Two Distinct Regions of the Immunophilin-like Protein XAP2 Regulate Dioxin Receptor Function and Interaction with hsp90

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The dioxin (aryl hydrocarbon) receptor is a ligand inducible transcription factor, which mediates cellular responses to a variety of xenobiotic compounds such as dioxins. In the absence of ligand the receptor is associated with the molecular chaperone hsp90 and the tetratricopeptide repeat (TPR) containing immunophilin-like protein XAP2. XAP2 has been implicated in regulation of the intracellular localization of the dioxin receptor and protection of the receptor against degradation. In this study a series of XAP2 mutants has been generated in order to identify the structural motif(s) mediating interaction with the dioxin receptor-hsp90 complex and modulation of receptor function. Immunoprecipitation experiments demonstrated that the C-terminal part of XAP2, including the TPR motifs and the region outside the TPR motifs, was required to directly contact hsp90. The N-terminal part of XAP2 was required for the stability of the ternary dioxin receptor-hsp90-XAP2 complex. In addition, the integrity of the N-terminal region of XAP2 was essential for XAP2 to regulate the intracellular localization of the dioxin receptor. In conclusion, these data demonstrate that two distinct regions of XAP2 modulate dioxin receptor function and interaction with hsp90, illustrating the complexity in regulation of dioxin receptor signaling by the hsp90 molecular chaperone machinery.

Cellular responses to a variety of xenobiotic compounds including 2,3,7,8-tetrachlorodibenzo-p-dioxin and related environmental pollutants are mediated by the dioxin (aryl hydrocarbon) receptor. The dioxin receptor belongs to the basic helix-loop-helix (bHLH)/Per-Arnt-Sim domain (PAS) family of transcription factors. These proteins are characterized by two conserved domains, the N-terminal bHLH DNA binding domain and the PAS domain, which spans two hydrophobic repeats termed PAS-A and PAS-B. The dioxin receptor is unique among bHLH/PAS proteins in that it contains a ligand-binding region located in the C-terminal part of the PAS domain including the PAS-B motif (for a review, see Ref. 1). In the absence of ligand, the non-activated dioxin receptor is associated with the molecular chaperone hsp90 (2), the co-chaperone p23 (3, 4), and the immunophilin-like protein XAP2, also known as ARA9 and AIP (5–7). Upon ligand binding the dioxin receptor accumulates in the cell nucleus where it forms a transcriptionally active complex with the bHLH/PAS transcription factor Arnt, which, in turn, induces release of hsp90 from the receptor (4, 8, 9). The dioxin receptor-Arnt heterodimer activates transcription of target genes by specifically binding to dioxin inducible transcriptional control elements, XREs, which are located in regulatory regions of a network of genes encoding drug-metabolizing enzymes such as cytochrome P-4501A1, glutathione S-transferase Ya and quinone oxidoreductase (for a review, see Ref. 1).

The molecular chaperone complex hsp90 plays an important role in dioxin receptor signaling. Hsp90 interacts with two spatially distinct motifs of the dioxin receptor, the ligand-binding PAS-B and the bHLH domains (10, 11). hsp90 is required to maintain a high affinity ligand binding conformation of the receptor (12–14). The hsp90-associated molecular co-chaperone p23 appears to play a role in stabilizing dioxin receptor-hsp90 interaction (4). Another hsp90-binding protein, the 38-kDa immunophilin-like protein XAP2, was originally identified as the hepatitis B virus protein X-associated protein in yeast two-hybrid studies (15) and later this protein has been characterized as a constituent of the non-activated dioxin receptor complex (5–7). XAP2-dioxin receptor interaction is mediated by a C-terminal portion of the PAS domain of the receptor including the PAS-B motif (16, 17). XAP2 contains regions of homology with the immunophilins FKBP12 and FKBP52 (5–7). In addition, XAP2 contains three tetratricopeptide repeats (TPRs), a degenerated sequence motif of 34 amino acids, which play a role in mediating protein-protein interactions (18). TPR motifs of XAP2 display a high degree of homology with those of the steroid hormone receptor-interacting immunophilin FKBP52 (5–7). Unlike immunophilins, however, XAP2 does not bind FK506 (19). We and others (16, 17, 20) have observed that XAP2 has a stabilizing effect on dioxin receptor protein levels and has a role in regulation of the intracellular localization of the dioxin receptor by an uncharacterized cytoplasmic retention mechanism (17, 21). The exact physiological function of XAP2 in dioxin receptor-mediated signaling remains largely unknown. In the present study we have identified the structural motifs by which XAP2 regulates function of the dioxin receptor-hsp90 complex. To this end we have generated a series of XAP2 mutants and tested their ability to interact with the dioxin receptor-hsp90 complex and to modulate subcellular localization of the non-activated receptor complex. These experiments demonstrate that the N-terminal region of XAP2 confers stability on the ternary XAP2-dioxin receptor-hsp90 complex, which is a critical prerequisite for XAP2-dependent regulation of dioxin receptor signaling, whereas the C-terminal domain of XAP2 mediates direct interaction with hsp90.
MATERIALS AND METHODS

