Clathrin-mediated Endocytosis of the β-Adrenergic Receptor Is Regulated by Phosphorylation/Dephosphorylation of β-Arrestin1*

Fang-Tsyr Lin, Kathleen M. Krueger, Humphrey E. Kendall, Yehia Daaka, Zoey L. Fredericks, Julie A. Pitcher, and Robert J. Lefkowitz‡

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

β-Arrestins serve a dual regulatory role in the life cycle of G protein-coupled receptors such as the β2-adrenergic receptor. First, they mediate rapid desensitization by binding to G protein-coupled receptor kinase-phosphorylated receptors. Second, they target the receptors for internalization into endosomal vesicles, wherein receptor dephosphorylation and resensitization occur. Here we report that phosphorylation of a carboxy-terminal serine (Ser-412) in β-arrestin1 regulates its endocytotic but not its desensitization function. Cytoplasmic β-arrestin1 is constitutively phosphorylated and is recruited to the plasma membrane by agonist stimulation of the receptors. At the plasma membrane, β-arrestin1 is rapidly dephosphorylated, a process that is required for its clathrin binding and receptor endocytosis but not for its receptor binding and desensitization. Once internalized, β-arrestin1 is rephosphorylated. Thus, as with the classical endocytic adaptor protein complex AP2, β-arrestin1 functions as a clathrin adaptor in receptor endocytosis which is regulated by dephosphorylation at the plasma membrane.

Endocytosis of many cell-surface receptors including those for epidermal growth factor, insulin, and transferrin is mediated by classical clathrin-coated vesicle mechanisms (1). G protein-coupled receptors such as the β2-adrenergic receptor, M1 muscarinic cholinergic receptor, LH/HCG receptor, gastrin releasing peptide receptor, and others also utilize this pathway (2–5). In the case of tyrosine kinase receptors, endocytosis involves clustering of the receptors in coated pits formed by the recruitment and assembly of clathrin and associated molecules such as the AP2 complex and dynamin on the plasma membrane (6, 7). The heterotetrameric AP2 complex is a structural component of clathrin-coated pits on the plasma membrane that triggers the assembly of clathrin cages (8–10). It serves as an adaptor linking receptors to the structure of clathrin cages.

In the case of G protein-coupled β2-adrenergic receptors, recent in vitro evidence has suggested that β-arrestins may play a role in linking the receptors to clathrin-coated pits (11–13). β-Arrestins were originally discovered in the context of homologous or agonist-specific desensitization of β2-adrenergic receptors (14, 15). Following phosphorylation of the agonist-occupied receptors by β-adrenergic receptor kinase, β-arrestins bind to the receptors, thereby interdicting signal transduction to heterotrimeric G proteins (16). The arrestin family includes visual arrestin, β-arrestin1 (arrestin2), β-arrestin2 (arrestin3), and other splicing variants (17). Arrestin functions specifically in inactivation of rhodopsin (18, 19), whereas β-arrestin1 and β-arrestin2 exhibit similar functions in desensitization of non-visual G protein-coupled receptors (15).

When β-arrestin1 or -2 are overexpressed in cells, not only is desensitization of β2-adrenergic receptors augmented, but their sequestration or internalization is promoted as well (20). Moreover, a “dominant negative” mutant of β-arrestin1 (V53D) impairs receptor endocytosis (20). β-Arrestin1 and -2 have been shown in vitro to bind with high affinity to clathrin cages, whereas visual arrestin does not, and β-arrestin/arrestin chimeras defective in either receptor or clathrin binding do not support agonist-dependent internalization of the β2-adrenergic receptor (11–13).

Despite these data suggesting a role for β-arrestin1 in clathrin-mediated β2-adrenergic receptor internalization, its role as an adaptor linking the receptors to clathrin cages has not been demonstrated in cells. Nor is anything known of how the putative association of β-arrestins and clathrin-coated pits on the plasma membrane might be regulated. Here we demonstrate that agonist-promoted recruitment and dephosphorylation of β-arrestin1 on the plasma membrane transforms it into a clathrin adaptor and thus controls the process of receptor endocytosis.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Site-specific Mutagenesis—A 1.26-kb EcoRV fragment containing the entire β-arrestin1 coding sequences was digested from pVL1393/arr1 and subcloned into the SmalI site of pKK223-3, a bacterial expression vector (Pharmacia Biotech Inc.). Then an oligonucleotide 5′-CATCACCATCACCATCAC-3′, encoding six histidine residues, was engineered at the end of the carboxy-terminal coding sequences by the polymerase chain reaction (PCR) and designated pKK/arr1-6xHis. This PCR product was verified by DNA sequencing. To express His-tagged β-arrestin1 in mammalian cells, a 0.5-kb 5′ EcoRI fragment of β-arrestin1 from pCMV5/arr1 (15) was ligated to a 0.8-kb 3′ EcoRI/SalI fragment of β-arrestin1–6xHis from pKK/arr1–6xHis and was then inserted into the EcoRI/SalI sites of pCMV5 to create pCMV5/arr1–6xHis.

