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The hsp90 Co-chaperone XAP2 Alters Importin β Recognition of the Bipartite Nuclear Localization Signal of the Ah Receptor and Represses Transcriptional Activity*

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The mouse aryl hydrocarbon receptor (mAhR) is a ligand-activated transcription factor that exists in a tetrameric, core complex with a dimer of the 90-kDa heat shock protein, and the hepatitis B virus X-associated protein 2 (XAP2). Transiently expressed mAhR-YFP (yellow fluorescent protein fused with the mAhR) localizes throughout cells, with a majority occupying nuclei. Co-expression of XAP2 with mAhR-YFP results in a distinct redistribution to the cytoplasm. We have utilized several approaches to attempt to identify the mechanism by which XAP2 modulates the sub-cellular localization of the mAhR. The nuclear export inhibitor, leptomycin B, was used to demonstrate that XAP2 inhibits ligand-independent nucleocytoplasmic shuttling of the receptor. Results from cytoskeletal disruption and the addition of an alternate nuclear localization sequence (NLS) to mAhR-YFP suggest that XAP2 does not physically tether the complex in the cytoplasm. The use of a rabbit polyclonal antibody raised against a portion of the bipartite NLS of the mAhR revealed that XAP2 does not appear to block access to the NLS. However, XAP2 hinders importin β binding to the mAhR complex, suggesting that XAP2 alters the conformation of the bipartite NLS of mAhR. XAP2 also represses the transactivation potential of the AhR, in contrast to previously published reports, perhaps by stabilizing the receptor complex and/or blocking nucleocytoplasmic shuttling of the AhR complex.

The aryl hydrocarbon receptor (AhR)[1] is a ligand-activated transcription factor that binds to a diverse group of compounds, with TCDD being the most studied and highly toxic AhR ligand (1–3).

The TCDD·AhR complex mediates a wide range of biological responses in rodents, such as a wasting syndrome, hepatotoxicity, teratogenesis, and tumor promotion. The unliganded AhR exists in cytosolic extracts as a core tetrameric complex, composed of a ligand binding subunit (AhR), a dimer of hsp90, and the immunophilin homolog XAP2 (4–6). Upon binding ligand, the AhR translocates to the nucleus where it forms a heterodimer with ARNT, concurrent with the loss of hsp90 from the complex (7). The AhR·ARNT heterodimer is capable of regulating transcription of a number of genes, such as CYP1A1, CYP1B1, and NADPH quinone oxidoreductase, upon binding to DREs in their enhancer regions. The ability of XAP2 to interact with the AhR was originally established independently by three laboratories (6, 8, 9). XAP2, also referred to as AIP (for AhR-interacting protein), or ARA9 (for AhR-associated protein 9), is a tetratricopeptide repeat motif domain containing immunophilin homolog that has significant sequence homology with FKBP52. The immunophilin FKBP52 is found bound to hsp90 and also exists in larger complexes with certain steroid receptors, including the glucocorticoid and progesterone receptors (10). Subsequent studies have established that XAP2 is able to stabilize and enhance cellular levels of the mAhR (11), which may occur through protection from proteolysis (12). In contrast, FKBP52 was unable to modulate AhR levels (13). Interestingly, transient expression of XAP2 also leads to sequestration of the mAhR in the cytoplasm (12, 14, 15). Regulation of intracellular movement of the AhR both in the presence and absence of ligand is a potentially important aspect of Ah receptor function that warrants further investigation.

For many proteins larger than 40 kDa, import or export across the nuclear envelope is controlled by the presence of nuclear localization sequences (NLS) and leucine-rich nuclear export signals (NES). An NLS is usually a short cluster of basic amino acids, such as those found in SV40 large T antigen, or it can be two clusters of basic amino acids separated by 10–12 amino acids, known as a bipartite NLS (16). The NLS is recognized by importin α, and this complex is then recognized by importin β, which mediates docking of the ternary complex to the cytoplasmic face of the nuclear pore complex. However, importin β is capable of binding directly to arginine-rich NLS of a number of proteins and mediate nuclear import (17, 18). The NES are short, hydrophobic, leucine-rich amino acid sequences (*XLXXLXXLXLX*) and are found in a variety of proteins (*e.g.* p53, IκBα, and glucocorticoid receptor). In the nucleus chromosomal region maintenance protein 1 (CRM-1) binds to the NES, followed by cooperative binding of RanGTP, which results in a complex competent for export (17). Proteins that carry both an NLS and NES often dynamically shuttle between the cytoplasm and the nucleus by a process termed nucleocytoplasmic

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[1] The abbreviations used are: AhR, aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachloro-*p*-dioxin; XAP2, hepatitis B virus X-associated protein 2; ARNT, aryl hydrocarbon receptor nuclear translocator; CFP, cyan fluorescent protein; CYP, cytochrome P450; CRM-1, chromosomal region maintenance protein 1; DRE, dioxin-responsive enhancer; FKBP52, 52-kDa FK506-binding protein; hsp90, 90-kDa heat shock protein; mAb, monoclonal antibody; NES, nuclear export signal(s); NLS, nuclear localization signal(s); GFP, green fluorescent protein; YFP, yellow fluorescent protein; PVDF, polyvinylidene difluoride; GR, glucocorticoid receptor; mAhR, murine aryl hydrocarbon receptor; hAhR, human aryl hydrocarbon receptor; PBS, phosphate-buffered saline; ARA9, AhR-associated protein 9; GST, glutathione *S*-transferase; DTT, dithiothreitol; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; MOPS, 4-morpholinepropanesulfonic acid.

shuttling. In these cases, the relative localization seen is the result of the relative rate of import and export combined. Shuttling provides a rapid and reversible means to regulate protein localization through protein-protein interactions, phosphorylation, or other post-translational modifications, in the microenvironment of the cytoplasm or nucleus. An example is the regulation of FKHRL1, a member of the Forkhead family of transcription factors, which is retained in the cytoplasm after Akt phosphorylation and subsequent 14-3-3 protein binding (18).

A bipartite NLS between amino acid residues 13 and 39 of the human AhR (hAhR) has been identified using a GFP fusion protein expression system (19). In addition, a NES has been identified between residues 55 and 75 in helix 2 of the helix-loop-helix domain of the hAhR and has been shown to interact with CRM-1 (20). Considering that the AhR has both a NLS and a NES, it is not surprising that it can undergo nucleocytoplasmic shuttling; indeed, this has been demonstrated through the use of the nuclear export inhibitor, leptomycin, and microinjection techniques (20–22). The primary objective of the studies presented here was to examine the mechanism of cytoplasmic retention of the mAhR in the presence of XAP2. The initial observation in this study indicated that XAP2 effectively blocks ligand-independent nucleocytoplasmic shuttling. There are a number of hypothetical mechanisms that could explain the ability of XAP2 to retain the mAhR in the cytoplasm. The most likely hypotheses are that XAP2 blocks importin access to the NLS, XAP2 locks the bipartite NLS of AhR into an unfavorable conformation for importin binding, XAP2 sequesters the mAhR in the cytoplasm by binding to cytoskeletal matrix proteins, or XAP2 may enhance nuclear export, leading to apparent cytoplasmic localization. The results outlined in this report demonstrate that XAP2·mAhR complexes are not sequestered in the cytoplasm through docking on tubulin or actin filaments. In addition, any other type of anchoring seems unlikely, as XAP2 did not promote sequestration of mAhR-YFP-Nuc, which contains a NLS distinct from the one present in the native receptor. Interestingly, XAP2 prevented active nucleocytoplasmic shuttling of the mAhR, which would suggest that XAP2 may block the NLS or alter the conformation of the bipartite NLS. Antibodies directed against the NLS were capable of binding to mAhR·XAP2 complexes, indicating that the NLS of mAhR is not blocked by the presence of XAP2. However, XAP2 does reduce importin β binding to the mAhR·hsp90 complex *in vitro*. These studies taken together would suggest that XAP2 alters the ability of importin β to recognize the bipartite NLS sequence of the mAhR, and this appears to be the primary mechanism of XAP2-mediated redistribution of the mAhR to the cytoplasm. Another hypothesis that we wanted to test is whether blocking of nucleocytoplasmic shuttling could lead to a repression of AhR transcriptional activity. Transient transfection experiments indicated that XAP2 is capable of repressing AhR activity. In summary, these studies would suggest that XAP2 cause a functionally significant alteration in the conformation of the mAhR.

