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The role of chaperone proteins in the aryl hydrocarbon receptor core complex

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Abstract

The aryl hydrocarbon receptor (AhR) exists in the absence of a ligand as a tetrameric complex composed of a 95–105 kDa ligand binding subunit, a dimer of hsp90, and the immunophilin-like X-associated protein 2 (XAP2). XAP2 has a highly conserved carboxy terminal tetratricopeptide repeat domain that is required for both hsp90 and AhR binding. Hsp 90 appears to be involved in the initial folding of newly synthesized AhR, stabilization of ligand binding conformation of the receptor, and inhibition of constitutive dimerization with ARNT. XAP2 is capable of stabilizing the AhR, as well as enhancing cytoplasmic localization of the receptor. XAP2 binds to both the AhR and hsp90 in the receptor complex, and is capable of independently binding to both hsp90 and the AhR. However, the exact functional role for XAP2 in the AhR complex remains to be fully established.

Keywords: Chaperone proteins; Aryl hydrocarbon receptor core complexes; X-associated protein 2

1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-activated member of the basic-helix–loop–helix/Per–Arnt–Sim (bHLH/PAS) family of transcription factors [1]. It was discovered as a component of hepatic cytosol that bound specifically and with high affinity to a [^{3}H]-labeled analog of the persistent environmental contam-

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inant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, also commonly referred to as TCDD or dioxin) [2]. Correlations between the binding affinity of a number of dioxin congeners and induction of cytochrome P450 (CYP) enzyme activity were explained by the demonstration that this component of hepatic cytosol was a ligand-activated receptor that transcriptionally up-regulated CYP 1A1 and CYP 1A2, as well as a number of other gene products, many of which are involved in xenobiotic metabolism.

Today, the AhR[2] is known to bind to a wide variety of structurally dissimilar compounds with varying affinity [3]. Most notable are the polyhalogenated dibenzo-*p*-dioxins, polyhalogenated dibenzofurans, some polycyclic aromatic hydrocarbons, a variety of flavone derivatives, and co-planar members of the polyhalogenated biphenyl family. Compounds that bind the receptor with the highest affinity tend to be hydrophobic, planar, and of a defined size that is thought to facilitate occupation of a hydrophobic ligand binding pocket. However, AhR ligands are a diverse group of compounds that can structurally diverge from the requirements for maximal binding affinity. Exposure to the potent AhR ligand, TCDD, results in a wide array of biological responses. Some of the commonly observed responses to TCDD exposure include enzyme induction, immune suppression, thymic involution, chloracne, cellular hyperplasia, teratogenicity, and acute lethality which results from a poorly understood wasting syndrome. One of the hallmarks of TCDD toxicology is a wide spectrum of species sensitivity and variability in the observed biological responses. Not all endpoints are seen in any one species, and some species are much more sensitive to the production of a given biological response than others. A classic example is the dose of TCDD required to cause acute lethality, which varies by several orders of magnitude between the very sensitive guinea pig (LD_{50} $\sim$1 μg/kg), and the relatively tolerant golden Syrian hamster (LD_{50} $\sim$5000 μg/kg) [4–6]. The mechanism underlying the high degree of inter-species variability remains unclear.

Currently, it is widely accepted that the AhR mediates many, if not all, of the diverse biochemical, biological, and toxicological responses that are observed following exposure to TCDD and related compounds [7]. A large number of studies have consistently correlated binding affinity with activation of the receptor and subsequent production of a biological response [8]. The regulation of AhR activation has been examined primarily from the context of the role of protein–protein interactions. A number of proteins have been shown to be capable of interacting with the AhR including; heat shock protein 90 (hsp90), hepatitis B virus (HBV) X-associated protein 2 (XAP2), retinoblastoma (Rb), receptor interacting protein 140 (RIP140) and other coactivators, and Rel A [9–14]. In this review, we will focus on the role of chaperone proteins that interact with the unliganded AhR.

[2] Throughout this review AhR refers to the mouse AhR, except where otherwise noted.