The β-arrestin1–6xHis coding sequences, removed from pKK/arr1–6xHis by KpnI and HindIII digestions, were subcloned into pBlueScript KS−1. This plasmid was then linearized at the XhoI or XbaI site of the polylinkers as a template for mutagenesis by recombination PCR (21). The nucleotides TCT, encoding serine at amino acid 412, were replaced with GCT or GAT which mutate the serine to alanine or aspartic acid. The PCR

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† To whom correspondence should be addressed: Howard Hughes Medical Institute, Deps. of Medicine and Biochemistry, Duke University Medical Center, Box 3821, Durham, NC 27710. Tel.: 919-684-2974; Fax: 919-684-8875.

1 The abbreviations used are: kb, kilobase pair; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; β2-AR, β2-adrenergic receptors; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride.

2 H. Attredamadal and R. J. Lefkowitz, unpublished data.
products were verified by DNA sequencing. To express mutant β-arrestin1, the 0.3-kb 3' SnaI fragment of pCMV5/arr1–6xHis was replaced with a corresponding fragment containing the mutated site and designated pCMV5/S412A/arr1–6xHis or pCMV5/S412D/arr1–6xHis.

**Transfection for Metabolic Labeling**—The plasmids of interest were transfected into HEK 293 cells by calcium phosphate precipitate. Stable cell lines were generated by co-transfecting empty vector or different β-arrestin1–6xHis expression plasmids with PhevNeo at a 10:1 ratio and were then selected with 400 μg/ml G418 for 2–3 weeks. Overexpression of wild-type or mutant β-arrestin1 was determined by Western blot analysis using an antibody specific to β-arrestin1 (15) and was visualized by enhanced chemiluminescence assay (ECL, Amer sham Corp.).

For metabolic labeling, HEK 293 cells stably transfected with pCMV5/arr1–6xHis were starved in phosphate-free Dulbecco's modified Eagle's medium (Life Technologies, Inc.) for 30 min, labeled for 2 h in the same medium containing [32P]orthophosphate (1 μCi/ml), and harvested for β-arrestin1 purification. The stoichiometry of [32P]-labeled β-arrestin1 was determined as described (22).

**Subcellular Fractionation**—Cells incubated with or without 10 μM (-)-isoproterenol were washed with ice-cold phosphate-buffered saline (PBS), incubated with 250 μg/ml concanavalin A in PBS on ice for 20 min, incubated with 0.25 M sucrose, 10 mM Tris, pH 7.4, 1 mM EDTA, and disrupted by Dounce homogenization. Differential centrifugation was carried out as described (23). Nuclei and unbroken cells were removed by centrifugation at 1000 × g for 10 min. A crude plasma membrane fraction was precipitated by centrifugation of the supernatant at 3000 × g for 15 min. Then centrifugation of the resulting supernatant at 300,000 × g for 30 min gave rise to a pellet composed of the vesicles and other subcellular organelles as well as a supernatant containing cytosol. The cytosol was dialyzed in 1 × binding buffer (20 mM Tris, pH 7.9, 5 mM imidazole, and 0.5 mM NaCl) containing 50 mM NaF, 10 mM sodium pyrophosphate, and a mixture of protease inhibitors for β-arrestin1 purification.

**Purification of β-arrestin1 by Nickel Affinity and Heparin-Sepharose Chromatography**—The cell pellets were lysed in 1 × binding buffer containing a mixture of protease inhibitors and 0.2% Nonidet P-40. To purify phospho-β-arrestin1, 50 mM NaF and 10 mM sodium pyrophosphate were added to inhibit phosphatase activity. β-Arrestin1 was purified by nickel affinity chromatography following the manufacturer's protocol (Novagen) except that following washing with 20 mM Tris, pH 7.9, containing 30 mM imidazole and 0.5 mM NaCl, it was eluted with 100 mM sodium imidazole in the same buffer.