EXPERIMENTAL PROCEDURES

Construction and Sources of Expression Vectors—The vector pcDNA3-βmAhR provided by Oliver Hankinson (University of California, Los Angeles, CA) was used for mammalian expression of the mouse AhR (23). As a control for importin binding experiments, amino acid residues 13–15 of the mAhR were mutated to alanine residues using pcDNA3-βmAhR and a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The vector was designed as pcDNA3-βmAhR/M-NLS. The nucleotide sequence corresponding to the amino acids 31–44 of the mAhR/FLAG was deleted using a site-directed mutagenesis approach. Two alanine residues were inserted between amino acids 30 and 45, and this construct was designated as pcDNA3-βmAhR/FLAG/ΔNLS. The plasmids pEYFP-N1, pEYFP-actin, pEYFP-tubulin, pEYFP-Nuc, and pECFP-Nuc were obtained from Clontech (Palo Alto, CA). The pCI-XAP2, pCI-XAP2-FLAG, and pCI-XAP2-G272D-FLAG vectors were previously prepared in our laboratory (11, 13). The pEYFP-mAhR and pEYFP-mAhR-K13A were constructed as previously described (14). The pEYFP-mAhR-YFP-Nuc and pEYFP-mAhR-K13A-YFP-Nuc were constructed by excising mAhR-YFP and mAhR-K13A-YFP from the vectors described above with *Nhe*I and *Bsr*GI and inserted directly into the *Nhe*I/*Bsr*GI sites of pEYFP-Nuc. The DRE-driven reporter vector pGudLuc 6.1 was obtained from Mike Denison (University of California, Davis, CA). Human importin β-myc-pET30a and GST-importin β and α constructs were obtained from Stephen Adam (Northwestern University Medical School, Chicago, IL).

Cell Culture—Cells were grown in α-minimal essential medium supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT), 100 IU/ml penicillin, and 0.1 mg/ml streptomycin (Sigma) at 37 °C in 95% air, 5% CO_2.

Fluorescence Microscopy—Fluorescence micrographs were obtained directly from cells grown in six-well microplates that were transfected with 1.5 μg of DNA using LipofectAMINE with PLUS reagent (Invitrogen) according to the manufacturers instructions. Approximately 18 h following transfection, fluorescence was visualized with a Nikon TE300 inverted microscope with TE-FM epifluorescence attachment using a Nikon Pan Fluor 60X objective and a SPOT RT Color model 2.2.0 cooled CCD camera. For examination of cytoskeletal disruption, cells grown on glass cover slips in six-well culture dishes were transfected with 2 μg of DNA using LipofectAMINE (Invitrogen) according to the instructions from the manufacturer. Approximately 18 h after transfection, cells were treated with either 5 μM colchicine or 10 μM cytochalasin B (Sigma) for 1 h. Before visualization, cells were rinsed twice with PBS, fixed for 15 min in 4% formaldehyde/PBS at room temperature, and rinsed twice with PBS, and inverted coverslips were mounted onto microscope slides with Vectashield mounting medium (Vector Laboratories Inc., Burlingame, CA). Fluorescence micrographs were obtained with a SPOT SP100 cooled CCD camera fitted to a Nikon Optiphot-2 upright microscope with EFD-3 episcopic fluorescence attachment using a Nikon Pan Fluor 100× oil immersion objective. All cells in micrographs are representative of >80% of the transfected cell population.

Production of Affinity-purified Rabbit Polyclonal Anti-AhR-NLS Antibodies—A 15-residue peptide corresponding to the C-terminal portion of the bipartite NLS of the AhR (amino acids 31–44) (19) was synthesized with an N-terminal cysteine residue (H_2N-CK-SNPSKRHRDRLNT-COOH) by New England Peptide (Fitchburg, MA). The peptide was conjugated to keyhole limpet hemocyanin and injected into rabbits using standard techniques at New England Peptide. The AhR-NLS peptide (1 mg) was conjugated to 3 ml of Sulfolink resin (Pierce) following the instructions from the manufacturer. A 3-ml column was washed and stored at 4 °C in M/N buffer (20 mM MOPS, 0.02% sodium azide) until use. Affinity purification was carried out entirely at 4 °C. Before use, the column was washed with three volumes of ice-cold M/N buffer. Rabbit serum (6 ml) was applied to the column by gravity flow, collected, and reapplied. The column was then washed as follows: 10 volumes of M/N, 5 volumes of M/N + 500 mM NaCl, and 2 volumes of M/N. The antibody was eluted with two volumes of 0.1 M glycine-HCl, pH 2.5, and 1-ml fractions were collected into 1.5-ml microcentrifuge tubes containing 100 μl of 1 M Tris, pH 8.0. Protein content of fractions was determined using the BCA assay (Pierce). The specificity of this peptide antibody was assessed by *in vitro* translating pcDNA3-βmAhR/FLAG and pcDNA3-βmAhR/FLAG/ΔNLS in the presence of [^{35}S]methionine, which were subjected to SDS-PAGE, transferred to PVDF membrane. The presence of the AhR was visualized using either mAb RPT 1 or anti-AhR-NLS peptide antibody and goat anti-mouse peroxidase conjugate. The ability of the anti-AhR-NLS polyclonal antibody bound to protein G-Sepharose to immunoprecipitate *in vitro* translated mAhR/FLAG or mAhR/FLAG/ΔNLS was assessed using standard techniques.

Cytosol Preparation and Immunoprecipitations—Transfected COS-1 cells grown in 100-mm plates were harvested by trypsinization, rinsed with PBS, and mechanically homogenized with 30 strokes of a stainless steel Dounce homogenizer. Immunoprecipitations were carried out for 1 h at 4 °C in IP buffer (MENG (25 mM MOPS, 2 mM EDTA, 0.02% NaN_3, 10% glycerol, pH 7.4) with 20 mM sodium molybdate, 50 mM NaCl, 2 mg/ml bovine serum albumin, 2 mg/ml ovalbumin), using rabbit polyclonal anti-AhR-NLS antibodies bound to protein G-Sepharose (Pierce). Immunoprecipitates were rinsed three times with IP buffer, twice with wash buffer (MENG with 20 mM sodium molybdate, 50 mM NaCl), resolved by Tricine SDS-PAGE, and electroblotted to PVDF membrane (Millipore, Bedford, MA) as previously described (13). The AhR and XAP2 were visualized by Western blot analysis using RPT1

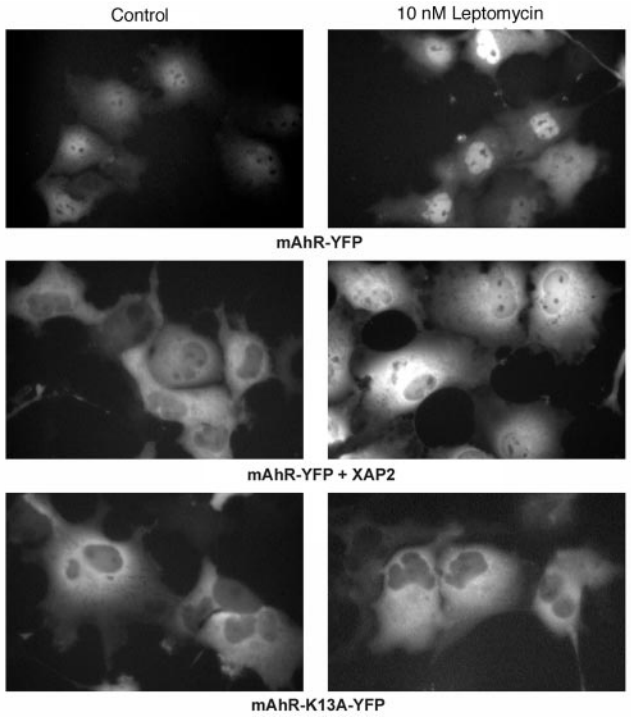

FIG. 1. **XAP2 blocks ligand-independent nucleocytoplasmic shuttling of the mAhR-YFP.** COS-1 cells grown in six-well microplates were transiently transfected with either pEYFP-mAhR, pEYFP-mAhR + pCI-XAP2, or pEYFP-mAhR-K13A. Control (carrier solvent) and 10 nM leptomycin B treated cells were visualized after 1 h of treatment by fluorescence microscopy. The mAhR-YFP alone localized throughout cells and showed nuclear retention following 1 h treatment with the export inhibitor leptomycin B. Co-expression of XAP2 resulted in cytoplasmic localization, and leptomycin B-mediated nuclear accumulation was inhibited. A mutation in the NLS abolished nuclear accumulation of the AhR-YFP in the absence of XAP2 and was unaffected by treatment with leptomycin B.