Recombinant Plasmids—pGEM/XAP2 encoding full-length human XAP2 was a generous gift from Edward Seto (University of South Florida, Tampa), and pSUTHK/FKB52 encoding full-length human immunophilin FKB52 was generously provided by David Smith (Mayo Clinic, Scottsdale, AZ). To create expression vectors pCMV2/FLAG-XAP2 and pCMV2/FLAG-XAP2Δ113–161, pCMV2/FLAG-FKB52, pCMV2/FLAG-FKB52ΔDCR, pCMV2/FLAG-FKBP2DCR, and pCMV2/FLAG-FKB52XDCR, DNA fragments containing XAP2 and FKB52 fragments were amplified by PCR using the vectors pCMV2/FLAG-XAP2 and pSUTHK/FKB52 as templates, respectively. PCR products were subcloned, in-frame with the FLAG epitope, into pCMV2/FLAG (Kodak). 

The fidelity of the PCR-generated DNA sequences has been confirmed by automated sequencing. pCMX/DR-GFP was kindly provided by Jacqueline McGuire (Karolinska Institute, Stockholm). The GST-dioxin receptor fusion protein expressing pc/DR vector was kindly provided by Pilar Carrero (Karolinska Institute, Stockholm).

Sequence Analysis—Primary sequence alignment between human XAP2 (15) and human FKB52 (22) was performed by using the LALIGN program ((23), version 2.0u, found at the web site www.ch.embnet.org/cgi-bin/LALIGN_form_parser.

Cell Extracts and Immunoblot Assays—For in vivo immunoprecipitation experiments, COS7 cells were grown in 10-cm diameter dishes.

Expression vectors encoding GST-tagged dioxin receptor (4 µg) and FLAG-tagged proteins (2 µg) were transiently transfected using LipofectAMINE (Invitrogen) according to the manufacturer’s recommendations. To prepare whole cell extracts, cells were washed twice with cold phosphate-buffered saline, collected by centrifugation, and resuspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 50 mM NaCl, 0.5% Tween 20, 10 mM Na3VO4) supplemented with a protease inhibitor mixture (Complete-Mini, Roche Molecular Biochemicals). Cell suspensions were sonicated by two 4-s bursts. Lysates were cleared by centrifugation for 1 h at 5,000 rpm. Immunoprecipitated proteins were collected by centrifugation, and resuspended in lysis buffer supplemented with 1% SDS-PAGE and 50 mM Tris-HCl, pH 7.4. Lysates (200 µg) were immunoprecipitated overnight at 4 °C with 5 µg of anti-FLAG (Sigma, dilution 1:500), murine anti-FLAG (Sigma, dilution 1:2000) or rabbit polyclonal anti-hsp90 (Ref. 24, dilution 1:500) antibodies in phosphate-buffered saline, collected by centrifugation, and resuspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 50 mM NaCl, 0.5% Tween 20, 10 mM Na3VO4) supplemented with a protease inhibitor mixture (Complete-Mini, Roche Molecular Biochemicals). Cell suspensions were sonicated by two 4-s bursts. Lysates were cleared by centrifugation for 30 min at 13,000 × g at 4 °C. 600–800 µg of total cellular protein were incubated with anti-FLAG (Sigma) or anti-GST (Amerham Biosciences) antibodies at 4 °C for 2–3 h. Immunocomplexes were precipitated by adding 25 µL of a 50% slurry of protein A-Sepharose (Amerham Biosciences) followed by incubation at 4 °C under slow rotation for 90 min. After rapid centrifugation, the resulting pellets were washed four times with 1 mL of cold lysis buffer. Immunoprecipitated proteins and whole cell extracts were analyzed by 7.5% or 12% SDS-PAGE and transferred to nitrocellulose membranes. Immobilized proteins were incubated for 2 h at 25 °C with primary rabbit anti-dioxin receptor (Biosciences), mouse anti-FLAG ( Sigma, dilution 1:2000) or rabbit polyclonal anti-hsp90 (Ref. 24, dilution 1:500) antibodies in blocking solution (5% nonfat milk in phosphate-buffered saline). Horseradish peroxidase-conjugated anti-rabbit (Dako) or anti-mouse (Amerham Biosciences) immunoglobulins were used as secondary antibodies diluted 1:500–1000 in blocking solution. After extensive washing in phosphate-buffered saline, 0.2% Tween 20, immunocomplexes were visualized using enhanced chemiluminescence reagents (Amerham Biosciences).