2. Composition of the unliganded AhR core complex

The subunit composition of the AhR took years to resolve, due to studies being hampered by low levels of receptor expression (e.g. 100–1000 fmol per mg protein) combined with an inherent instability. The ability of the AhR to exist in a complex with hsp90 was initially established in two laboratories in 1988 [9,15]. In 1992, Perdew described chemical cross-linking studies that demonstrated the receptor existed in a heterotetrameric complex, composed of the AhR, and three additional components with M_r of approximately 88, 96, and 46 kDa [16]. This was determined in Hepa 1 cell cytosol pre-incubated with the photo-affinity ligand, [^{125}I]2-iodo-3-azido-7,8-dibromo-*p*-dioxin ([^{125}I]N_3Br_2DpD), followed by incubation with a homobifunctional cross-linking reagent, which resulted in a number of partially cross-linked complexes (Fig. 1). The minor band a′, shown in Fig. 1, is probably the low molecular weight protein bound directly to the AhR. Indeed, the ability of the low molecular weight protein to bind directly to AhR has been recently established [17]. The 88 and 96 kDa components were later shown to be isoforms of hsp90 [18], while the remaining component would not be identified for several years. Although, it is important to point out that other proteins may exist in these complexes but did not efficiently cross-link to components of the core complex. In addition, considering that detailed cross-linking studies have only been published for the AhR in Hepa 1 cells, it is quite possible that in other cell types, or species, other protein(s) could interact with the AhR. The domains directly involved in hsp90/AhR interac-

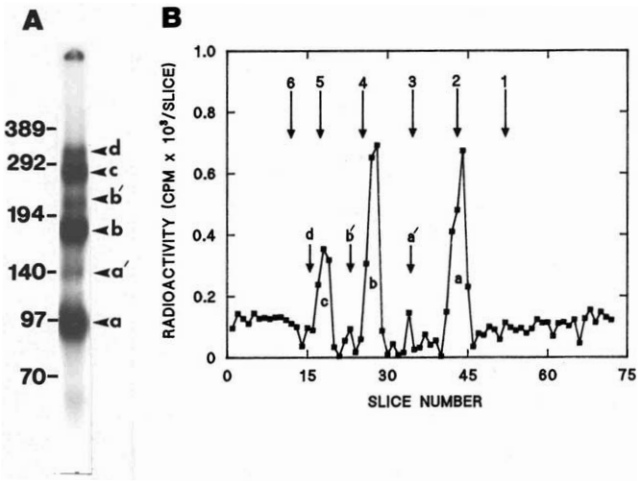

Fig. 1. Polyacrylamide gel electrophoretic analysis of cross-linked [^{125}I]N_3Br_2DpD-labeled Hepa 1 cell cytosolic extracts. Hepa 1 cytosolic extracts were incubated with [^{125}I]N_3Br_2DpD (photoaffinity ligand) and exposed to UV light. The cytosol was then incubated with dimethyl pimelimidate for 1 h and subjected to polyacrylamide gel electrophoresis as described [16]. Autoradiogram of the gel (panel A), the gel was cut into 2 mm slices and counted (panel B). The M_r for each band is: a, 97; a′, 140; b, 185; b′, 227; c, 281; d, 327 kDa.

tion have been mapped. The AhR binds to the middle portion of hsp90 (amino acid residues 272–617) as does the estrogen receptor [17]. Hsp90 binds to both the bHLH and PAS domains of the AhR, this latter binding domain appears to be adjacent or is an integral part of to the ligand binding domain [19–21]. Considering that two distinct domains of AhR are capable of binding to hsp90, it is possible that the AhR interacts with both proteins in the hsp90 dimer, however, this possibility has not been firmly established.

The role of hsp90 in the AhR complex has been examined in a number of reports that suggest hsp90 is required for the proper folding and stability of the AhR. Ligand occupation leads to down-regulation of AhR protein levels, both in cultured cells as well as in vivo [22–25]. This effect may be due to destabilization of the hsp90/AhR complex, although this has not been firmly established. Treatment of cells with geldanamycin, a benzoquinone ansamycin that binds to the ATP binding site of hsp90, leads to rapid proteolytic turnover of the AhR [26]. In fact, the AhR has been demonstrated to be more sensitive to disruption of hsp90 function than several other proteins chaperoned by hsp90 (e.g. glucocorticoid receptor, c-raf). Dissociation of hsp90 in vitro leads to an inability of the AhR to bind TCDD, suggesting that hsp90 maintains the AhR in a conformation required for ligand binding [27,28]. However, this effect appears to be species-dependent, as the guinea pig and rabbit AhR exhibit little change in ligand binding upon salt induced dissociation of hsp90 [29]. This may be in part due to species-specific differences in the relative stability of hsp90/AhR complexes. For example, mouse AhR/hsp90 complex is quite stable, while the human AhR/hsp90 complex requires molybdate to stabilize the complex [30,31]. Nevertheless, hsp90 appears to be critical to obtaining the proper ligand binding conformation after initial synthesis. An additional property of hsp90 is to repress the ability of the AhR to heterodimerize with ARNT [27,32]. This is particularly significant considering that, after binding ligand the AhR translocates into the nucleus bound to hsp90 [33]. Thus, the role of hsp90 in the AhR complex is quite similar to that which has been established for the glucocorticoid receptor [34].