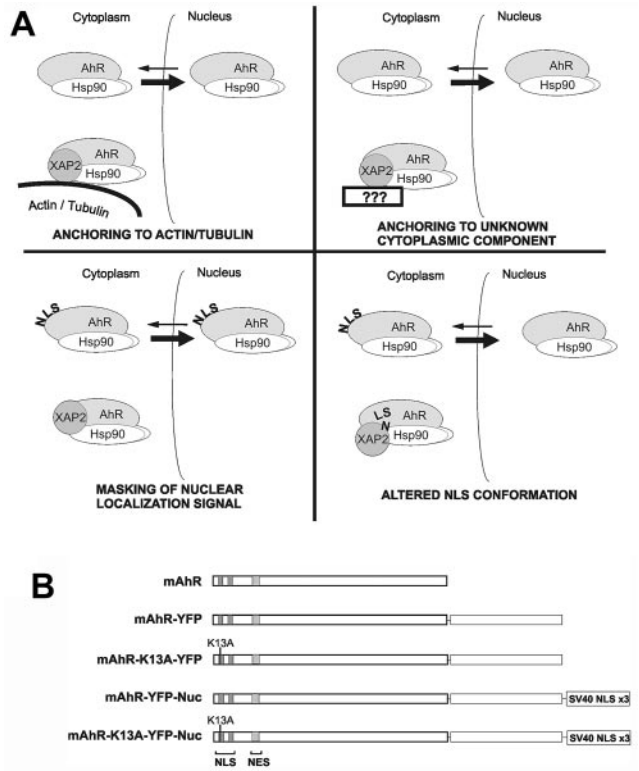

FIG. 2. **Possible mechanisms by which XAP2 may mediate cytoplasmic localization of the mAhR.** *A*, (i) XAP2 may physically anchor the mAhR to actin/tubulin-based components of the cytoskeleton; (ii) XAP2 may physically anchor the mAhR to some unknown cytoplasmic component; (iii) XAP2 may block access to the NLS of the mAhR; (iv) XAP2 may stabilize a conformation of the receptor in which the bipartite NLS is incapable of recognition by importin molecules. *B*, schematic representation of mAhR constructs used to examine the role of XAP2 in mediating cytoplasmic localization of the AhR complex.

mAb (24), and anti-ARA9 mAb (Novus Biologicals, Littleton, CO), respectively. Primary antibodies were detected with either ^{125}I-labeled goat anti-mouse IgG or ^{125}I-labeled donkey anti-rabbit polyclonal IgG (Amersham Biosciences), visualized by autoradiography, and quantitated with a phosphorimager.

Importin/AhR Interaction Assay 1—COS-1 cells in three 100-mm dishes were transfected with pcDNA3-βmAhR/FLAG, with pcDNA3-βmAhR/FLAG and pCI-XAP2, or with pcDNA3-βmAhR/FLAG-M-NLS (as a importin specificity control) using LipofectAMINE PLUS™ transfection method as described by the manufacturer (Invitrogen). After 24 h cells were trypsinized and washed three times with PBS. Cells from each set of three plates were lysed in 1 ml of MENG containing 20 mM $NaMoO_4$, 1% Nonidet P-40, protease inhibitor mixture (Sigma), and 1 mM DTT for 15 min at 4 °C, and centrifuged at 100,000 × *g* for 30 min at 4 °C. The lysate (~350 μl) was treated with TCDD to a final concentration of 10 nM or Me_2SO for 30 min at ambient temperature followed by 10 min on ice. To the TCDD- or Me_2SO-treated lysate 350 μl of immunoprecipitation buffer (MENG containing 20 mM $NaMoO_4$, 300 mM NaCl, 10 mg/ml bovine serum albumin, 5 mg/ml ovalbumin, 1 mM DTT) was added and then transferred to 25 μl of pre-washed anti-FLAG M2-agarose (Sigma). The immunoprecipitations were incubated with agitation for 1 h at 4 °C and washed three times with MENG containing 100 mM NaCl and 20 mM $NaMoO_4$. The FLAG-tagged proteins were displaced by incubating with 200 μg of FLAG peptide (Sigma) in 125 μl of 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl and 0.1% Nonidet P-40 for 15 min at ambient temperature. The displacement was repeated once more, and supernatants were pooled. Each of the displaced FLAG-tagged proteins (200 μl) was transferred into 200 μl of importin-binding buffer (PBS containing 10 mg/ml bovine serum albumin, 5 mg/ml ovalbumin, 0.2% Nonidet P-40, 10% glycerol, and 1 mM DTT), and 22 μg of GST-importin β was added. The mixture was incubated for 30 min on ice, transferred to 25 μl of pre-washed glutathione-Sepharose (Amersham Biosciences), incubated with agitation for 1 h at 4 °C, and washed three times with 1 ml of PBS. GST-importin β was displaced from the resin by incubating with 80 μl of 20 mM glutathione in 50 mM Tris-HCl, pH 7.5, plus 1 mM DTT for 5 min at ambient temperature. The displacement was repeated once more, and the pooled supernatant was subjected to Tricine SDS-PAGE. Protein was transferred onto PVDF membrane (Millipore, Bedford, MA) as previously described. The mAhR, XAP2, and GST-importin β were visualized by protein blot analysis using mAb RPT1, mAb anti-ARA9 (Novus Biologicals), and mAb B-14 anti-GST (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), respectively. Primary antibodies were detected with ^{125}I-labeled goat anti-mouse IgG (Amersham Biosciences), visualized by autoradiography, and quantitated with a phosphorimager and/or a γ counter.

Importin/AhR Interaction Assay 2—COS-1 cells (100-mm dishes) were transfected with pcDNA3, pcDNA3-βmAhR/FLAG, or pcDNA-βmAhR/FLAG and pCI-XAP2 using LipofectAMINE PLUS™ transfection method as described by the manufacturer (Invitrogen). After 24 h cells were harvested and lysed in MENG + 20 mM sodium molybdate + 1% Nonidet P-40 and centrifuged at 100,000 × *g* for 30 min. The supernatants were transferred to three tubes, each containing 50 μl of M2-agarose (Sigma), and incubated with agitation for 90 min. The immunoprecipitations were washed twice with MENG + 20 mM sodium molybdate, followed by two additional washes with 20 mM MOPS, 50 mM potassium acetate, 50 mM NaCl, 2 mM magnesium acetate, 0.02% NaN_3, pH 7.4 (binding buffer). The human importin β-myc-pET30a construct was transcribed and translated in a TnT coupled translation system (Promega, Madison, WI) in the presence of [^{35}S]methionine. To the immunoprecipitates 5 μl of *in vitro* translated importin β and 45 μl of binding buffer was added. Each sample incubated for 20 min at 30 °C and gently mixed every 5 min, followed by incubation at 4 °C for 45 min. The immunoprecipitates were washed quickly three times and incubated with 50 μl of FLAG peptide (5 mg/ml), and these mixtures were incubated for 30 min at room temperature. After centrifugation the FLAG peptide-displaced AhR-FLAG present in the supernatant was collected. This displacement was repeated once more and the pooled supernatants subjected to Tricine SDS-PAGE. Proteins were transferred to PVDF membrane, and the presence of [^{35}S]methionine-labeled importin β was visualized and

