β-Arrestin1 and β-arrestin2 play a key role in the regulation of G protein-coupled receptor-mediated signaling, whereas the subcellular distribution of β-arrestin1 and β-arrestin2 has been shown to be quite different. In this study, we found that although both β-arrestin1 and β-arrestin2 are able to interact with ubiquitin-protein isopeptide ligase (E3) Mdm2, only expression of β-arrestin2 leads to the relocalization of Mdm2 from the nucleus to the cytoplasm. Further study reveals that β-arrestin2 but not β-arrestin1 shuttles between the cytoplasm and nucleus in a leptomycin B-sensitive manner. A hydrophobic amino acid-rich region (VAXXFX-XXLX) at the C terminus of β-arrestin2 was further demonstrated to serve as a nuclear export signal responsible for the extranuclear localization of β-arrestin2. In the corresponding region of β-arrestin1, there is a single amino acid difference (Glu instead of Leu in β-arrestin2), and mutation of Glu to Leu conferred to β-arrestin1 similar subcellular distribution to that of β-arrestin2. Moreover, data from a series of deletion mutations demonstrated that the N domain (residues 1–185) was indispensable for the nuclear localization of both β-arrestins, and the results from a Val to Asp point mutation in the N domain also supported this notion. In addition, our data showed that nucleocytoplasmic shuttling of β-arrestin2 was required, via protein/protein interaction, for the cytoplasmic relocalization of Mdm2 and JNK3, another well known β-arrestin2-binding protein. Our study thus suggests that both the nuclear export signal motif and the N domain of β-arrestins are critical for the regulation of their subcellular localization and that β-arrestin2 may modulate the function of its binding partners such as Mdm2 and JNK3 by alteration of their subcellular distribution.

β-arrestins, mediate many physiological functions. Agonist binding to GPCRs results in both the initiation of cellular signaling and the receptor phosphorylation by G protein-coupled receptor kinases. Arrestins were originally discovered to bind to the phosphorylated GPCRs and function in regulation of the desensitization and internalization of these receptors (1, 2). To date, at least four members of the arrestin gene family have been identified: visual arrestin, cone arrestin, and two β-arrestins (β-arrestin1 and β-arrestin2). Visual arrestin and cone arrestin have specialized functions due to their limited localization to the retina, whereas β-arrestins are ubiquitously expressed in various tissues (3–6). β-Arrestin1 and β-arrestin2 are two highly homologous proteins (sharing 78% identity in amino acid composition), and both of them rapidly translocate from the cytoplasm to cell plasma membrane when GPCRs are stimulated. Binding of either β-arrestin to the activated GPCRs can disassociate G proteins from the receptors and thus quench the receptor signaling. In addition, both β-arrestins effectively regulate the internalization of many GPCRs by direct interaction with AP2 and clathrin (7, 8).

Although β-arrestin1 and β-arrestin2 perform similar functions in the regulation of GPCR signaling, some differences between them have been reported such as in their binding affinity to different classes of GPCRs (9) and in their association with different binding partners (10). One of the well-established differences is their subcellular localization. It is reported that when expressed in HEK293 and HeLa cells, β-arrestin1 is localized in the cytoplasm and nucleus, but β-arrestin2 is predominantly distributed in the cytoplasm (9, 11). In addition, a very recent study (11) shows that two β-arrestins shuttle differentially between the nucleus and cytoplasm due to the presence of a two-leucine nuclear export signal (NES) in β-arrestin2 that is absent in β-arrestin1. However, the molecular determinant of their nuclear import remains to be further investigated.