3. XAP2 is present in the unliganded AhR core complex

The low molecular weight subunit of the AhR complex was recently identified as a 38 kDa protein that shares significant homology to the immunophilins FKBP12 and FKBP52. Interestingly, FKBP52 is an analogous component of unliganded glucocorticoid receptor complexes [34]. The 38 kDa component of the AhR core complex was independently identified by three laboratories as AIP (AhR interacting protein) [35], ARA9 (AhR associated protein 9) [36], and as a previously cloned protein known as XAP2 (HBV X-associated protein 2) [10]. XAP2 was discovered using a yeast two-hybrid assay due to its ability to associate with the X-protein of the HBV, and was found to repress the X-protein's transciptional activity [37]. Throughout this review, this protein will be referred to as XAP2, the name assigned this protein by the first group to clone and characterize this protein [37]. Thus, the

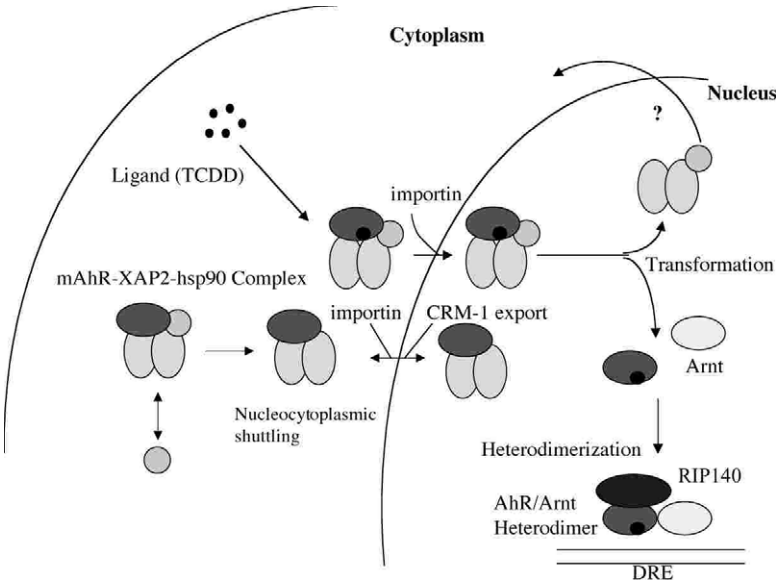

Fig. 2. Model of ligand-dependent and -independent movement of the AhR between the cytoplasm and the nucleus.

unliganded, inactive, cytoplasmic, AhR exists as a multimeric core complex that includes the AhR (ligand binding subunit), a dimer of the 90 kDa heat shock protein (hsp90), and the immunophilin homolog XAP2. Knowledge of the function of hsp90, and recent experimental evidence, suggest that a number of co-chaperones, such as p23, hsp70, and p60, may also associate with this complex at various stages during the lifetime of the receptor [38,39]. However, neither the presence of these components in the AhR complex from cell extracts nor a functional role for them has yet been established.

Being relatively hydrophobic in nature, AhR ligands are thought to enter cells by simple diffusion. Ligand binding to the AhR is a reversible event that induces a poorly defined process, commonly referred to as transformation. The transformation process is temperature dependent, and results in a ligand becoming bound with much higher affinity [40]. Numerous studies have suggested that the transformation process is a ligand-induced conformational change that confers upon the receptor the ability to translocate to the nucleus and heterodimerize with ARNT (AhR Nuclear Translocator also known as HIF-1β). The transformation process is complete upon formation of a heterodimer between the AhR and ARNT, a transcription factor complex that possesses high affinity for specific enhancer regions in DNA, termed dioxin responsive enhancers (DREs), which contain a core recognition motif. A model for the AhR pathway is presented in Fig. 2. Two theories exist as to the path the liganded AhR takes towards the formation of this heterodimer. The initial model is that ligand induced dissociation of the core complex occurs in the cytoplasm, which is followed by nuclear uptake of the free

AhR subunit and subsequent heterodimerization with ARNT. The second view point, supported by a growing body of evidence, suggests that ligand binding induces nuclear translocation of the entire core complex, which then dissociates only upon interaction with the ARNT protein [32]. However, recent observations made in our laboratory suggest that XAP2 does not remain bound to the AhR/hsp90 complex prior to nuclear translocation in the absence of ligand (unpublished observation, Perdew).