RESULTS

The Distal C-Terminal Region of XAP2 Is Critical for Interaction between XAP2 and hsp90—Recent studies have indicated that interaction between the immunophilin-like protein XAP2 and hsp90 requires the presence of TPRs, which are located in the C-terminal portion of XAP2 (26, 27). However, the TPR motifs alone appear to be insufficient to mediate the interaction between the XAP2 homolog AIP and the dioxin receptor complex (26). In addition to the TPR motifs, this interaction has been reported to require an additional C-terminal amino acid region, which is located outside the TPR domain, including the very last five amino acids of AIP (326GIFSH330), which have been proposed to directly contact the dioxin receptor (26). Given this background it is important to elucidate the potential role of the distal C-terminal region (DCR) located C-terminally from the TPR motifs of XAP2 in creating a substrate-specific recognition site for the dioxin receptor. To address this question, we examined if the DCR of XAP2 will be able to act as an independent interaction interface for the dioxin receptor when fused to the immunophilin FKB52, which specifically interacts with steroid hormone receptors (for a review, see Ref. 28), but does not form a stable interaction with the dioxin receptor (19). We generated FLAG epitope-tagged fusion constructs spanning wild-type XAP2, wild-type FKB52, DCR deletion constructs of XAP2 and FKB52, respectively, and chimeric XAP2-FKB52 proteins in which the DCR motifs were reciprocally exchanged (schematically represented in Fig. 1A). These constructs were transiently expressed in COS7 cells (Fig. 1B, upper panel) together with the GST-tagged dioxin receptor or GST tag alone (Fig. 1B, lower panel). Protein complexes bound to the FLAG-tagged XAP2 and FKB52 constructs were immunoprecipitated from whole cell extracts (WCEs) by anti-FLAG antibodies. As shown in Fig. 1C, wild-type XAP2 was specifically immunoprecipitated together with the dioxin receptor (upper panel, compare lanes 1 and 3) and hsp90 (lower panel, compare lanes 1–3). XAP2 weakly interacted with hsp90 in the absence of the dioxin receptor, while in the presence of the receptor, XAP2 association with hsp90 was markedly increased (lower panel, compare lanes 2 and 3). The DCR deletion construct of XAP2 (XAP2ΔDCR), however, failed to interact with both the dioxin receptor (lane 5 in upper panel) and hsp90 (lanes 4 and 5 in lower panel). Interestingly, fusion of the DCR domain from FKB52 to XAP2ΔDCR fully restored the interaction of XAP2 with both the dioxin receptor (lane 7 in upper panel) and hsp90 in a receptor-dependent manner (lanes 6 and 7 in lower panel). As expected (19), wild-type FKB52, as well as the FKB52ΔDCR, did not interact with the dioxin receptor (upper panel, lanes 9 and 11, respectively). On the other hand, both constructs interacted with hsp90 with a similar potency. Consistent with earlier observations (19), the dioxin receptor had no stabilizing effect on association of the FKB52 constructs with hsp90 (lower panel, lanes 8–11). The DCR domain of XAP2, which contains the putative dioxin receptor contact site (326GIFSH330) (26), however, failed to mediate interaction with the dioxin receptor complex when fused to FKB52 (lane 13 in upper panel) and did not enhance the association between FKB52 and hsp90 (lower panel, lanes 12 and 13). These experiments suggest that the DCR domain of XAP2 is required for direct association between XAP2 and hsp90 rather than mediating specificity in XAP2-dioxin receptor interaction.