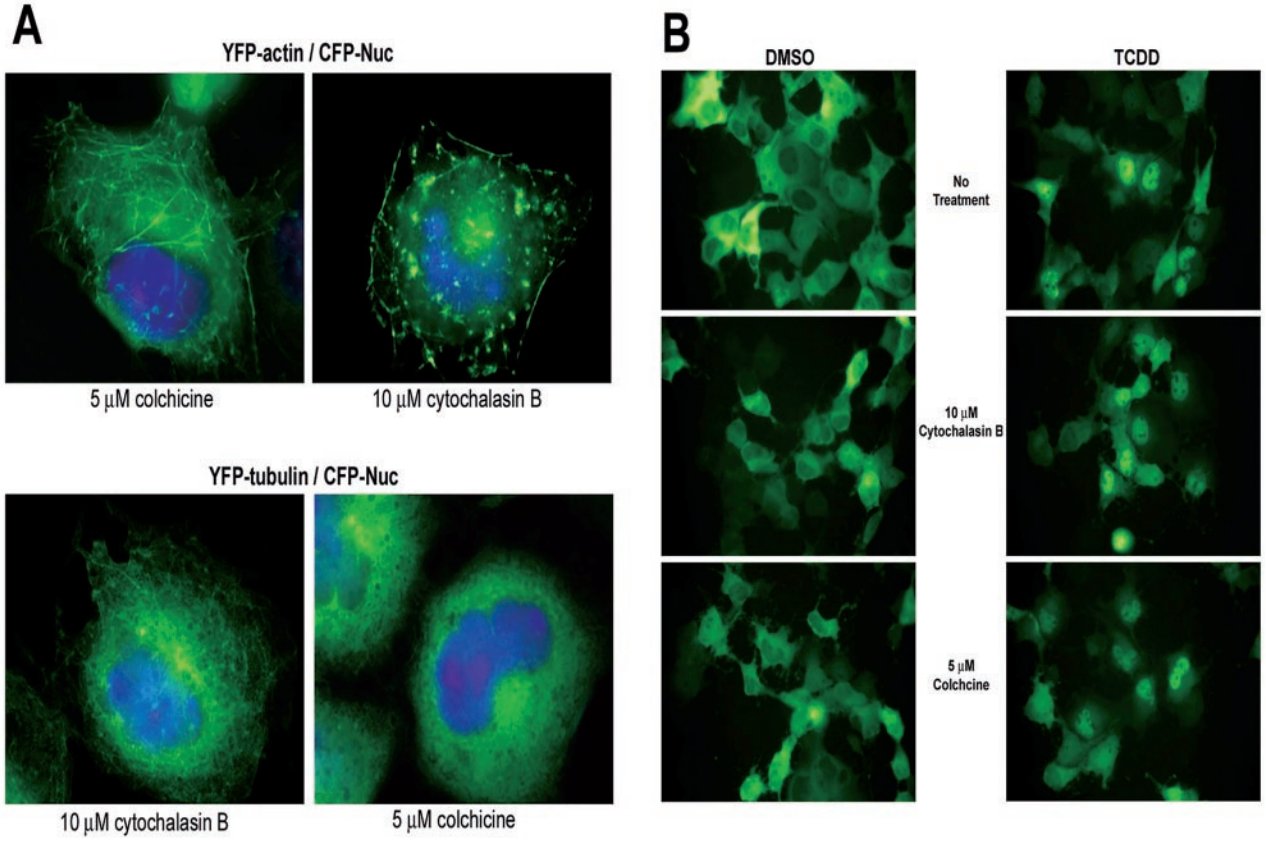

FIG. 3. **Role of actin and tubulin structures in the localization and movement of the mAhR in COS-1 cells.** *A*, COS-1 cells expressing YFP-actin and CFP-Nuc or YFP-tubulin and CFP-Nuc were treated for 1 h with either 5 μM colchicine to disrupt tubulin or 10 μM cytochalasin B to disrupt actin. In each case, the agent was specific and did not appear to adversely influence the integrity of the nuclear envelope. *B*, COS-1 cells expressing mAhR-YFP and XAP2 were treated with either colchicine or cytochalasin B in the presence or absence of 10 nM TCDD for 1 h. Treatment with cytoskeletal disrupting agents did not affect localization of mAhR-YFP, and TCDD treatment resulted in nuclear accumulation in a timeframe similar to that for control-treated cells. DMSO, Me_2SO.

quantitated by phosphorimaging. The presence of XAP2 and AhR was detected using the monoclonal antibodies described above and peroxidase conjugated to goat anti-mouse IgG as the secondary antibody. The presence of peroxidase was visualized with a Vector VIP peroxidase kit (Vector Laboratories, Burlingame, CA).

Luciferase Reporter Gene Assay—COS-1 cells in 100-mm plates were co-transfected using LipofectAMINE PLUS™ (Invitrogen) with 0.5 μg of pcDNA3-βmAhR, 100 ng of luciferase reporter construct pGudLuc 6.1, 30 ng of pCMV-βGalactosidase, either 2 or 4 μg of pCI-XAP2, and pCI was added for a total of 8 μg of DNA. Transfected cells after 9 h were transferred to 12-well plates; 13 h later cells were treated with iodoflavone. Cells were lysed after an 8-h exposure to ligand, and reporter activity was assayed using a Turner TD-20e luminometer and a luciferase assay system (Promega).

RESULTS

XAP2 Inhibits Ligand-independent Nucleocytoplasmic Shuttling of the mAhR—COS-1 cells were utilized in this study because of their ability to be transfected with greater than 50% transfection efficiency; additionally, they have low levels of endogenous AhR, and our previous studies examining regulation of mAhR localization were performed in this cell line (14). COS-1 cells were transiently transfected with pEYFP-mAhR, pEYFP-mAhR + XAP2, or pEYFP-mAhR-K13A (containing a single point mutation that renders the NLS nonfunctional) and treated with either vehicle (MeOH) or 10 nM leptomycin B (Fig. 1). As previously reported (14), control-treated cells expressing mAhR-YFP showed localization of the receptor throughout cells, with a higher level in cell nuclei than in the cytoplasm. Co-expression of XAP2 resulted in redistribution of the wild-type receptor to the cytoplasm, whereas mAhR-K13A-YFP is cytoplasmic with or with out co-expression of XAP2 (19). Treatment with leptomycin B, a specific inhibitor of CRM-1-mediated nuclear export, resulted in enhanced nuclear accumulation of mAhR-YFP, suggesting that the unliganded receptor is capable of undergoing nucleocytoplasmic shuttling as has previously been suggested using N-terminal fragments of the human AhR fused to glutathione *S*-transferase and GFP (20). Nucleocytoplasmic shuttling was eliminated by mutation of the NLS of the AhR, but quite unexpectedly, co-expression of XAP2 with mAhR-YFP also resulted in inhibition of nucleocytoplasmic shuttling. When XAP2 was co-expressed with mAhR-YFP and cells treated with leptomycin B, nuclear accumulation was not observed after 1 h (Fig. 1), and little or no increase in nuclear accumulation was seen at the longest observed time point of 6 h (data not shown). Inhibition of nucleocytoplasmic shuttling of the mAhR by XAP2 suggests that the observed effect of XAP2 on mediating cytoplasmic retention of mAhR-YFP is not caused by either an enhanced rate of nuclear export or an inhibition of nuclear retention. This information has allowed us to narrow down the possible hypotheses to explain XAP2-mediated sequestration of the mAhR; these hypotheses are schematically represented in Fig. 2*A*.

XAP2 Does Not Promote Anchoring of the mAhR to the Cytoskeletal Components Actin or Tubulin—We hypothesized that XAP2 may sequester the mAhR in the cytoplasm through interaction with components of the cytoskeleton. To examine this possibility, COS-1 cells were co-transfected with either pEYFP-actin and pECFP-Nuc or pEYFP-tubulin and pECFP-Nuc, followed by treatment with either 10 μM cytochalasin B or 5 μM colchicine (Fig. 3*A*). Initial experiments were conducted to (*a*)

demonstrate specific disruption of the cytoskeleton by each agent, and (*b*) demonstrate that the integrity of the nuclear membrane was maintained following each treatment. The actin cytoskeleton was disrupted by 10 μM cytochalasin B, but was unaffected by 5 μM colchicine, whereas the tubulin cytoskeleton was disrupted by 5 μM colchicine, but was unaffected by 10 μM cytochalasin B. In each case, nuclear retention of CFP-Nuc (nuclear localized cyan fluorescent protein) was preserved. COS-1 cells were next co-transfected with pEYFP-mAhR and pCI-XAP2 and subjected to treatment with cytoskeletal disruption agents in the presence and absence of TCDD (Fig. 3*B*). Control cells showed mAhR-YFP localization to cell cytoplasm, whereas 1-h treatment with 10 nM TCDD resulted in nuclear uptake of the receptor. Treatment of cells with cytochalasin B or colchicine did not result in altered subcellular localization of the unliganded receptor and did not disturb ligand-dependent nuclear accumulation, which occurred at a similar rate in control-treated cells. These results suggest that actin or tubulin cytoskeletal systems are not involved in mediating the translocation of the AhR complex.