Novel functions of β-arrestins, such as regulation of the cytoskeletal reorganization via association with Ral guanine nucleotide dissociation stimulator (Ral-GDS) (12) and enhancement of the CXCR4-mediated chemotaxis (13), have been reported recently. One of the most exciting findings is that β-arrestins can serve as a scaffold protein to regulate the functions of ERK and JNK3 cascades by directly interacting with these kinases. For example, β-arrestin2 can simultaneously associate with ERK and its upstream Raf or JNK3 and its upstream ASK1 and thus effectively promotes GPCR-mediated activation of these kinases. Moreover, the interaction of β-arrestin2 with signal-regulated kinase; DAPI, 4’,6-diamidino-2-phenylindole; HA, hemagglutinin; GFP, green fluorescent protein; EGFP, enhanced GFP; βarr1, β-arrestin1; βarr2, β-arrestin2; HEK, human embryonic kidney; MAPKK, mitogen-activated protein kinase kinase.
its binding partners such as ERKs and JNK3 can lead to the relocation of the kinases in the cytoplasm (14). Consequently, the cytoplasmic retention of ERKs inhibits activation of the transcription factor Elk (15). Recently, Mdm2, a well known ubiquitin-protein isopeptide ligase (E3) for p53, has been shown to regulate the trafficking of β 2-adrenergic receptor via its direct interaction with β-arrestin2 (16). However, whether the interaction of Mdm2/β-arrestin2 affects the subcellular distribution of Mdm2 remains unclear.

**EXPERIMENTAL PROCEDURES**

**Materials**—Leptomycin B (LMB) and DAPI were obtained from Sigma. Mouse anti-hemagglutinin (HA) monoclonal antibody (12CA5), recognizing influenza HA epitope, was purchased from Roche Molecular Biochemicals. Texas Red-conjugated goat anti-mouse IgG secondary antibody was purchased from Molecular Probes. Protein-A-Sepharose was from Amersham Biosciences. The anti-β-arrestin polyclonal antibody was produced in our lab as described (18). **Plasmid Constructs**—Construction of β-arrestin1 and β-arrestin2 expression vectors was described previously (18). pEGFP-N (GFP fused to the N terminus of the targeted protein) was generated by inserting the EGFP coding sequence into HindIII and BamHI sites of pcDNA3. pEGFP-C (EGFP fused to the C terminus of the targeted protein) was generated by inserting the EGFP coding sequence into XhoI and Xhol sites of pcDNA3. All GFP fusion proteins used were constructed using these two plasmids as vector. **Bar2-GFP** was generated by subcloning the full-length cDNA fragment of human β-arrestin2 into the BamHI and NotI sites of pEGFP-N. **β-arrestin1** was generated by subcloning full-length human β-arrestin1 cDNA fragments into the BamHI and XhoI sites of pEGFP-N. HA-tagged β-arrestins were generated by subcloning the full-length human β-arrestins cDNA fragments into the pcDNA3-containing coding sequence of HA epitope.

Mutant constructs were made by mutating Ile-386, Val-387, Phe-388, Ile-393, Leu-394, Leu-396, and Met-399 of β-arrestin1 to Ala by a PCR-based strategy. To detect the subcellular localization of different parts of β-arrestins, each fragment was amplified by PCR using specific primers that contained recognition sequences for specific restriction enzymes. The PCR products were purified and digested with restriction enzymes and inserted into the BamHI and NotI sites of pEGFP-C.

The GFP-Mdm2 construct was created by inserting the mouse Mdm2 cDNA fragment (amplified from mouse brain by reverse transcription-PCR (RT-PCR) strategy) into EcoRI and XhoI sites of pEGFP-N. An NES mutant of Mdm2 was made by mutating two critical leucines of its NES (Leu-190 and Leu-192) to alanine by PCR-based strategy. GFP-JNK3 construct was made by inserting the JNK3 cDNA fragment into BamHI and XhoI sites of pEGFP-N. All constructs were verified by sequencing.

**Cell Culture and Transfection**—Human Saos2 (osteosarcoma) and HeLa cells were cultured in Dulbecco’s modified Eagle’s medium plus 10% (v/v) heat-inactivated fetal bovine serum. Human embryonic kidney (HEK293) cells were grown in Eagle’s minimal essential medium with Earle’s salt supplemented with 10% (v/v) heat-inactivated fetal bovine serum. All cells were cultured at 37 °C in a humidified 5% CO2 incubator. Plasmids were transiently transfected in the cells using the calcium phosphate method as described previously (19).