To purify β-arrestin1 to the highest homogeneity, the 100 mM imidazole buffer was replaced with a stepwise gradient in 4, 5, and 6 M urea in 20 mM Tris, pH 7.9, containing 0.2 M NaCl and applied to heparin-Sepharose as described (24). β-Arrestin1 was purified with a linear gradient of NaCl from 0.2 to 1 M (a regular procedure) or directly eluted with 1 M NaCl (as shown in Fig. 3) and was desalted in 20 mM Tris, pH 7.4, and 1 mM EDTA. All fractions were analyzed by SDS-PAGE. The purity of β-arrestin1 was determined by either Coomassie Blue staining or silver staining (Bio-Rad) of the gel.

**Phosphorylated Peptides by Reverse-phase HPLC—Phosphorylated β-arrestin1 was purified by nickel affinity chromatography, electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon PVDF, Millipore), and then eluted (25). Proteins were hydrolyzed in 6 N HCl for 1 h at 110 °C. The hydrolysates were lyophilized, combined with phosphoamino acid standards, and fractionated by one-dimensional thin-layer electrophoresis as described (26). Phosphoamino acid standards were stained with ninhydrin, and radioiodinated phosphoamino acids were detected by autoradiography.

Proteins transferred to PVDF membranes were digested in situ with modified sequencing-grade trypsin (Boehringer Mannheim) and purified by reverse-phase HPLC as described (27). Radioiodinated peptides were collected for amino acid sequencing using an Applied Biosystems model 477A protein sequencer with an in-line 120A PTH-analyzer (Protein Chemistry Core Facility, Baylor College of Medicine).

**Receptor Binding and Desensitization Assays—**HEK 293 cells were transiently co-transfected with pDNA1/FLAG-β2-AR (28) and either empty vector (mock) or plasmid encoding β-arrestin1 (wild-type or β-arrestin1 mutated at Ser-412) (29). Two days after transfection, cells were incubated with [3H]isoproterenol to define nonspecific binding. The agonist-promoted receptor sequestration was determined as the percentage of radiolabeled 2-AR endocytosis, we established a line of human embryonic kidney (HEK) 293 cells stably overexpressing β-arrestin1, tagged at its carboxyl terminus with hexahistidine. Purification of the β-arrestin1 to >95% homogeneity could be achieved by a two-step procedure as follows: a first step on a nickel-chelating column, followed by a heparin-Sepharose column (Fig. 1, A and B).

To determine if cellular β-arrestin1 is a phosphoprotein, HEK 293 cells overexpressing β-arrestin1 were metabolically labeled with [32P]P, and then β-arrestin1 was purified from whole cell extracts by nickel affinity chromatography and was subjected to SDS-PAGE. As shown in Fig. 2, the 50-kDa β-arrestin1, which was not present in the mock transfected, was clearly phosphorylated. The stoichiometry of phospho-β-arrestin1 from whole cell extracts was ~0.84 mol of 32P/mol of protein, suggesting a potential physiological relevance of this modification.

These results are consistent with our previous observations that the immunoprecipitated native β-arrestin1 from overexpressing COS-7 cells is a phosphoprotein. Moreover, we have determined that the function of the hexahistidine-tagged β-arrestin1 is equivalent to that of native β-arrestin1 as assayed for 10 min. The expression levels of wild-type or mutant β-arrestin1 were determined by Western blot analysis using 5% of the lysates from a 10-cm plate. For the rest of the samples, the FLAG-tagged β-adrenergic receptor was immunoprecipitated with the M2 antibody directed against the FLAG epitope (Rakdal). Then the immunoprecipitates were subjected to SDS-PAGE, and Western blot analysis was performed using the antibody specific to β-arrestin1.

For desensitization assays, the cells described above were incubated in serum-free medium either alone (control cells) or in the presence of 10 μM (-)-isoproterenol for 5 min at 37 °C (desensitized cells). After washing with cold PBS on ice, cells were scraped in lysis buffer (10 mM Tris, pH 7.5, 5 mM EDTA, 1 μg/ml aprotinin, and a mixture of protease inhibitors), homogenized, and centrifuged at 500 × g for 10 min to pellet nuclei and debris. Crude membranes were isolated by centrifugation of the supernatant at 40,000 × g for 30 min and resuspended in 75 mM Tris, pH 7.5, 2 mM EDTA, 15 mM MgCl2, and protease inhibitors. Cyclase assays were performed as described (30). Samples containing 10 μg of membrane protein, 30 mM Tris, pH 7.5, 0.8 mM EDTA, 6 mM MgCl2, 120 μM [3H]ATP (50 μCi/ml), 100 μM cAMP, 55 μM GTP, 2.7 mM phosphoenolpyruvate, 20 units/ml myokinase, 4 units/ml pyruvate kinase, and either 10 μM forskolin or 10 μM (-)-isoproterenol were incubated at 37 °C for 25 min. Assays were terminated by placing the samples on ice and adding 1 ml of 0.3 mM ATP and 0.3 mM [14C]cAMP.