Because the DCR domain of FKB52 fully reconstituted the interaction between XAP2 and the dioxin receptor complex, it

* PCR primer sequence information and cloning details are available upon request.
was of interest to test whether this region of FKBP52 was also able to restore the function of XAP2 in regulating intracellular localization of the dioxin receptor. We therefore co-expressed various FLAG-tagged XAP2 or FKBP52 constructs together with GFP-tagged dioxin receptor in HeLa cells and examined the intracellular localization of the dioxin receptor-GFP by fluorescent microscopy (17, 21). In agreement with our earlier observations (17, 21), the dioxin receptor-GFP fusion protein was evenly distributed in both cell cytoplasm and nucleus when co-expressed together with the FLAG epitope alone. In contrast, upon co-expression of wild-type XAP2, we observed relocation of dioxin receptor-GFP to the cytoplasmic compartment of the cell (Fig. 2). Importantly, accumulation of the dioxin receptor-GFP in the cell cytoplasm was also mediated by the chimeric form of XAP2 (XAP2FDCR), which harbors the DCR domain of FKBP52 (Fig. 2). The other tested XAP2 and FKBP52 constructs, including XAP2ΔDCR, wild-type FKBP52 and the FKBP52XDCR, which did not associate with the dioxin receptor complex in the co-immunoprecipitation experiment (Fig. 1), had no effect on the intracellular localization of the dioxin receptor-GFP (Fig. 2).

Role of the N-terminal Region of XAP2 in Stabilization of the Ternary XAP2-Dioxin Receptor-hsp90 Complex—Next we examined the role of the N-terminal part of XAP2 in formation of the ternary XAP2-dioxin receptor-hsp90 complex. We generated a series of FLAG-tagged N-terminal deletion constructs of XAP2 (schematically presented in Fig. 3A) and the FLAG-tagged N-terminal fragment of XAP2, XAP2(1–169). These XAP2 mutants, as well as wild-type XAP2, were transiently expressed together with the GST-dioxin receptor in COS7 cells (Fig. 3B). The GST-dioxin receptor complex was immunoprecipitated by anti-GST antibodies, and FLAG-tagged XAP2 constructs were detected in immunoblot assays with anti-FLAG antibodies. As shown in Fig. 3C, deletion of the first 148 amino acids from the N terminus of XAP2 did not inhibit the binding of XAP2 to the dioxin receptor complex (lanes 4–7). Notably, the XAP2(84–330) construct was expressed at low levels, and it was very unstable in WCE preparations unless the dioxin receptor was present (see below). However, this XAP2 mutant efficiently interacted with both the dioxin receptor complex (lane 4) and hsp90 (see below). Further amino acid deletions from the N-terminal part of XAP2, however, completely abolished association between XAP2 and the dioxin receptor complex (Fig. 3C, lanes 8 and 9). The N-terminal fragment of XAP2 alone, XAP2(1–169), on the other hand, was unable to bind to the dioxin receptor (Fig. 3C, lane 10) because it lacks the TPR- and DCR-containing C-terminal structures critical for interaction with hsp90 (Refs. 26 and 27 and Fig. 1). It is noteworthy that among the tested XAP2 mutants none had a stabilizing effect on dioxin receptor protein levels. On the contrary, some of the N-terminal truncation mutants of XAP2 induced a decrease of GST-dioxin receptor expression levels (Fig. 3B, lower panel).

We also tested the ability of the various XAP2 N-terminal deletion mutants to interact with the molecular chaperone hsp90. The XAP2 mutants, as well as wild-type XAP2, were expressed in COS7 cells in the presence or absence of GST-dioxin receptor (Fig. 4A). FLAG-tagged proteins were immunoprecipitated by anti-FLAG antibodies (Fig. 4B), and immunocomplexes were visualized with anti-hsp90 antibodies in immunoblot assays. As already observed in the previous experiment (Fig. 1C), specific interaction between XAP2 and hsp90 was markedly increased in the presence of the dioxin receptor (Fig. 4B, lanes 1–3). This receptor-dependent stabilizing effect

