-FLAG affinity beads, and proteins resolved by PAGE. The amount of NHERF in the immune complex (upper panel) and cell lysate (lower panel) was detected with an anti-HA antiserum (IB: HA-NHERF). Results are representative of three experiments.

It has been reported previously that the NHERF binds via a PDZ domain to the same region of the β<sub>2</sub>AR tail as is demonstrated here for NSF (27, 28). NSF does not contain a PDZ domain, and therefore it must be interacting with the tip of the β<sub>2</sub>AR tail through a wholly different mechanism than that utilized by NHERF. In support of this, sequential mutation analysis of the β<sub>2</sub>AR tail has revealed different residue requirements for NHERF and NSF binding; in particular, an alanine at position 412 does not affect NHERF binding while it decreases NSF binding, and an alanine at position 410 ablates NHERF binding but does not affect NSF binding (shown here by cellular coimmunoprecipitations in Fig. 3, B and C and by in vitro overlay in Ref. 27). If the sites of interaction of NHERF and NSF do indeed overlap, we would expect that the binding of one protein to the β<sub>2</sub>AR tail should inhibit the binding of the other. We tested this by measuring what effect overexpression of NHERF has on the ability of NSF to coimmunoprecipitate with the β<sub>2</sub>AR (Fig. 4). Coexpression of both NHERF and NSF with the β<sub>2</sub>AR leads to a complete loss of NSF from receptor immunoprecipitates, while NHERF is now detected. This is supportive of the concept that NSF and NHERF share an overlapping binding domain within the tail of the β<sub>2</sub>AR and that NHERF is capable of competing with NSF for binding to this site.

Having demonstrated that NSF can bind to the tail of the β<sub>2</sub>AR, we then investigated the sites at which the interaction occurs in cells using a GFP-tagged NSF molecule and fluorescent-labeled antibodies for detecting the epitope-tagged β<sub>2</sub>AR. In unstimulated HEK293 cells, the majority of the β<sub>2</sub>AR is located at the cell surface in the plasma membrane, while NSF
is evenly distributed throughout the cytosol with a small amount colocalizing with the \( \beta 2 \)-AR at the plasma membrane-cytosol interface (Fig. 5). After agonist stimulation with (-)-isoproterenol, the \( \beta 2 \)-AR undergoes endocytosis into the cell to collect in small puncta, presumably endocytic vesicular structures that enlarge with prolonged exposure of the cells to agonist. The initial weak colocalization of the receptor with NSF becomes more pronounced with continuing exposure to agonist and occurs mainly at the vesicular structures within the cytosol (Fig. 5, lower panels). As would be expected for a mutant receptor that is unable to interact with NSF, no colocalization of the \( \beta 2 \)-AR-412A and NSF is observed (Fig. 6). Prior to stimulation with agonist, the same pattern of receptor distribution is seen for \( \beta 2 \)-AR-412A as for the wild type receptor; \( \beta 2 \)-AR-412A is located primarily at the cell surface, and NSF is uniformly distributed in the cytosol. Upon agonist stimulation, \( \beta 2 \)-AR-412A also undergoes endocytosis and collects in small vesicular structures; even after prolonged exposure to agonist, however, NSF fails to colocalize with the receptor at these sites.