**Subcellular Localization Analyses and Immunofluorescence**—For the subcellular localization studies of the different GFP fusion proteins, cells were plated onto coverslips in 12-well dishes 16 h before transfection. The cells were transfected with 1–2 μg of expression vectors for the different GFP fusions. To examine the subcellular localization by DAPI staining, the cells were fixed and permeabilized in 4% paraformaldehyde, 0.1% Triton X-100 for 20 min at 4 °C, and the DNA was stained with 0.5 μg/ml DAPI for 2 min at room temperature. HA-tagged arrestins were detected using a monoclonal anti-HA antibody, 24 h after transfection, and the cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. The fixed cells were incubated with 2% bovine serum albumin in phosphate-buffered saline for 1 h at room temperature before incubation with the anti-HA antibody (diluted 1:200 in blocking solution) for 2 h at room temperature. The immunostaining was developed using Texas Red-conjugated goat anti-mouse IgG secondary antibody (diluted 1:100 in blocking solution). Visualization was performed with an Olympus microscope (×60 oil or ×100 oil immersion lens) equipped for epifluorescence. Images were captured with a CCD camera and analyzed with the Spot Advanced software.

**Coimmunoprecipitation**—Various HA-β-arrestin constructs were co-transfected with GFP-Mdm2 plasmid. 48 h after transfection, the cells were lysed in Lysis buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl, 20 mM NaF, 0.5% Nonidet P-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride) for 1.5 h at 4 °C. Cell extracts were cleared by centrifugation at 12,000 × g for 10 min, and supernatants were incubated at 4 °C with 1 μg of anti-HA antibody for 2 h. Immune complexes were immobilized on protein-A-Sepharose beads for 3 h, washed three times with Lysis buffer, and heated in SDS sample buffer in 50 °C water bath for 20 min.

**Western Blotting Analysis**—Lysates from cells (48 h after transfection) were boiled for 5 min in Sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, and 50 mM dithiothreitol). Aliquots containing 30 μg of protein were subjected to 10% SDS-PAGE, and the proteins resolved were electroblotted onto nitrocellulose membrane. The membrane was probed with anti-HA primary and peroxidase-conjugated secondary antibodies. The immune complexes were visualized using enhanced chemiluminescence detection (Amersham Biosciences) according to the manufacturer’s protocol.

**RESULTS**

**Expression of β-Arrestin2 but not β-Arrestin1 Relocalized Mdm2 from the Nucleus to the Cytoplasm**—The full length of human β-arrestin1 and β-arrestin2, respectively, fused to the GAL4 DNA-binding domain was used as bait in a yeast two-hybrid system to screen a mouse fetal brain complementary DNA library, and Mdm2 was thus identified as a binding partner of both β-arrestin1 and β-arrestin2 (data not shown). Coimmunoprecipitation assay demonstrated the abilities of both β-arrestin1 and β-arrestin2 to bind Mdm2 in intact cells (Fig. IA), consistent with the recent report (18). To investigate the potential biological significance of Mdm2/β-arrestin interaction, Mdm2 was fused to GFP at the N terminus (GFP-Mdm2), and the effects of Mdm2/β-arrestin interaction on the subcellular localization of Mdm2 was examined. GFP-Mdm2 was predominantly localized in the nucleus when expressed in...
Saos2 cells (Fig. 1B); β-arrestin1 was distributed in both the cytoplasm and nucleus, whereas β-arrestin2 was mainly localized in the cytoplasm (data not shown). No change in the subcellular localization of Mdm2 was observed when Mdm2 was coexpressed with β-arrestin1. However, in strong contrast, coexpression of Mdm2 with β-arrestin2 resulted in a dramatic redistribution of Mdm2 from the nucleus to the cytoplasm (Fig. 1C).