![Image](attachment:11797.png)
The present data suggest that the N terminus of XAP2 serves as a site of interaction with the dioxin receptor complex and seems to be required for the stability of the ternary XAP2-dioxin receptor-hsp90 complex. To further test this hypothesis, we generated the two internal deletion mutants of XAP2, XAP2-(1–162) and XAP2-(162–330) (schematically depicted in Fig. 5A), which does not stably interact with the dioxin receptor by itself. FLAG-tagged XAP2-(162–330) and XAP2-(112–330) were expressed in COS7 cells in the presence or absence of GST-dioxin receptor (Fig. 5B). The GST-dioxin receptor complex was immunoprecipitated by anti-GST antibodies and analyzed by immunoblotting using anti-FLAG antibodies (Fig. 5C). As already observed above (Fig. 3C), GST-dioxin receptor specifically interacted with wild-type XAP2 (Fig. 5C, lanes 2 and 3) but no stable interaction was detected between the dioxin receptor and XAP2-(162–330) (Fig. 5C, lanes 4 and 5). Interestingly, specific interaction with the GST-dioxin receptor was mediated by XAP2-(186–161) (Fig. 5C, lane 7). In a similar fashion, XAP2-(113–161) also interacted with the dioxin receptor (Fig. 5C, lane 9). In addition, despite the lower expression levels of XAP2-(113–161), this construct was recovered with a greater efficiency as compared with XAP2-(186–161) (compare lanes 7 and 9 in Fig. 5, B and C). Notably, although the internal deletion mutants interacted with the dioxin receptor, these constructs displayed a rather destabilizing effect on protein levels of the receptor (Fig. 5B, lower panel). A similar effect was
produced by N-terminal truncation mutants of XAP2. The anti-FLAG immunoprecipitation experiment (Fig. 5D), performed in parallel to the anti-GST experiment, showed that all tested XAP2 constructs specifically interacted with hsp90 (lanes 1–9). As observed above (Fig. 4), interaction between wild-type XAP2 and hsp90 was markedly increased by the presence of the dioxin receptor in the complex (compare lanes 2 and 3), whereas the receptor-dependent stabilizing effect on XAP2-hsp90 interaction was completely lost in the case of XAP2-(162–330) (lanes 4 and 5). On the other hand, the dioxin receptor had only a marginal stabilizing effect on the interaction between hsp90 and the two internal deletion mutants of XAP2, XAP2-Δ86–161 and XAP2-Δ113–161, respectively. Taken together, these data support our notion that the N-terminal part of XAP2 serves as an interaction site for the dioxin receptor complex and plays an important role in conferring stability upon the ternary XAP2-dioxin receptor-hsp90 complex.

Regulation of Dioxin Receptor Function by XAP2—The molecular mechanisms underlying XAP2-induced relocalization of the dioxin receptor to the cytoplasmic compartment of the cell are unknown. To clarify this question it would be important to identify the structural region(s) within XAP2 that are required to regulate the intracellular localization of the dioxin receptor. We therefore examined effects of the N-terminal mutants of XAP2 on intracellular localization of the dioxin receptor by co-expressing these proteins together with the GFP-tagged dioxin receptor in HeLa cells. The intracellular localization of the dioxin receptor-GFP was monitored by fluorescence microscopy, and quantitative evaluations of green fluorescent cells were performed as described earlier (21, 25) and under “Materials and Methods.” Representative images of green fluorescent cells are presented in Fig. 6. Upon co-expression of XAP2-(84–330), dioxin receptor-GFP displayed an unusual perinuclear localization pattern very different from the pattern induced by wild-type XAP2, where the dioxin receptor-GFP is evenly distributed within the cytoplasmic compartment of the cell (Ref. 17 and Figs. 2 and 6). Mutants XAP2-(112–330) and XAP2-(121–330) (and XAP2-(148–330); not shown), however, failed to induce cytoplasmic relocalization of dioxin recep-
tor-GFP (Fig. 6). Moreover, according to the statistical evaluation of the data from these experiments, these constructs resulted in an increase in the number of cells displaying the predominantly nuclear localization pattern of dioxin receptor-GFP. More specifically, predominant nuclear localization (corresponding to categories C–N and N, as outlined under “Materials and Methods”) was observed in 10–15% of control cells, whereas this intracellular localization pattern was observed in 50–60% of cells expressing mutants XAP2-(112–330) and XAP2-(121–330) (data not shown). We suggest that this may be due to a depletion of the dioxin receptor from the cell cytoplasm rather than facilitation of ligand-independent nuclear import of the receptor, because we observed a decrease in protein levels of the dioxin receptor in the presence of XAP2-(112–330), XAP2-(121–330) and XAP2-(148–330) (Fig. 3). Notably, XAP2-(162–330) did not have any detectable effects on the intracellular localization pattern of dioxin receptor-GFP (Fig. 6), in agreement with the observation that this XAP2 mutant does not stably interact with the dioxin receptor complex (Fig. 3). In the presence of the internal-deletion constructs of XAP2, XAP2Δ86–161 and XAP2Δ113–161, we observed perinuclear accumulation of dioxin receptor-GFP. A similar intracellular distribution pattern was also observed in the presence of XAP2-(84–330) (Fig. 6). Thus, despite the ability of a number of the deletion mutants of XAP2 to interact with the dioxin receptor, i.e. XAP2-(84–330), XAP2-(112–330), XAP2-(121–330), XAP2Δ86–161, and XAP2Δ113–161, these constructs failed to induce the homogenous cytoplasmic localization pattern of the dioxin receptor-GFP in the cell cytoplasm. Thus, our results show that deletions of the N-terminal region of XAP2 compromised the function of this protein in regulating intracellular localization of the dioxin receptor. In conclusion, our data suggest that the N-terminal part of XAP2 is required not only to mediate the interaction with the dioxin receptor but is also of critical importance for XAP2 function.