The primary function ascribed to NSF is as an ATPase that acts as a key regulator of membrane fusion events occurring during vesicle trafficking and exocytosis (33–35). The interaction of NSF with the cytoplasmic tail of the \( \beta 2 \)-AR therefore suggests that it may also play a more specific role in the trafficking of those vesicles containing the \( \beta 2 \)-AR. Ablation of NSF binding to the tail of the \( \beta 2 \)-AR might then be expected to lead to alterations in the rates of internalization or recycling of the receptor. To test this, the wild type \( \beta 2 \)-AR and the mutant \( \beta 2 \)-AR-412A, which shows decreased binding to NSF, were expressed in the absence or presence of overexpressed NSF, and the rate and extent of agonist-induced receptor internalization was measured. Subsequent treatment with the membrane-permeable antagonist propranolol to displace agonist from the internalized receptors then allowed the rate of recycling to be determined (Fig. 7). The cells rapidly sequester the wild type \( \beta 2 \)-AR following isoproterenol treatment, reaching 24% of the cell-surface receptor being internalized after 30 min. Overexpression of NSF enhances both the rate and the total amount of internalized receptors (to 38%) after 30 min of stimulation (Fig. 7A). Subsequent treatment of the cells with propranolol leads to rapid recycling of internalized receptors, with 90% of the receptors being detectable on the cell surface within 30 min (Fig. 7A). In the presence of overexpressed NSF, both the rate and extent of recycling are enhanced; 90% of the receptors are detectable on the cell surface just 10 min after antagonist treatment, and all the receptors are detectable on the cell surface after 30 min (Fig. 7A). This is accompanied by the complete loss of all the sites of colocalization of NSF and receptor at intracellular vesicles (Fig. 7C). A smaller fraction of the mutant receptor \( \beta 2 \)-AR-412A is internalized following isoproterenol treatment, reaching only 63% of the level of internalization of the wild type \( \beta 2 \)-AR after 30 min (Fig. 7A). Overexpression of NSF with \( \beta 2 \)-AR-412A does not enhance the rate of receptor internalization, and nor does it lead to colocalization of the receptor and NSF (Fig. 7, A and D and previously in Fig. 6B). It should also be noted that mutation of the \( \beta 2 \)-AR at this site does not affect the level of receptor expression at the plasma membrane prior to agonist stimulation. However, the most striking effect of perturbing the NSF-\( \beta 2 \)-AR interaction is seen after the addition of propranolol to the cells; the mutant \( \beta 2 \)-AR-412A is completely inhibited in its recycling, with NSF overexpression having no discernable ability to rescue this effect (Fig. 7, A and D).

It has been reported that NHERF binding to the tail of the \( \beta 2 \)-AR plays a role in determining the rate and amount of receptor that is recycled to the cell surface following internalization (28). The data presented here in Fig. 7A indicates that the binding of NSF to the tail of the \( \beta 2 \)-AR is also important for receptor recycling. In fact, the recycling-deficient mutant \( \beta 2 \)-AR-412A can still bind to NHERF (see Ref. 27 and Fig. 3, B and C), suggesting that NHERF binding may not be involved in \( \beta 2 \)-AR recycling. To test further whether both NHERF and NSF are involved in receptor recycling, we compared the recycling capabilities of the wild type \( \beta 2 \)-AR and the two mutant receptors that bind to only one of these two proteins: \( \beta 2 \)-AR-412A that binds NHERF but not NSF, and \( \beta 2 \)-AR-410A that binds...
NSF but not NHERF (Fig. 7B). The wild type receptor and NSF-binding D410A receptor both show equivalent levels of internalization after 30 min of agonist treatment, and both recycle back to the cell surface with the subsequent addition of the antagonist propranolol. However, β2AR-412A poorly internalizes and is unable to recycle following propranolol treatment. These data imply that the direct binding of the β2AR to NSF, but not to NHERF, is a required event for correct trafficking of the receptor during agonist-induced internalization and recycling.

**DISCUSSION**

The balance struck between the resensitization (recycling) and the down-regulation of desensitized GPCRs determines the magnitude and endurance of a cell’s response to subsequent exposures to ligand. Studies have indicated that this balance varies greatly between receptors and is determined, in part, by signals within the receptor molecules themselves (15–17, 22). These signals are presumed to be domains through which receptors interact with protein factors whose functions are to direct the sorting of internalized receptors for either recycling or degradation. Thus, through understanding the interactions that any given receptor makes with these factors it may be possible to determine its fate following internalization.

Here, we set out to identify proteins that, through direct interactions with the carboxyl terminus of the β2AR, could be serving such fate-determining functions. We identified one such candidate, the membrane fusion regulatory ATPase, NSF. Through its interaction with αSNAP (soluble NSF attachment protein), NSF binds vesicular membrane and target membrane-specific proteins (αSNAP receptors, SNAREs) to form a complex called the “20S particle” (33, 34). This complex is a critical component of the machinery necessary to perform membrane fusion events during the processes of intracellular membrane trafficking and exocytosis.

It has been shown in a number of studies that following agonist stimulation the β2AR is one of the most swiftly internalized and recycled of the GPCRs (3, 10–12). The ability of NSF to directly bind the β2AR may provide the basis of the mechanism underlying this rapidity; vesicles containing the internalized β2AR would have associated with them large quantities of NSF and the other 20S particle proteins, and thus they would preferentially be targeted for rapid trafficking. This is supported here by the demonstration that overexpression of NSF enhances the rates of internalization and recycling of the wild type β2AR but not of a mutant receptor that no longer binds NSF (Fig. 7). In fact, the failure of the mutated β2AR to recycle at all suggests that the binding of NSF to the receptor is essential for this process.