The molecular determinant of the differential effects of β-arrestin1 and β-arrestin2 on the subcellular localization of Mdm2 was analyzed next. At first, we tested whether the relocalization of Mdm2 by expression of β-arrestin2 was a result of the enhancement of the Mdm2 export from the nucleus by its interaction with β-arrestin2. Mutant Mdm2 with L190A/L192A substitutions (Mdm2NES−), which greatly impairs export of Mdm2 from the nucleus (20), was used in this study. Mdm2NES− was mainly localized in the nucleus when expressed alone (data not shown). However, coexpression with β-arrestin2 caused the significant relocalization of the mutated Mdm2 from the nucleus to cytoplasm (Fig. 2). This result clearly indicates that the relocalization of Mdm2 by β-arrestin2 was independent of the nuclear export of Mdm2. Next, we tested whether the relocalization of Mdm2 by expression of β-arrestin2 is affected by LMB, an antifungal antibiotic, which binds directly and irreversibly to CRM1 and inhibits NES-mediated active nuclear export (21, 22). As shown in Fig. 2, Mdm2 was accumulated in the cells coexpressing β-arrestin2 and Mdm2 following LMB treatment since β-arrestin2 was detained in the nucleus by the LMB treatment (Fig. 2). This result strongly suggests that interaction of β-arrestin2 and Mdm2 modulates the nucleus/cytoplasm equilibrium of Mdm2.

**Difference between β-Arrestin2 and β-Arrestin1 in the Nucleocytoplasmic Shuttling**—To further study the nucleocytoplasmic shuttling of β-arrestins, we fused GFP to the C termini of β-arrestin1 (βarr1-GFP) and β-arrestin2 (βarr2-GFP). When transiently transfected into Saos2, βarr1-GFP was distributed in both cytoplasm and nucleus, whereas βarr2-GFP was excluded from the nucleus in most of the transfected cells. Treatment with 5 ng/ml LMB induced the significant nuclear accumulation of βarr2-GFP fusion protein. In contrast, LMB exerted no significant effect on the subcellular distribution of βarr1-GFP (Fig. 3A). A similar result was also obtained using HA-β-arrestins in HEK293 cells (data not shown). These results suggest that β-arrestin2 but not β-arrestin1 shuttles between the nucleus and cytoplasm in an LMB-sensitive manner.

In addition, the nucleocytoplasmic shuttling of native β-arrestins in HeLa cells was also investigated through immunostaining with the specific anti-β-arrestin antibody that recognizes both β-arrestins. Under resting conditions, endogenous β-arrestins were distributed throughout the HeLa cells with a preferential cytoplasmic localization. LMB treatment induced significant nuclear accumulation of β-arrestins (Fig. 3B), indicating that endogenous β-arrestins constitutively shuttle between the nucleus and cytoplasm by a mechanism involving a functional cis-acting, LMB-sensitive NES or via an NES-containing interaction partner.

**Presence of a Typical NES Motif at the C Terminus of β-Arrestin2 but Not β-Arrestin1**—Usually, nuclear export in an LMB-sensitive manner depends on a functional NES, and a typical NES possesses a leucine- or other hydrophobic residue-rich motif (23). When we compared the amino acid sequence of β-arrestin2 with the reported functional NES sequences (Fig. 4A), a 10-residue region (residues 385–396) in β-arrestin2 was revealed to constitute a hydrophobic motif similar to the reported NES. To test whether this region is a functional NES of β-arrestin2, we attached the C-terminal peptide (residues 384–409) of β-arrestin2 containing this putative NES to the C terminus of GFP (GFP-C26). As shown in Fig. 4B, GFP, when expressed alone, was distributed throughout the nucleus and cytoplasm with slightly more accumulation in the nucleus. In contrast, GFP-C26 showed an evident nuclear exclusion of fluorescence in the cells, and this was reversed by the LMB treatment (Fig. 4B). Moreover, deletion of the C terminus (residues 384–409) of β-arrestin2 resulted in a significant nuclear accumulation of β-arrestin2 (data not shown).

To define the critical residues in the NES motif of β-arrestin2 in addition to the two leucine residues known (11), each of seven hydrophobic residues in the putative NES was replaced with alanine (Fig. 4C). The β-arrestin2 mutant with Phe-391, Leu-394, or Leu-396 replaced failed to shuttle to the cytoplasm, whereas replacement of Ile-386, Phe-388, or Met-399 produced no change in localization of β-arrestin2 (Fig. 4D). The V387A

**Fig. 2. Relocalization of Mdm2 by β-arrestin2 was independent of the nucleocytoplasmic shuttling of Mdm2.** 36 h after transfection, Saos2 cells co-transfected with plasmids encoding HA-β-arrestin2 and GFP-Mdm2 or GFP-Mdm2NES were treated with or without 5 ng/ml LMB overnight. The cells were stained with anti-HA antibody to detect β-arrestins (red) and DAPI to visualize nuclei (blue). Green fluorescence represents GFP-Mdm2. The subcellular localization of Mdm2 and β-arrestins was analyzed by fluorescent microscopy.