XAP2 Does Not Alter Nuclear Accumulation of the mAhR Directed by an Alternate NLS—To examine whether XAP2 was capable of modulating the localization of the mAhR directed by an alternate NLS, two constructs were generated. They consisted of the mAhR-YFP and mAhR-K13A-YFP with three repeats of the NLS from SV40 large T-antigen added at the C terminus of YFP (Fig. 2*B*). The resulting localization of these constructs transiently expressed in COS-1 cells is summarized in Fig. 4. As has been previously demonstrated, the mAhR-YFP localized mostly in nuclei, and co-expression of XAP2 resulted in redistribution of the mAhR-YFP to the cytoplasm, whereas mAhR-K13A-YFP remained in cytoplasm. Addition of an NLS to the C terminus of mAhR-YFP resulted in strong nuclear localization; in addition, co-expression of XAP2 did not alter nuclear accumulation of the AhR in either case, suggesting that XAP2 is only able to modulate localization of the mAhR directed by its endogenous NLS. Thus, XAP2 does not have an "anchoring" effect on the mAhR complex. YFP-Nuc, lacking the mAhR, shows essentially complete localization to nuclei (data not shown).

XAP2 Does Not Block the Ability of an Anti-mAhR NLS Antibody to Bind to the mAhR—To determine whether XAP2 plays a role in altering/masking the mAhR NLS, we used a rabbit polyclonal antibodies directed against the C-terminal portion of the bipartite NLS, amino acid residues 31–44, of the mAhR. The critical residues of the NLS of AhR necessary for function are between amino acid residues 13 and 39 (19). The specificity of this polyclonal antibody was examined on a protein blot and was found to be highly specific for the AhR transiently expressed in COS 1 cells (Fig. 5*A*). In addition, this antibody fails to recognize the mAhR after the amino acids 31–44 are deleted (Fig. 5, *B* and *C*). Thus, this antibody is highly specific and only binds to the NLS sequence of the mAhR. These antibodies were bound to protein G-Sepharose and used to immunoprecipitate the mAhR from transiently transfected COS-1 cells. The COS-1 cells were transfected with either pcDNA3-βmAhR (3 μg) and pCI-XAP2 (6 μg) or pcDNA3-βmAhR (3 μg) alone. Cytosolic extracts from control cells and cells treated with TCDD (10 nM, 1 h) were incubated for 1 h on ice with protein G-Sepharose-bound anti-AhR-NLS antibodies or protein G-Sepharose-bound mouse IgG as control. Immunoprecipitates were resolved by Tricine SDS-PAGE, electroblotted to PVDF membrane, and visualized by Western blot with mAbs to the AhR and XAP2 (Fig. 5*D*). Co-expression of XAP2 with the mAhR resulted in a clear increase in expression of the AhR; however, neither ligand treatment nor the presence

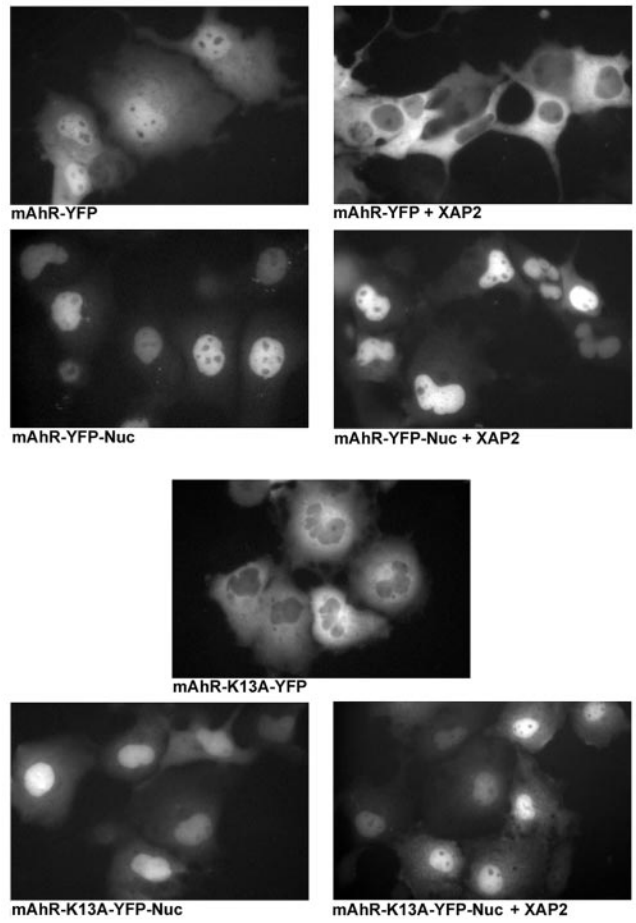

FIG. 4. **XAP2 does not influence mAhR-YFP nuclear accumulation directed by an alternate NLS.** COS-1 cells were transiently transfected with various constructs expressing the noted proteins. The mAhR-YFP localized predominantly in the nuclei and was retained in the cytoplasm upon co-expression of XAP2. The NLS mutant of mAhR-YFP (K13A) also localized exclusively to the cytoplasm. Addition of an NLS at the C terminus of the mAhR fusion protein produced a species that localized almost exclusively to cell nuclei with and without the NLS of mAhR (compare mAhR-YFP-Nuc with mAhR-K13A-YFP-Nuc). In both cases, co-expression of XAP2 had no visible effect on subcellular localization of the mAhR as directed by an alternate NLS.

of excess XAP2 resulted in significant differences in the ability of anti-AhR-NLS polyclonal antibodies to immunoprecipitate the receptor. The key observation to note is that these antibodies efficiently immunoprecipitate the AhR complexed with XAP2.

Importin β Binding to the mAhR Is Inhibited by XAP2—Two *in vitro* assays were developed to test whether or not the presence of XAP2 in the AhR-FLAG complex results in reduced ability to bind importin β. In the first approach, the ability of immunopurified mAhR·FLAG complexes isolated for transfected COS-1 cells to bind to GST-importin β immobilized on glutathione-agarose was assessed. The presence of XAP2 in the mAhR complex resulted in a 40% decrease in receptor binding to importin β (Fig. 6). The very low level of mAhR/M-NLS binding to GST-importin β indicates that the receptor interacts with importin β through its NLS. The second assay used a different approach and examined whether or not *in vitro* translated importin β could bind to immobilized AhR-FLAG. AhR-FLAG expressed alone or co-expressed with XAP2 in COS-1 cells was immunoprecipitated from cytosolic extracts with M2-agarose, and, after washing the agarose, *in vitro* translated importin β was added for 1h. After washing the immunoprecipitates, the isolated receptor complexes were eluted from the