The amount of cAMP generated was determined (31).

**RESULTS AND DISCUSSION**

**Constitutive Phosphorylation of β-Arrestin1 and Clathrin—**One 150-mm plate of transfected HEK 293 cells transfected with pCMV5-empty vector or β-arrestin1 (wild-type, S412A, or S412D) were homogenized and subjected to subcellular fractionation. After removing nuclei and unbroken cells by centrifugation at 1000 × g for 10 min, all of the membrane fractions were pelleted by centrifugation of the supernatant at 300,000 × g for 30 min and dissolved in lysis buffer (same as that used in receptor binding described above). β-Arrestin1 was immunoprecipitated using 25 μg of antibody directed against β-arrestin1 (15), and the proteins were resolved by SDS-PAGE. Western blot analysis was performed using a monoclonal antibody specific to the heavy chain of clathrin (ICN).
by the binding to β2-adrenergic receptors and the promotion of receptor desensitization and internalization (see below). Accordingly, since it is much easier to purify, the tagged β-arrestin1 was used for all subsequent studies.

**Agonist Stimulation Promotes Dephosphorylation of β-arrestin1**—To study the potential effects of agonist stimulation on the phosphorylation status of β-arrestin1, HEK 293 cells stably expressing β-arrestin1 were labeled with [32P]orthophosphate for 90 min, the cells were incubated with or without 10 μM (-)-isoproterenol for 5 min and subjected to subcellular fractionation. Lysates devoid of nuclei and unbroken cells were sequentially centrifuged at 3000 × g and 300,000 × g to obtain two pellets (P) and the supernatant (S), i.e., plasma membrane (PM), crude vesicles (CV), and the cytosol, respectively. Lysates from each fraction were subjected to the two-step purification as described under “Experimental Procedures.” Similar amounts of purified β-arrestin1 were fractionated by SDS-PAGE, transferred to nitrocellulose membranes, and detected by autoradiography as shown in the upper panel. The same blot was then subjected to Western blot analysis using the antibody specific to β-arrestin1 as shown in the lower panel.

If the plasma membrane is the site of β-arrestin1 dephosphorylation, then an obvious explanation for the agonist-promoted decrease of β-arrestin1 phosphorylation in crude vesicles is an agonist-promoted transit of dephosphorylated β-arrestin1 from plasma membrane to the internalized vesicles. This seemed a reasonable hypothesis since isoproterenol stimulates the association of β-arrestins with plasma membrane β2-adrenergic receptors, as judged by receptor desensitization as well as receptor translocation from plasma membrane to a vesicular fraction. To confirm this notion, we treated cells with isoproterenol for 5 min, isolated plasma membrane and vesicle fractions, and assessed their β-arrestin1 content by Western blot. As shown in Fig. 4, both fractions showed an agonist-promoted increase in β-arrestin1. Taken together, these data suggest that agonist stimulation of β2-adrenergic receptors promotes translocation of β-arrestin1 to the plasma membrane, which is the site of its dephosphorylation.
2-Adrenergic Receptors Are Not Affected by Ser-412 Mutation—To investigate how phosphorylation regulates the activities of β-arrestin1 within the cell, we utilized two mutant β-arrestin1 expression vectors where Ser-412 was replaced with Ala or Asp. The S412A mutant should simulate the unphosphorylated form of β-arrestin1, whereas S412D would be predicted to mimic its phosphorylated form. We then set out to investigate the biological properties of the wild-type and mutant proteins expressed in 293 cells. As shown in Fig. 7A (upper panel), the three proteins were expressed at similar levels.