**Fig. 3. β-Arrestin2 but not β-arrestin1 shuttled between the nucleus and cytoplasm in an LMB-sensitive manner.** The subcellular localization of β-arrestins was analyzed by fluorescent microscopy. As shown in A, Saos2 cells were transfected with GFP-β-arrestin1 or GFP-β-arrestin2 vectors. 36 h after transfection, the cells were treated with or without 5 ng/ml LMB overnight, and the subcellular localization of GFP-β-arrestins was analyzed by fluorescent microscopy. As shown in B, HeLa cells treated with or without LMB overnight were fixed, and the endogenous β-arrestins were detected with β-arrestin specific antiserum.
As shown in D, the differential nucleocytoplasmic shuttling of \( \beta \)-arrestin1 and \( \beta \)-arrestin2 was due to a single amino acid residue difference. Saso2 cells expressing GFP \( \beta \)-arrestins and their mutants were treated with 5 ng/ml LMB overnight, and the subcellular localization of these \( \beta \)-arrestins was analyzed by fluorescent microscopy.

**Fig. 4. Identification of the NES in \( \beta \)-arrestin2.** A, alignments of the putative NESs in arrestins from different species with the previously characterized NESs of MAPKK, protein kinase inhibitor (PKI), p53, and Mdm2. Important hydrophobic residues are in bold. As shown in B, Saso2 cells were transiently transfected with GFP or GFP-C26 and treated with or without 5 ng/ml LMB and analyzed under a fluorescent microscope. C, Ala replacement of the critical hydrophobic residues in the putative NES of \( \beta \)-arrestin2. WT, wild type; C, C terminus; N, N terminus. As shown in D, \( \beta \)-arrestin2 mutants as indicated were transiently transfected into Saso2 cells. 36 h after transfection, the cells were fixed, and the subcellular localization of \( \beta \)-arrestin2 mutants was examined under a fluorescent microscope.

\( \beta \)-arr2L394Q was accumulated in the nucleus, and its distribution was insensitive to LMB treatment (Fig. 5). In contrast to the wild type \( \beta \)-arrestin1, \( \beta \)-arr1Q394L was mainly localized in the cytoplasm, and LMB treatment induced its nuclear accumulation (Fig. 5). Thus, the exchange of a single amino acid between \( \beta \)-arrestin2 and \( \beta \)-arrestin1 can totally convert their subcellular localization, and this result is consistent with the very recent report (11) that Leu/Gln differences at the C terminus of \( \beta \)-arrestin plays a critical role in differential shuttling of \( \beta \)-arrestin2 and \( \beta \)-arrestin1.

The intact N domain of \( \beta \)-arrestins was required for their nuclear import—Proteins larger than 40–60 kDa cannot enter into the nucleus through the nuclear pore complex by passive diffusion. GFP-\( \beta \)-arrestins have an apparent molecular mass of about 82 kDa and are apparently too large to diffuse into the nucleus. Thus, it seems logical to assume that \( \beta \)-arrestins may contain a functional nuclear localization signal (NLS) or may be transported via interaction with a partner protein containing an NLS. To test the assumption, a series of deletion mutants of \( \beta \)-arrestin2 was created via a GFP fusion protein (Fig. 6A), and expression of those GFP-fused proteins was confirmed by Western blot analysis (data not shown). The \( \beta \)-arrestin2 mutants with deletion of its N terminus (residues 1–185) were primarily present in the cytoplasm whether with (data not shown) or without a functional NES (Fig. 6B). In strong contrast, the N-terminal fragment (residues 1–185) of \( \beta \)-arrestin2, which contains the intact N domain of \( \beta \)-arrestin2, was exclusively distributed in the nucleus, clearly indicating that the N terminus of \( \beta \)-arrestin2 contains an essential and sufficient structure for its nuclear localization. The results from \( \beta \)-arrestin1 mutants (\( \beta \)-arrestin1185 and \( \beta \)-arrestin1185–413) also supported the notion (Fig. 6C). The further attempt to map down the NLS in the region, however, failed to define a classical NLS structure with a motif no larger than 20 amino acids (Fig. 6B). The mutations of several Lys and Arg residues in the region did not provide any detailed information about the putative NLS (data not shown).