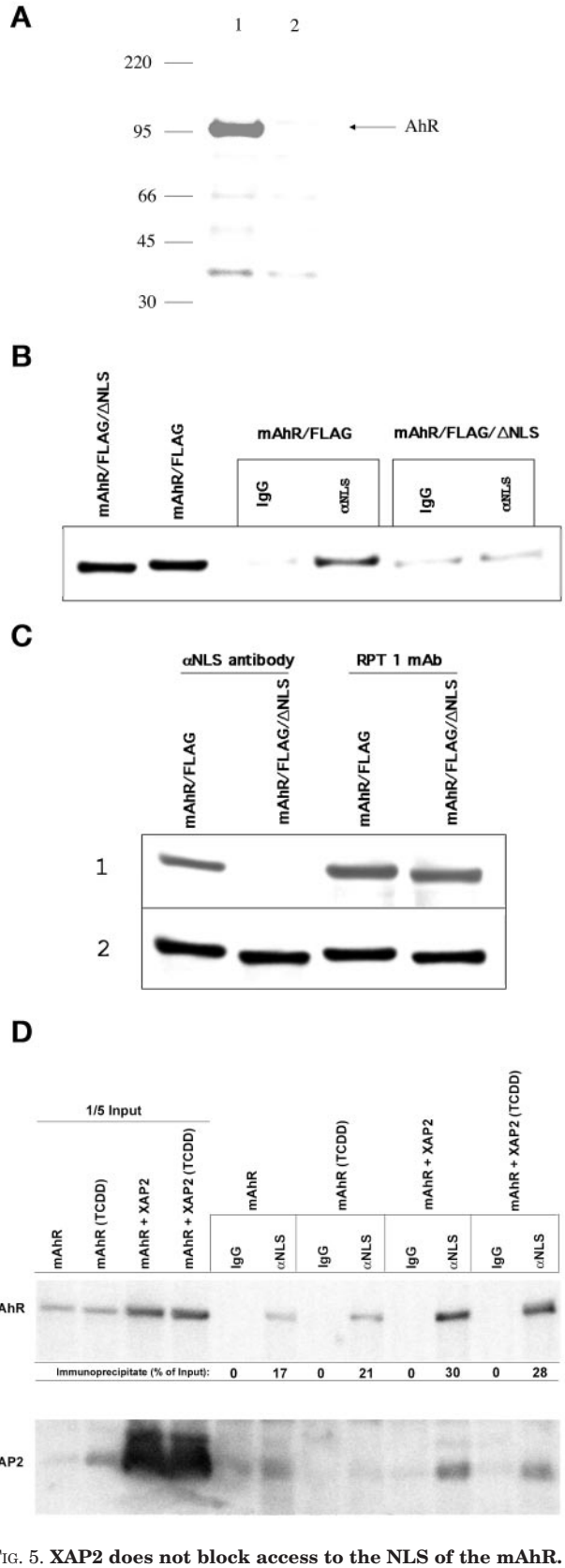

FIG. 5. **XAP2 does not block access to the NLS of the mAhR.** A rabbit polyclonal antibody directed against the C-terminal half of the bipartite NLS of the mAhR was produced and purified by affinity chromatography and was designated anti-AhR/NLS rabbit polyclonal antibody. *A*, cytosolic extracts from COS 1 cells transfected with pcDNA3/mAhR/FLAG (*lane 1*) or pcDNA3 (*lane 2*) were resolved by SDS-PAGE and transferred to membrane. The presence of the AhR was detected with the anti-AhR/NLS or control polyclonal antibodies. *B*, *in vitro* translated mAhR-FLAG or mAhR-FLAG/ΔNLS were subjected to immunoprecipitation analysis using anti-AhR/NLS polyclonal antibody. A 1/3 input of *in vitro* translated mAhR-FLAG/ΔNLS and mAhR-FLAG are shown in *lanes 1* and *2*. Immunoprecipitations were subjected to SDS-PAGE, transferred to membrane, and [^{35}S]methionine-labeled receptor visualized by autoradiography. *C*, detection of mAhR-FLAG but not mAhR-FLAG/ΔNLS by anti-AhR/NLS rabbit polyclonal antibody on membranes containing SDS-PAGE resolved *in vitro* translated [^{35}S]methionine-labeled proteins. *Panel 1*, immunochemical visualization of the AhR with anti-AhR/NLS rabbit polyclonal antibody or monoclonal antibody RPT 1. *Panel 2*, autoradiography of the *in vitro* translated [^{35}S]methionine-labeled AhR/FLAG and mAhR-FLAG/ΔNLS. *D*, the anti-AhR antibody (or mouse IgG control) bound to protein G-Sepharose was used to immunoprecipitate the mAhR from cytosol prepared from COS-1 cells transiently transfected with either the mAhR or the mAhR and XAP2, as well as in the presence or absence of TCDD. The *left lanes* show 20% of the input for each immunoprecipitation.

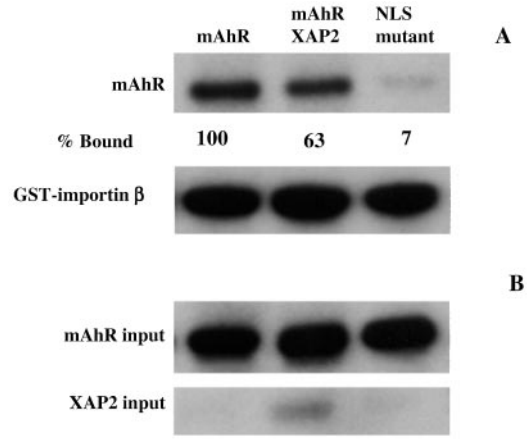

FIG. 6. **XAP2 inhibits importin β binding to the AhR.** COS-1 cells were transfected with pcDNA3-βmAhR-FLAG in the presence or absence of pCI-XAP2 and AhR-FLAG complexes were immunoprecipitated from cytosolic extracts and displaced from the M2 resin with FLAG peptide. The isolated AhR complexes were incubated with GST-importin β bound to glutathione-agarose. AhR·FLAG complexes were displaced from the glutathione-agarose with glutathione and subjected to SDS-PAGE followed by transfer to membrane. The presence of the AhR, GST-importin β, and XAP2 were visualized as described under "Experimental Procedures". *A*, *upper panel*, the presence of mAhR bound to GST-importin β; *lower panel*, the amount of GST-importin β in each sample. The percentage of mAhR bound values given has been corrected for the amount of GST-importin β and mAhR present or placed into each assay. *B*, *upper panel*, the relative amount of mAhR incubated with GST-importin β-glutathione-agarose in each assay, respectively. The *lower panel* shows the relative amount of XAP2 incubated with GST-importin β-glutathione-agarose in each assay. Assays were performed three times, and essentially the same results were obtained.

agarose with FLAG peptide. The eluted protein was subjected to Tricine SDS-PAGE, protein was transferred to membrane, and the presence of importin β, AhR, and XAP2 was assessed (Fig. 7). In preliminary experiments a higher level of importin β binding to the mAhR was obtained compared with importin α1 (data not shown). A 2.5-fold increase in binding of importin β to the AhR complex was detected relative to background binding. In contrast, AhR complexed with a relatively large amount of XAP2 exhibited 3-fold less specific binding of importin β to the AhR. Whether importin β is binding directly to the NLS of AhR or indirectly, by binding to importin α complexed with the receptor in cells was not determined. Nevertheless, this result, considered along with the inability of XAP2 to block access to the NLS of the receptor, suggests that XAP2 inhibits importin β binding by altering the conformation of the bipartite NLS of the mAhR, yielding an unfavorable conformation for importin binding.

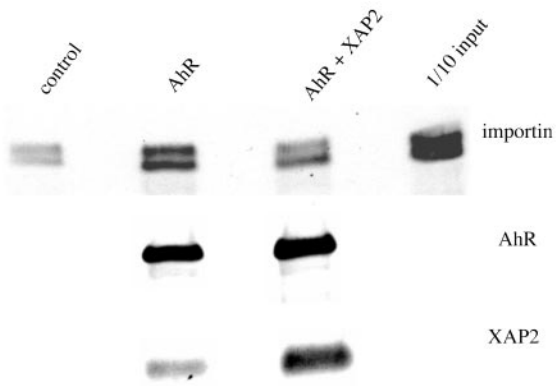

FIG. 7. **Importin β binding to the AhR is inhibited by XAP2.** COS-1 cells were transfected with pcDNA3-βmAhR-FLAG in the presence or absence of pCI-XAP2 and AhR-FLAG complexes were immunoprecipitated from cytosolic extracts. The isolated AhR complexes were incubated with *in vitro* translated [^{35}S]methionine-labeled importin β. The AhR-FLAG complexes were displaced from the agarose with an excess of FLAG peptide, resolved by Tricine SDS-PAGE, and transferred to PVDF membrane. The presence of [^{35}S]methionine-labeled β-importin was assessed by phosphorimaging, whereas the AhR and XAP2 were visualized using standard immunochemical techniques as described under "Experimental Procedures."

XAP2 Represses mAhR Transactivation Potential at Low Agonist Levels—Previous published reports from several laboratories have shown that transient expression of XAP2 in established cell lines increases AhR transcriptional activity upon treatment with saturating levels of TCDD (6, 8). Considering that XAP2 is able to stabilize the AhR and block nucleocytoplasmic shuttling, we wanted to explore whether under the appropriate conditions XAP2 can repress AhR transcriptional activity. In addition, it is reasonable to hypothesize that inhibition of ligand-mediated transformation of the AhR by XAP2 would be greater in the presence of a relatively weak ligand. To test this hypothesis, COS-1 cells were transfected with either pcDNA3/βmAhR or pcDNA3/βmAhR plus pCI/XAP2 at two different levels, followed by treatment with various concentrations of iodoflavone. Iodoflavone was chosen because it has intermediate affinity for the AhR (25). AhR transcriptional activity was assessed using a DRE-driven luciferase reporter. A significant level of inhibition of reporter activity is observed in the presence or absence of ligand upon co-transfection with XAP2 (Fig. 8, *A* and *C*). The *graph* in *panel A* (Fig. 8) reveals an inhibition of reporter activity except at relatively high ligand concentrations (*e.g.* 5 μM). This result is even more striking when you consider that, at high levels of co-transfected XAP2, receptor levels actually increase (Fig. 8*B*). In addition, expression of an intermediate amount of XAP2 leads to repression of iodoflavone-mediated mAhR activity even at a saturating dose of ligand (Fig. 8*C*). It is important to note that both constitutive and inducible mAhR activity are repressed by XAP2 expression. These results would indicate that XAP2 does repress transcriptional activity of the mAhR.