We first investigated the ability of each β-arrestin1 to interact with the agonist-occupied receptors. Cells expressing β2-adrenergic receptor and either wild-type or mutant β-arrestin1 were treated with isoproterenol for 5 min and subjected to reversible cross-linking with Dithiobis(succinimidyl propionate). The receptors were immunoprecipitated with an antibody directed at the FLAG epitope (see “Experimental Procedures”). After SDS-PAGE, co-immunoprecipitating β-arrestin1 was visualized by immunoblot. As shown in Fig. 7A (lower panel), isoproterenol treatment of cells strikingly increased β-arrestin1/receptor interaction. However, neither mutation of Ser-412 altered receptor/β-arrestin1 interaction. The failure of these mutations to alter receptor binding of β-arrestin1 is consistent with previous observations indicating that the carboxyl domain of β-arrestins is not involved in this interaction (33).

We next examined the ability of different β-arrestin1 proteins to desensitize the receptors. Previously it has been demonstrated that overexpression of β-arrestin1 enhances desensitization of G protein-coupled receptors (16). As shown in Fig. 7B, when expressed at equal levels, wild-type β-arrestin1 and its two mutants, S412A and S412D, equally augmented desensitization of the β2-adrenergic receptor. In view of the apparently unchanged receptor-binding ability of the two mutants (Fig. 7A), this finding is not surprising.

Ser-412 Phosphorylation Regulates Agonist-promoted Internalization of β2-Adrenergic Receptors—As noted earlier both β-arrestins appear to be bifunctional molecules, binding to and desensitizing receptors on the one hand and targeting them for internalization on the other (20). Accordingly, we next assessed the effects of phosphorylation of β-arrestin1 on its ability to promote receptor sequestration. These data are shown in Fig. 8. Overexpression of wild-type β-arrestin1 significantly in-
increased β2-adrenergic receptor sequestration, causing a doubling in the percentage of surface receptors internalized after a 30-min incubation with isoproterenol. Interestingly, the S412A mutant, which cannot be phosphorylated, enhanced receptor internalization to an even greater extent. In contrast, the S412D mutant, which would be predicted to mimic the phosphorylated form of β-arrestin1, failed to increase receptor sequestration and in fact acted as a dominant negative mutant and significantly reduced it. These data are consistent with the notion that dephosphorylation of β-arrestin1 is required for internalization of the β2-adrenergic receptor.

Ser-412 Phosphorylation Regulates the Ability of β-Arrestin1 to Serve as a Clathrin Adapter—Previously published data (11) have indicated that β-arrestins bind to clathrin cages in vitro and that the carboxyl-terminal 77 amino acid residues of β-arrestins are important for this interaction. Alanine scanning mutagenesis further localized the clathrin-binding domain to residues 371–379 of β-arrestin2 (12). It was also shown, in intact cells, that β-arrestin1 and clathrin co-localize by immunofluorescent microscopy after isoproterenol stimulation. Since Ser-412 is located within the carboxyl domain of β-arrestin1 previously shown to be involved in its in vitro binding to clathrin cages, we sought to investigate the potential regulatory role of Ser-412 phosphorylation in determining the ability of β-arrestin1 to serve as a clathrin adapter in intact cells.

A 300,000 × g particulate fraction from HEK 293 cells stably expressing wild-type or mutant β-arrestin1 at equivalent levels (Fig. 9, upper panel) was subjected to immunoprecipitation with anti-β-arrestin1 antibodies. After SDS-PAGE, the immunoprecipitates were blotted for clathrin heavy chain (Fig. 9, lower panel). Clathrin was detected only in the immunoprecipitated complex of S412A β-arrestin1. The failure to detect specific clathrin binding to the wild-type β-arrestin1 likely reflects the fact that it is already highly phosphorylated. S412D β-arrestin1, which mimics phosphorylated β-arrestin1, also did not co-immunoprecipitate with clathrin heavy chain.

A Model for β-Arrestin1 Function—Our data suggest the following model for the regulation of β2-adrenergic receptor desensitization and internalization by β-arrestin1. Prior to agonist stimulation, the bulk of β-arrestin1 is cytosolic and is a phosphoprotein. The phosphate is present almost exclusively on Ser-412. The nature of the kinase(s) that is responsible for this phosphorylation is currently unknown. Following agonist stimulation, β-arrestin1 is translocated to the plasma membrane where it binds tightly to agonist-occupied receptors that have been phosphorylated by a G protein-coupled receptor ki-
nase such as β-adrenergic receptor kinase. In association with its movement to the plasma membrane, β-arrestin1 is dephosphorylated by an as yet unknown phosphatase.