We then tested the subcellular distribution of mutant \( \beta \)-arrestin2 with a V54D point mutation in the N domain (\( \beta \)-arr2V54D), a widely used dominant negative mutant that inhibits the internalization of GPCRs. Although \( \beta \)-arr2V54D was predominantly distributed in the cytoplasm in Saso2 cells, the LMB treatment exerted no effect on its subcellular distribution even after longer treatment as compared with the wild type \( \beta \)-arrestin2 (Fig. 6D). This result not only supports that the structural integrity of N domain is important for the nuclear import of \( \beta \)-arrestin2 but also implies that the N domain of \( \beta \)-arrestin2 may interact with other partner proteins to facilitate its nuclear import.

**Subcellular Localization of \( \beta \)-arrestin2 Modulated the Distribution of Its Binding Partner Mdm2 and JNK3**—Whether differential nucleocytoplasmic shuttling of two \( \beta \)-arrestins contributes to their different effects on the localization of Mdm2 was further examined. Unlike its wild type counterpart,
**Fig. 6.** The intact N domain of β-arrestins was required for nuclear import of β-arrestins. A, schematic representation of GFP-fused β-arrestin2 and its deletion mutants. C, C terminus; N, N terminus. B and C, HEK293 cells transiently expressing GFP or GFP-fused β-arrestins. As shown in D, Saos2 cells transfected with GFP-βarr2V54D mutant were treated with or without LMB overnight. The cells were fixed and examined by fluorescent microscopy.

βarr2L394Q was localized in the nucleus; coexpression of βarr2L394Q with Mdm2 resulted in accumulation of Mdm2 in the nucleus, in contrast to what was observed following coexpression of the wild type β-arrestin2 (Fig. 7A). This is unlikely to be a result of the impairment of the interaction between βarr2L394Q and Mdm2 since their interaction was not affected by the mutation (data not shown). In addition, the coexpression of βarr1Q394L with Mdm2 also evidently relocalized Mdm2 in the cytoplasm in contrast to the wild type β-arrestin1 (Fig. 7A).

It was reported that the binding to β-arrestin2 keeps JNK3 outside the nucleus (14). We therefore studied the effect of the nuclear import and export of β-arrestin2 on the subcellular localization of JNK3. When expressed alone, JNK3 was distributed evenly in the nucleus and cytoplasm, and coexpression with β-arrestin2 caused the significant extranuclear localization of JNK3 (Fig. 7B), in good agreement with a previous report (14). When coexpressed with βarr2V54D, JNK3 translocated to the cytoplasm, where it co-localized with βarr2V54D, whereas coexpression of βarr2L394Q did not change the JNK3 localization (Fig. 7B). Thus, our data indicate that the nucleocytoplasmic shuttling of β-arrestins modulates subcellular distribution of its binding partner Mdm2 and JNK3.

**Fig. 7.** Subcellular localization of Mdm2 and JNK3 was affected by β-arrestin distribution. As shown in A, HA-tagged β-arrestin or its mutant (βarr2L394Q or βarr1Q394L) was cotransfected with GFP-Mdm2 into Saos2 cells. As shown in B, HA-tagged β-arrestin2 or its mutant (βarr2L394Q or βarr2V54D) was coexpressed with GFP-JNK3 in HEK293 cells. The cells were fixed and stained with anti-HA antibody to detect β-arrestins (red). Green fluorescence was used to determine subcellular distribution of GFP-Mdm2 or JNK3. The samples were examined under a fluorescent microscope.