DISCUSSION

Upon transient expression in cells, the mAhR erroneously localizes to the nuclear compartment of cells. This apparent artifact is corrected upon co-expression of the XAP2, resulting in a dramatic re-localization of the mAhR exclusively to the cytoplasm (12, 14, 15). The mechanism by which XAP2 modulates the subcellular localization of the mAhR is unknown, but may result from a number of possible scenarios. Prior to undertaking these studies, some of the possibilities included, but were not limited to, the following. 1) XAP2 may enhance the nuclear export of the mAhR, leading to apparent cytoplasmic localization; 2) XAP2 may inhibit nuclear retention of the mAhR; 3) XAP2 may mediate sequestration or tethering of the mAhR in the cytoplasm by an unknown mechanism; 4) XAP2 may physically mask the NLS of the mAhR, thereby inhibiting nuclear translocation pending a ligand-induced conformational change that results in exposure of the NLS and subsequent binding of the appropriate NLS recognition molecules; 5) XAP2 may stabilize the mAhR in a conformation in which the NLS is exposed, but not recognized by import machinery. A variety of experiments based on the above hypotheses were performed and are presented in this report.

Leptomycin B is a specific inhibitor of CRM-1-mediated nuclear export; CRM-1 has been demonstrated to be responsible for nuclear export of the AhR (20). The experiments documented in Fig. 1 examined the possibility that XAP2 might enhance nuclear export of the mAhR, thus resulting in cytoplasmic localization of the mAhR. As a possible mechanism underlying the re-localization of the mAhR by XAP2, this theory was quickly eliminated. As can be seen in Fig. 1, co-expression of XAP2 with mAhR-YFP resulted in a strong inhibition of ligand-independent nucleocytoplasmic shuttling. The mAhR-YFP localized throughout cells with higher concentrations in the nucleus, a further increase in nuclear accumulation followed after 1 h of treatment with 10 nM leptomycin B. Co-expression of XAP2 resulted in cytoplasmic localization of mAhR-YFP, and receptor localization remains largely unaffected after 1 h of leptomycin B treatment. The cells were monitored for 6 h following treatment, and no significant nuclear accumulation was observed (data not shown). This result has been recently observed in similar experiments performed in HeLa cells (22). As a control, mAhR-K13A-YFP (mutation in NLS) was also examined. Lack of a functional NLS resulted in strong cytoplasmic localization, and no nuclear accumulation was observed following leptomycin B treatment, suggesting that the NLS of the mAhR is required for nucleocytoplasmic shuttling. The observation that the mAhR undergoes rapid ligand-independent nucleocytoplasmic shuttling strongly suggests that the NLS of the mAhR is exposed and can be recognized by components of the nuclear import pathway in the absence of ligand. Both GST-GFP fusions of an N-terminal fragment of the hAhR as well as the endogenous mAhR have been reported to undergo ligand-independent nucleocytoplasmic shuttling (19, 22).

We have reported that transiently expressed mAhR, in the absence of co-expressed XAP2, is capable of existing in a core complex that lacks the XAP2 component, which we hypothesized may somehow lack the ability to be retained in the cytoplasm (14). This observation, together with the inhibitory effect of XAP2 on nucleocytoplasmic shuttling, suggests that shuttling may only be seen in complexes lacking XAP2, and further raises the possibility that the mAhR may exist in multiple forms. In addition, XAP2 probably exists in a dynamic equilibrium with mAhR complexes, with XAP2 dissociation enhancing receptor translocation to the nucleus, whereas hsp90 probably remains bound to the mAhR upon ligand-dependent and -independent translocation into the nucleus (26). The occurrence of ligand-independent nucleocytoplasmic shuttling itself suggests that we need to modify how we look at the mechanism of action of the mAhR. The rapid nucleocytoplasmic shuttling of the mAhR would indicate that the observed localization of the receptor at any given point in time is actually a snapshot of a dynamic process. Thus, the relative rates of import and export will determine the apparent subcellular distribution observed. The traditional view that ligand binding initiates nuclear translocation seems to be represented more realistically by a shift in the equilibrium to favor accumulation in the nuclear

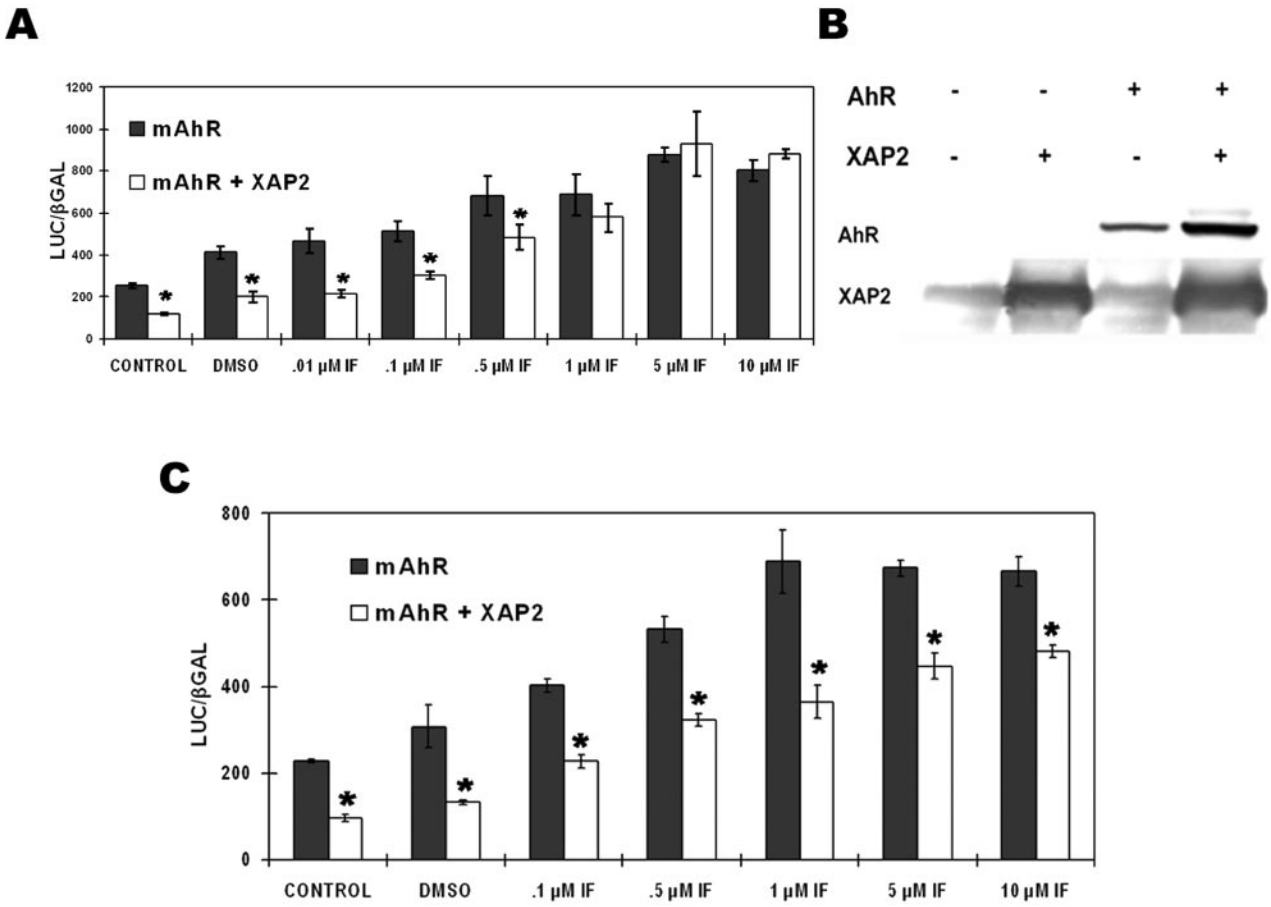

FIG. 8. **XAP2 represses AhR transcriptional activity at low agonist levels.** COS-1 cells in 100-mm plates were co-transfected with 0.5 μg of pcDNA3-βmAhR, 100 ng of pGudLuc 6.1, 30 ng of pCMV-βGal, either 2 or 4 μg of pCI/XAP2, and pCI was added, for a total of 8 μg of DNA. After 9 h transfected cells were transferred to 12-well plates; 13 h later cells were treated with iodoflavone. After 8 h in the presence of ligand, cells were lysed, and reporter activity was assessed. *A*, COS-1 cells were co-transfected with 4 μg of pCI-XAP2. *B*, the level of AhR and XAP2 in cells transfected with the same vector composition as in *A* was assessed by protein blot analysis. *C*, COS-1 cells were co-transfected with 2 μg of pCI-XAP2. An *asterisk* above the *bar* corresponds to a statistical difference ($p < 0.03$) using a paired Student's *t* test. The *error bars* represent the standard deviation obtained from triplicate samples.

compartment, as translocation appears to be continually occurring in the presence and absence of ligand. The nuclear accumulation that is observed in response to ligand binding is beyond the scope of this report, but may reflect either enhanced import of the receptor, diminished export, or a combination of both scenarios. Ligand binding may confer these changes in receptor movement by directly inducing a conformational change and/or by promoting an event such as phosphorylation/dephosphorylation that may mediate the altered localization.