It is not presently clear whether β-arrestin1 dephosphorylation precedes or follows its receptor binding. Even in the absence of receptor stimulation, plasma-membrane β-arrestin1 is dephosphorylated (Fig. 3). It is known that β-arrestin1 can bind to β2-adrenergic receptors even in the absence of agonist, albeit with lower affinity (Fig. 7A). Moreover, the concentrations of overexpressed β2-adrenergic receptors in our experiments are likely high enough to promote just such agonist-independent binding of β-arrestin1 to the receptors. On the other hand, as shown in Fig. 7A, phosphorylation of Ser-412 does not seem to regulate receptor binding of β-arrestin1. Thus, it is not necessary for the dephosphorylation to precede β-arrestin1 binding to the receptors. It seems plausible that such receptor binding of β-arrestin1 positions it in proximity to the relevant phosphatase or alters its conformation such that it becomes a substrate for the phosphatase. Since receptor binding of β-arrestin1 does not appear to require its dephosphorylation, it is not surprising that receptor desensitization is also unaffected by the phosphorylation status of β-arrestin1 (Fig. 7B).

Once β-arrestin1 has bound to the β2-adrenergic receptor and the receptors have become functionally uncoupled from G proteins, they move to clathrin-coated pits and become internalized. As shown here, the dephosphorylated form of proteins, they move to clathrin-coated pits and become inter-

FIG. 9. Phosphorylation of β-arrestin1 at Ser-412 regulates its interaction with clathrin. One 150-mm plate of HEK 293 cells stably transfected with pCMV5 vector (mock) or one of the β-arrestin1 expression plasmids (wild-type, S412A, and S412D) was subjected to subcellular fractionation. After removing nuclei and broken cells, all of the membrane fractions were pelleted by centrifugation at 300,000 × g and dissolved in lysis buffer (see “Experimental Procedures”). Five percent of total proteins were fractionated by SDS-PAGE and subjected to Western blot analysis using an antibody specific to β-arrestin1, which is shown in the upper panel. From the rest of the samples, equal amounts of proteins were immunoprecipitated with anti-β-arrestin1 antibody, fractionated by SDS-PAGE, and subjected to Western blot analysis using an antibody specific to the heavy chain (HC) of clathrin as shown in the lower panel.

Why should β-arrestin1 dephosphorylation be necessary for its clathrin interaction? Previous in vitro studies have revealed that the carboxyl terminus of β-arrestin1 interacts with clathrin (11). Presumably dephosphorylation of the very carboxyl-terminal Ser-412 alters the conformation and/or charge of this region of the β-arrestin1 molecule so that the acidic carboxyl terminus can bind to clathrin cages. It is of further interest that Ser-412 is not present in the other members of the arrestin family. Thus, they must be regulated either by phosphorylation at other sites or by totally different mechanisms.

Analogies between β-Arrestin1 and the AP2 Complex—β-Arrestin1 seems to function as an adaptor linking activated β2-adrenergic receptors, and presumably other G protein-coupled receptors, to clathrin cages. Recently, comparisons have been made between the clathrin adaptor functions of β-arrestins and the AP2 complex (13). A number of distinctions were drawn between these two molecules. First, the AP2 complex is a large multi-subunit entity containing four distinct proteins, whereas β-arrestins are monomeric. Second, AP2 was shown to bind to clathrin at two distinct sites, whereas β-arrestins bind a single site (13, 36, 37). Finally, unlike the AP2 complex, which supports clathrin-coat assembly in vitro, β-arrestins do not. However, it should be pointed out that β-arrestins might recruit additional as yet unrecognized proteins into their complexes with clathrin.

However, there are clear analogies between β-arrestins and AP2 as well. These include the fact that both β-arrestins and AP2 bind to clathrin and to the receptors. In the case of the AP2 complex, these receptors include the low density lipoprotein receptor, epidermal growth factor receptor, asialoglycoprotein receptor, and mannose 6-phosphate receptor (38–41). Another analogy, revealed by our study, is that only dephosphorylated β-arrestin1 can bind to clathrin, just as in vitro clathrin binding assays have shown that only dephosphorylated AP2 can bind to clathrin (42). In addition, the β2 subunit of the cytosolic AP2 complex is phosphorylated on serine residues, whereas membrane-bound AP2 is unphosphorylated (42). These data suggest that adaptor phosphorylation may generally regulate adaptor-clathrin interaction on the plasma membrane. This mechanism would provide a potential means for bringing such endocytic processes under the control of receptor-mediated signaling pathways.

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