NSF also interacts with the βarrestins, two proteins that mediate GPCR desensitization by binding to agonist-occupied phosphorylated GPCRs and thereby inhibiting their coupling to G proteins (36). A further function of the βarrestins is as adaptors/scaffolds for recruiting a wide range of accessory proteins to GPCRs (37–43). While NSF is likely to directly interact...
with only a small number of GPCRs, its interaction with arrestins may represent a mechanism by which it can influence the trafficking of all members of this receptor class. Whether recruitment of NSF to a receptor through direct binding or via a βarrestin scaffold results in differences in receptor fate awaits further investigation.

We also define a site at the tip of the β2AR tail that mediates binding of the receptor to NSF. This is overlapping with the site previously reported to mediate receptor binding to NHERF (27). However, while NHERF binds to the β2AR via a PDZ domain, NSF lacks such a domain so it must bind by an alternative mechanism. This is supported here by the demonstration that a mutation at position L412A in the receptor, which is known not to affect NHERF binding, ablates NSF binding. In addition, a mutation at position D410A that ablates NHERF binding does not affect NSF binding (27). The β2AR-NHERF interaction has been reported to affect postendocytic sorting of the receptor (28). Perturbation of the interaction is reported to prevent the receptor from recycling and to increase the proportion that is degraded after it is internalized. Here we show that the mutant β2AR-412A, which does not bind NSF but does still bind NHERF, is also defective in receptor recycling. Furthermore, we demonstrate that the mutant β2AR-410A, which still can bind NSF but cannot bind NHERF, recycles normally. Thus, NSF might in fact mediate some of the effects on receptor sorting that the β2AR-NHERF interaction has been previously reported to have (28).

It also has been recently reported that, like the β2AR, the human leukotizing hormone receptor (hLHR) undergoes rapid recycling (44). This process was shown to be dependent on the presence of a specific stretch of residues found near the tip of the receptor tail (GTALL), a sequence missing from the tail of the poorly recycling rat LH receptor (rLHR). Addition of this sequence, or the last four residues found at the tip of the β2AR tail (DSLl), to the rLHR bestowed on it hLHR recycling characteristics. Furthermore, this study demonstrated that NHERF does not play a role in hLHR recycling as manipulations that have been reported to disrupt NHERF function in β2AR recycling fail to affect hLHR recycling. It is interesting to note the sequence similarity that exists between the hLHR and the β2AR recycling determinants (GTALL and SLL, respectively). Both sequences contain a leucine dimer and a hydroxyl-containing residue (Thr or Ser) and may represent a conserved sequence through which NSF might bind and mediate receptor recycling. However, it remains to be shown whether NSF can bind the hLHR and mediate recycling.

The effect of NSF on β2AR recycling that we describe here parallels a recently revealed and hitherto unknown function for NSF in neurons where it acts as a chaperone during trafficking of the α-amino-3-hydroxy-5-methyl-isoxazolepropionate (AMPA) glutamate receptor (45–47). Rapid and continuous cycling of AMPA receptors to and from the postsynaptic membranes regulates the responsiveness of neurons to the presynaptic release of glutamate. Like for the β2AR, the recycling of the AMPA receptor is dependent on the direct interaction of the GluR2 subunits of the receptor with NSF (48, 49). Manipulations that inhibit exocytosis or that specifically block the interaction between the GluR2 subunits and NSF prevent AMPA receptor recycling. This leads to a rapid reduction in receptor density in the postsynaptic membrane and a subsequent reduction in glutamate responsiveness of the neuron. The direct binding of NSF to both the AMPA receptor and the β2AR, therefore, is critical to allow these receptors to undergo rapid recycling, a process that is important for sustaining the signaling potential of the cells in which they are expressed. There is also evidence suggesting that the mechanism of membrane fusion underlying AMPA receptor recycling is somewhat divergent from that utilized by the usual 20S-mediated pathway. While both NSF and eSNAP can be copurified with the AMPA receptor, SNAREs do not appear to be present in the same complex (47). Such an alternative SNARE-independent mechanism may only be employed for the trafficking of membrane proteins where rapidity of recycling is crucial for their correct function as is the case for both the AMPA receptor (50–52) and the β2AR (3, 11, 12).