**DISCUSSION**

The immunophilin-like protein XAP2 is a recently identified immunophilin component of the dioxin receptor-hsp90 complex (5–7), whereas the homologous immunophilin FKBP52 specifically interacts with steroid hormone receptors (see Ref. 28 for a review) and does not form a stable complex with the dioxin receptor (Ref. 19 and Fig. 1 in this study). The C-terminal part of XAP2 contains three TPR motifs, which share a high degree of homology with the immunophilin FKBP52 (5). In analogy to FKBP52, the TPR region of XAP2 is critical for interaction with hsp90 (26, 27). It is still unclear how immunophilin proteins such as FKBP52 and XAP2 recognize their cognate target hsp90-bound complexes. Interestingly, a comparison between the immunophilins FKBP52 and FKBP51 with regard to their ability to recognize the progesterone receptor-hsp90 complex indicated that the C-terminal region of FKBP51, located outside the TPR motifs, plays a role in conferring receptor specificity (29). In the case of the XAP2 homolog AIP it has been shown that, in addition to TPR motifs, C-terminal amino acids located outside the TPR region are required for interaction with the dioxin receptor complex (26). Intriguingly, the very last five amino acids of AIP (326GIFSH330) have been implicated in mediating a direct contact between the immunophilin-like protein and the dioxin receptor. On the other hand, our data indicate that the DCR region located outside the TPR motifs of XAP2 can be functionally substituted by the analogous region from FKBP52. Notably, immunoprecipitation experiments suggest that this region of XAP2 is more likely to play a role in mediating direct association with hsp90 rather than mediating preferential binding to the dioxin receptor complex. However, our results do not exclude the possibility that the DCR domain of XAP2 may contribute to direct association between XAP2 and the dioxin receptor.

The alignment of primary sequences revealed a 28.9% amino acid identity between the N-terminal part of XAP2 (amino acids 9–90) and the N-terminal part of FKBP52 (amino acids 29–107) which also overlaps with its peptidyl-prolyl isomerase domain (22). It is presently not clear whether the peptidyl-prolyl isomerase-like domain of XAP2 has a catalytic activity. Our experiments on deletion mutants of XAP2 suggest that the N-terminal portion of XAP2 contains an additional site of interaction with the dioxin receptor or dioxin receptor-hsp90 complex. Thus, this structure acts in concert with the C-terminally located TPR motifs and the distal C-terminal region of XAP2 in recognition of the dioxin receptor complex. In addition, the N-terminal region of XAP2 seems to confer stability to the ternary XAP2-dioxin receptor-hsp90 complex, because association between the N-terminal truncation/internal deletion mutants of XAP2 and hsp90 was poorly stabilized by the presence of the dioxin receptor. In contrast, wild-type XAP2 binds hsp90 very tightly in the presence of the receptor. Importantly, the loss of the ability of XAP2 to stabilize a ternary complex with the dioxin receptor and hsp90 correlates with the failure of XAP2 to induce a homogeneous, predominantly cytoplasmic localization of the non-activated dioxin receptor complex. Notably, none of the tested N-terminal deletion mutants of XAP2 showed any stabilizing effects on dioxin receptor protein levels. In fact, we observed a decrease in dioxin receptor protein levels upon co-expression of some of these XAP2 mutants. Two plau-

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**FIG. 6. The integrity of the N-terminal part of XAP2 is critical in regulating intracellular localization of the dioxin receptor.** GFP-fused dioxin receptor was expressed together with FLAG-tagged wild-type XAP2 and FLAG-tagged XAP2 mutants including N-terminal truncations of XAP2 (XAP2 84–330, XAP2 112–330, XAP2 121–330 and XAP2 162–330), and internal deletion mutants of XAP2 (XAP2Δ86–161 and XAP2Δ113–161). Co-expression of the FLAG peptide alone was used in control experiments. Intracellular localization of the dioxin receptor-GFP was examined by fluorescent microscopy as described under “Materials and Methods.” Representative images of the green fluorescing cells expressing dioxin receptor-GFP together with the indicated XAP2 constructs are shown.
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