**DISCUSSION**

β-Arrestin1 and β-arrestin2 play a critical role in the regulation of the signaling of GPCRs, and accumulating evidence demonstrates that the functions of β-arrestins depend on their nucleocytoplasmic shuttling. The current study clearly demonstrated that β-arrestins, either endogenous or exogenous, could effectively enter the nucleus, and this is likely mediated by its interaction with an NLS-containing partner since no classical nuclear import sequence was identified in β-arrestins. A similar mechanism has been reported for other regulatory proteins. For example, β-catenin is imported into the nucleus via its interaction with the lymphoid enhancer factor/T-cell factor (LEF/TCF) transcription factor (25, 26). In addition, our study also established that the N terminus of β-arrestins is essential and sufficient for their nuclear localization. Therefore, it can be speculated that the assumed partner with a functional NLS binds to the N terminus of β-arrestins, and thus, the complex is imported into the nucleus. However, these partners of β-arrestins remain to be identified.

The crystal structures of visual arrestin and β-arrestin2 show that a typical arrestin molecule has a central polar core flanked by the N and C domains and a C-terminal tail connecting the two domains (27, 28). Although the crystal structure of β-arrestin2 is not yet available, it is very likely to possess a similar conformation since two arrestins share a high homology in their amino acid sequences. Our current study has shown that the N terminus of β-arrestins, which contains the intact N domain (residues 6–172 in β-arrestin1), is fully responsible for their nuclear localization. It has been shown that the N domain is more flexible and shares a higher degree of homology than other domains or whole proteins among different arrestins. Moreover, the N domain contains the binding sites for several β-arrestin partners, including a GPCR-binding site. For example, c-Src has been demonstrated to bind to the proline-rich regions at the N domain of β-arrestins (29). In addition, our recent data revealed that Mdm2 also binds to the N domain of β-arrestins (data not shown). Thus, the structural integrity of the N domain within the N terminus of β-arrestins is important for their subcellular localization.

Results from our current study and the very recent report from another laboratory show that β-arrestin2, but not β-arrestin1, contains a functional NES at its C terminus (42). It is well established that a typical NES is typically comprised of a 10-amino-acid motif with at least 4 conserved hydrophobic residues. In this study, we identified the hydrophobic motif...
VXXXFXLXL as a functional NES for β-arrestin2 in addition to the two leucines reported previously (11). More interestingly, the alignment of arrestin amino acid sequences from different species clearly reveals that the typical NES in β-arrestin2 is highly conserved not only in human and rat β-arrestin2 but also in fruit fly, trout, and locust in which only one nonviral arrestin exists (Fig. 4A). This suggests the nucleocytoplasmic shuttling is very important for the functions of these arrestins.

β-Arrestins are originally identified as a negative regulator of GPCR signaling involved in the desensitization and internalization of the receptors. Recent findings of new binding partners of β-arrestins have expanded our knowledge about their functions (14). Although the function of β-arrestins as endocytic proteins in the nucleus is still unclear, several other endocytic proteins, such as Epsin1, the clathrin assembly endocytic proteins, such as Epsin1, the clathrin assembly

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Subcellular localization of proteins is vital to their functions. The nuclear localization of Mdm2 has been known to be important for its ability to degrade p53. Impairment of nuclear import of Mdm2, as well as in the cases of the mutant or the alternative spliced form of Mdm2, greatly reduces its ability to degrade p53 (39). However, the enhancement of Mdm2 nuclear targeting, such as phosphorylation of Mdm2, can increase its function (40). In the present study, β-arrestin2 was found to be able to shift the subcellular localization of Mdm2 from the nucleus to the cytoplasm via its nucleocytoplasmic shuttling, and thus, it is anticipated that β-arrestin2 likely modulates the functions of Mdm2 as well as p53. In fact, our recent data (41) indeed provided the evidence for the expectation and demonstrated that β-arrestin2 reduces the Mdm2-mediated ubiquitination and degradation of p53 and subsequently enhances p53-induced apoptosis. Moreover, it has been reported (16) that β-arrestin2 but not β-arrestin1 can mediate ubiquitination of β-AR. From the results of the current study, the specificity of the effect of β-arrestins can be reasonably explained since only β-arrestin2 can shift the subcellular localization of Mdm2 from nucleus to cytoplasm although both β-arrestins interact well with Mdm2.

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