We have described here the identification of NSF as a β2AR-interacting protein and present evidence for a critical role of this interaction in the recycling of the receptor. Thus, NSF represents a new member of a class of proteins whose binding to receptors determines their fate after agonist-induced internalization.

Acknowledgments—We thank Dr. Richard T. Premont and Dr. Randy Hall for helpful discussions, Millie MacAdams and Judy Phelps for DNA sequencing, and Donna Addison, Mary Holben, and Julie Turnbough for assistance with the preparation of this manuscript.

REFERENCES
1. Freedman, N. J., and Lefkowitz, R. J. (1996) Recent Prog. Horm. Res. 51, 319–353
2. Pitcher, J. A., Freedman, N. J., and Lefkowitz, R. J. (1998) Annu. Rev. Biochem. 67, 653–692
3. Pippig, S., Andezerling, S., and Lohse, M. J. (1995) Mol. Pharmacol. 47, 666–676
4. Krueger, K. M., Daaka, Y., Pitcher, J. A., and Lefkowitz, R. J. (1997) J. Biol. Chem. 272, 5–8
5. Hauforder, W. P., Caron, M. G., and Lefkowitz, R. J. (1999) FASEB J. 13, 2881–2889
6. Koenig, J. A., and Edwardsen, J. M. (1997) Trends Pharmacol. Sci. 18, 266–277
7. Tsao, P., Cao, T., and von Zastrow, M. (2001) Trends Pharmacol. Sci. 22, 91–96
8. Futter, C. E., Pearse, A., Hewlett, L. J., and Hopkins, C. R. (1996) J. Cell Biol. 132, 1011–1023
9. Moore, B. R., Tuffaha, A., Millman, E. E., Dorn, H. S., Dickey, B. F., and Knoll, B. J. (1999) J. Cell Sci. 112, 329–338
10. Zhang, J., Barak, L. S., Winkler, K. E., Caron, M. G., and Ferguson, S. S. (1997) J. Biol. Chem. 272, 27742–27747
11. Morrison, K. J., Moore, R. H., Carsrud, N. D., Trial, J., Millman, E. E., Tuvim, M., Clark, R. B., Barber, R., Dickey, B. F., and Knoll, B. J. (1996) Mol. Pharmacol. 50, 692–699
12. Hertel, C., and Staehelin, L. (1983) J. Cell Biol. 97, 1538–1543
13. Garland, A. M., Grady, E. F., Lovett, M., Vigna, S. R., Frucht, M. M., Krause, J. E., and Bunnett, N. W. (1996) Mol. Pharmacol. 49, 438–446
14. Fonseca, M. L., Buitten, D. C., and Brown, R. D. (1995) J. Biol. Chem. 270, 8902–8909
15. Innamorati, G., Sadeghi, H. M., Tran, N. T., and Birnbaumer, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2222–2226
16. Oakley, R. H., Laporte, S. A., Holt, J. A., Barak, L. S., and Caron, M. G. (1999) J. Biol. Chem. 274, 32248–32257
17. Innamorati, G., Le Gouill, C., Balamotis, M., and Birnbaumer, M. (2001) J. Biol. Chem. 276, 13996–13103
18. Tsao, P. I., and von Zastrow, M. (2000) J. Biol. Chem. 275, 11130–11140
19. Hoxie, J. A., Ahuja, M., Belmonte, E., Pizarro, S., Parton, R., and Brass, L. F. (1998) J. Biol. Chem. 273, 2216–2221
20. Trejo, J., and Coughlin, S. R. (1999) J. Biol. Chem. 274, 2216–2224
21. Ho, L. A., Tang, Y., Miller, W. E., Cong, M., Lai, A. G., Lefkowitz, R. J., and Hall, R. A. (2000) J. Biol. Chem. 275, 38659–38666
22. Fraser, I. D., Cong, M., Kim, J., Rollins, E. N., Daaka, Y., Lefkowitz, R. J., and Scott, J. D. (2000) Curr. Biol. 10, 409–412
23. Hall, R. A., Premont, R. T., and Lefkowitz, R. J. (1999) J. Biol. Chem. 144, 31–43
24. Trejo, J., and Coughlin, S. R. (1999) J. Biol. Chem. 274, 2216–2224
25. Cao, T. T., Deacon, H. W., Reczek, D., Bretscher, A., and von Zastrow, M. (1999) J. Biol. Chem. 274, 9896–9892