The clear inhibition of nucleocytoplasmic shuttling of the mAhR by XAP2 revealed the possibility that XAP2 might promote or mediate association of the mAhR with cytoskeletal components. XAP2-mediated association of the receptor with cytoskeletal components is a possible mechanism for the altered subcellular localization that is induced by XAP2, as well as the inhibition of nucleocytoplasmic shuttling. This type of a role has been ascribed to FKBP52, the immunophilin component of the glucocorticoid receptor (GR) complex, which has been shown to associate with microtubules and is required for nucleocytoplasmic trafficking of the GR along the cytoskeleton (27, 28). We chose to examine both tubulin and actin components of the cytoskeleton. Treatment of cells with the cytoskeletal disrupting agents did not result in altered localization of mAhR-YFP, nor was ligand-dependent nuclear translocation affected when cells were treated with TCDD. These results suggest that XAP2 is not modulating the subcellular localization of the mAhR via interaction with actin- or tubulin-based cytoskeletal components, although association with some other cytoplasmic cellular component remains a possibility at this point. In contrast, Berg and Pongratz (29) have recently reported that actin is necessary for XAP2-mediated retention of the AhR. The reason for this difference in the results they obtained compared with this report is unknown. Their experiments were performed in HeLa cells, whereas COS-1 cells were used in this study. Nevertheless, our results are also consistent with the inability of cytoskeletal disrupting agents to alter the induction of CYP1a1 in murine Hepa 1c1c7 cells by AhR ligands (27). The lack of an effect of cytoskeletal disruption on ligand-induced nuclear accumulation suggests that the mAhR does not rely on targeted movement along microtubules as has been reported for the glucocorticoid receptor (28). Disruption of microtubule, microfilament, and intermediate filament networks does not cause a significant reduction in the rate of nuclear accumulation of the GR. However, geldanamycin is able to markedly reduce the rate of translocation of steroid-bound GR, whereas disruption of cytoskeletal structures leads to no observed inhibition of the rate of GR translocation upon geldanamycin treatment (29). This would suggest that hsp90·GR complexes utilize the cytoskeleton during transport to the nucleus, yet transport to the nucleus does not require an intact cytoskeleton. Although geldanamycin induces translocation of the AhR to the nucleus (data not shown), geldanamycin does not induce translocation of the unliganded GR complex. However, geldanamycin does inhibit liganded GR movement

into the nucleus (30). Thus, despite the fact that both the GR and AhR are chaperoned by hsp90, regulation of nucleocytoplasmic shuttling appears to be distinct.

To further explore the possibility that XAP2 may facilitate a physical tethering of the mAhR in the cytoplasm, and to test whether or not the effect of XAP2 on mAhR localization was mediated via the NLS of the mAhR, we created mAhR-YFP-Nuc and mAhR-K13A-YFP-Nuc. These fusion proteins are identical to mAhR-YFP and mAhR-K13A-YFP, except for the addition of three tandem repeats of the NLS of SV40 large T antigen to the C terminus of YFP of each fusion protein. The results of transient expression of these constructs and the appropriate controls in COS-1 cells are shown in Fig. 4. Again, mAhR-YFP is found localized predominantly in the nucleus and to a lesser extent in the cytoplasm, whereas co-expression of XAP2 results in mostly cytoplasmic localization. The mAhR-K13A-YFP mutant localizes exclusively to the cytoplasm. Both mAhR-YFP-Nuc and mAhR-K13A-YFP-Nuc were found to localize to nuclei, demonstrating that the added NLS was capable of directing nuclear import of the receptor, even when the NLS of the mAhR is nonfunctional. Significantly, co-expression of XAP2 did not result in altered localization, as both forms of mAhR-YFP-NLS can be seen to clearly localize to nuclei. The lack of an effect of XAP2 on the cellular distribution of mAhR-K13A-YFP-Nuc argues against a tethering mechanism underlying cytoplasmic sequestration. Taking into consideration these results, some type of structural modulation of the receptor such as altering the conformation of the bipartite NLS, or a physical masking of the NLS by XAP2 inclusion in the complex, becomes more attractive as a theory to explain XAP2-mediated cytoplasmic sequestration of the mAhR.

To explore the possibility that the presence of XAP2 in the core complex may result in physical masking of the NLS of the mAhR, a rabbit polyclonal antibody against the C-terminal portion of the bipartite NLS (amino acid residues 31–44) of the AhR was produced. The anti-NLS antibodies were then utilized to immunoprecipitate the AhR from cytosolic extracts of COS-1 cells, transiently expressing either the mAhR or mAhR together with XAP2. We have previously reported that, under the transfection conditions used, ~25% of transiently expressed mAhR complexes contain XAP2 when the mAhR is expressed alone, whereas co-expression of the mAhR with XAP2 results in XAP2 inclusion in nearly 100% of the mAhR complexes (14). The results summarized in Fig. 5*D* revealed that neither TCDD nor co-expression of XAP2 altered the ability of these antibodies to recognize the NLS-based epitope. Perhaps most important, the results clearly indicated that XAP2 is co-immunoprecipitated with the AhR. In addition, immunoprecipitation of the mAhR with the anti-AhR monoclonal antibody Rpt 9 (24), directed against the N-terminal portion of the NLS of the AhR (amino acid residues 12–31), resulted in co-immunoprecipitation of XAP2 (14), further suggesting that access to the N-terminal portion of the NLS is not blocked by XAP2. The lack of effect of XAP2 on the ability of AhR antibodies to bind the NLS of the mAhR clearly suggests that XAP2 does not physically mask the NLS when present in the complex. The mechanism of XAP2-mediated sequestration of the mAhR appears to be quite different from the model that has emerged explaining the mechanism of cytoplasmic sequestration of NF-κB subunit p65 by IκB. Using antibodies to the NLS of p65, several laboratories have clearly shown that IκB physically masked the NLS of p65 (31–33).

The results in Fig. 6 would indicate that the ability of importin β to bind to the NLS of the AhR is reduced when XAP2 is found complexed with the mAhR. This result suggests that XAP2 alters the conformation of the bipartite NLS of the AhR and leads to reduced recognition by importins. Considering that the site on the AhR required for XAP2 binding is near the ligand binding domain, it is tempting to speculate that the ligand binding pocket could also be altered by the presence of XAP2. Indeed this possibility is supported by the results in Fig. 8, which demonstrate that XAP2 represses the ligand-mediated activity of the mAhR, although this result may be because of an overall stabilization of the hsp90·mAhR complex or the blocking of nucleocytoplasmic shuttling in the presence of XAP2. Considering that XAP2 levels vary considerably from tissue to tissue, it is plausible that XAP2 could mediate tissue-specific differences in mAhR activity. In particular, the very low level of XAP2 in liver is consistent with the concept that the mAhR should be capable of being rapidly activated in response to dietary exposure to AhR ligands (9, 34). In summary, the data presented here support the assertion that XAP2 is capable of altering importin β recognition of the AhR, and this appears to be the primary mechanism of XAP2-mediated retention of the AhR in the cytoplasm. However, it is still possible that other mechanisms may also play a contributing role in the ability of XAP2 to block nucleocytoplasmic shuttling of the AhR. Finally, the work presented here is one of the first demonstrations of a clear functional role for an hsp90 co-chaperone protein in influencing the activity of a chaperoned soluble receptor.

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The hsp90 Co-chaperone XAP2 Alters Importin β Recognition of the Bipartite Nuclear Localization Signal of the Ah Receptor and Represses Transcriptional Activity

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