26. Tsao, P., Cao, T., and von Zastrow, M. (2001) J. Biol. Chem. 276, 932–937
27. Novick, P., Ferro, S., and Schekman, R. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 461–467
28. Lin, R. C., and Scheller, R. H. (1996) Annu. Rev. Cell Dev. Biol. 16, 19–49
29. Brugger, A. T. (2000) Curr. Opin. Neurobiol. 10, 293–302
30. Neuwald, A. F. (1999) Structure 7, 19–23
31. McDonald, P. H., Cote, N. L., Lin, P. T., Premont, R. T., Pitcher, J. A., and
37. Luttrell, L. M., Ferguson, S. S., Daaka, Y., Miller, W. E., Maudsley, S., Della Rocca, G. J., Lin, F., Kawakatsu, H., Owada, K., Luttrell, D. K., Caron, M. G., and Lefkowitz, R. J. (1999) Science 283, 655–661
38. Laporte, S. A., Oakley, R. H., Zhang, J., Holt, J. A., Ferguson, S. S., Caron, M. G., and Barak, L. S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3712–3717
39. Goodman, O. B., Jr., Krupnick, J. G., Santini, F., Gurevich, V. V., Penn, R. B., Gagnon, A. W., Keen, J. H., and Benovic, J. L. (1996) Nature 383, 447–450
40. McDonald, P. H., Chow, C. W., Miller, W. E., Laporte, S. A., Field, M. E., Lin, F. T., Davis, R. J., and Lefkowitz, R. J. (2000) Science 290, 1574–1577
41. Pierce, K. L., Luttrell, L. M., and Lefkowitz, R. J. (2001) Oncogene 20, 1532–1539
42. Luttrell, L. M., Roudabush, F. L., Choy, E. W., Miller, W. E., Field, M. E., Pierce, K. L., and Lefkowitz, R. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 2449–2454
43. Miller, W. E., and Lefkowitz, R. J. (2001) Curr. Opin. Cell Biol. 13, 139–145
44. Kishi, M., Liu, X., Hirakawa, T., Reetz, D., Bretscher, A., and Ascoli, M. (2001) Mol. Endocrinol. 15, 1624–1635
45. Nishimune, A., Isaac, J. T., Molnar, E., Noel, J., Nash, S. R., Tagaya, M., Collingridge, G. L., Nakanishi, S., and Henley, J. M. (1998) Neuron 21, 87–97
46. Song, I., Kamboj, S., Xia, J., Dong, H., Liao, D., and Huganir, R. L. (1998) Neuron 21, 393–400
47. Osten, P., Srivastava, S., Inman, G. J., Vilim, F. S., Khatri, L., Lee, L. M., States, B. A., Einheber, S., Milner, T. A., Hanso, P. I., and Ziff, E. B. (1998) Neuron 21, 99–110
48. Noel, J., Ralph, G. S., Pickard, L., Williams, J., Molnar, E., Uney, J. B., Collingridge, G. L., and Henley, J. M. (1999) Neuron 23, 365–376
49. Luscher, C., Xia, H., Beattie, E. C., Carroll, R. C., von Zastrow, M., Malenka, R. C., and Nicoll, R. A. (1999) Neuron 24, 649–658
50. Liao, D., Hessler, N. A., and Malinow, R. (1995) Nature 375, 400–404
51. Isaac, J. T., Nicoll, R. A., and Malenka, R. C. (1995) Neuron 15, 427–434
52. Benke, T. A., Luthi, A., Isaac, J. T., and Collingridge, G. L. (1998) Nature 393, 793–797
Binding of the β2 Adrenergic Receptor to N-Ethylmaleimide-sensitive Factor Regulates Receptor Recycling
Mei Cong, Stephen J. Perry, Liaoyuan A. Hu, Phyllis I. Hanson, Audrey Claing and Robert J. Lefkowitz

J. Biol. Chem. 2001, 276:45145-45152.
doi: 10.1074/jbc.M106087200 originally published online September 27, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106087200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 28 of which can be accessed free at http://www.jbc.org/content/276/48/45145.full.html#ref-list-1