4. Characterization of XAP2

XAP2 was first discovered due to its ability to associate with the X-protein of the HBV [37]. Soon afterwards, it was independently identified from a yeast two-hybrid screen with the AhR [35,36]. The mRNA encodes a protein with a calculated molecular weight of 38 kDa. XAP2 is expressed in all tissues that have been examined, with the highest levels in the spleen and thymus, and the lowest levels in the liver, lung, and kidney [36,10]. The low level of XAP2 in liver is particularly noteworthy, considering the high level of AhR in this organ, which plays an important role in xenobiotic metabolism [36,37]. Interestingly, in situ hybridization studies have demonstrated that XAP2 is widely expressed during early development, suggesting additional roles beyond influencing AhR function [41]. XAP2 has been reported to localize predominantly to the cytoplasm [37]. However, transient expression of a YFP–XAP2 fusion protein has been observed to localize to both cytoplasm and nuclei [42].

XAP2 shares regions of significant homology with immunophilins such as FKBP52 (52 kDa FK506 binding protein). Although, XAP2 is not an immunophilin itself because it lacks affinity for the immunosuppressant drugs FK506 and rapamycin [36]. The domain structure of XAP2 (Fig. 3A) includes a putative peptidylprolylisomerase domain and several tetratricopeptide repeat motifs (TPR). TPR motifs are involved in protein–protein interactions and are found in a variety of proteins [43–46]. The TPR domain near the carboxy terminus is highly conserved and similar to the TPR found in FKBP52 and the yeast transcription factor SSN6 (Fig. 3B). Although, examination of the proposed α-helical structure shown in Fig. 3B reveals that two of the TPR consensus residues in Helix A of XAP2 are not conserved. This observation may help explain differences in the binding partner specificity between FKBP52 and XAP2.

5. Assembly of XAP2 into the AhR core complex

In mammalian cells, XAP2 has been found to associate with hsp90 [10,35], however, in reticulocyte lysate, in the absence of AhR, XAP2 has been reported to only weakly associate with hsp90 [47], or not bind hsp90 at all [10]. Immunoprecipitation of hsp90 complexes from cells co-immunoprecipitated hsp70, p60, FKBP52 and p50, later shown to be Cdc37 [48,49]. While a protein corresponding to XAP2

was not detected, perhaps because a low percentage of the XAP2 pool formed a stable complex with hsp90 within a cell. In a reticulocyte lysate system, the association of XAP2 with the AhR appeared to be an hsp90-requiring process, which was both ATP-dependent, and sensitive to geldanamycin [47]. They concluded that hsp90 is required for XAP2–AhR binding, and that the AhR is required to stabilize XAP2–hsp90 binding. Therefore, it appears that the assembly of XAP2 into the core complex is a co-operative process that requires all components to be assembled in concert to give a properly formed, stable, unliganded receptor. There is, however, evidence that XAP2 is not absolutely required in the complex to yield a functional AhR. Assembly of the AhR in reticulocyte lysate, which does not contain XAP2, results in a functional receptor that can bind ligand, transform, dimerize with ARNT, and bind to DREs [10]. In addition, evidence for the presence of two pools of Ah receptor in cell lines, one containing and one lacking XAP2 has been reported [50]. This observation arose from the examination

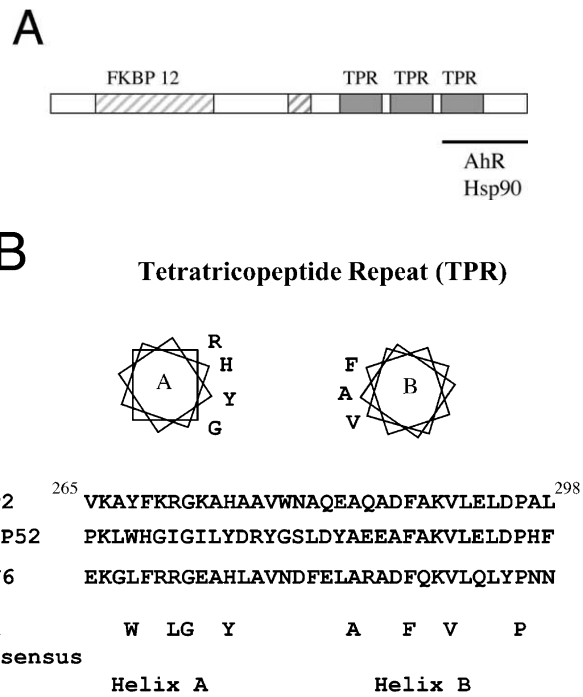

Fig. 3. Presence and structure of a conserved TPR motif in XAP2. There are three putative TPR motifs in XAP2, the third TPR, nearest to the carboxy terminus, is required for both AhR and hsp90 binding (Panel A). Regions of XAP2 with significant homology with FKBP12 are also shown. Alignment of XAP2 carboxy-terminal TPR, TPR3 of FKBP59, and TPR4 of SSN6 are shown in Panel B. The consensus TPR was generated from CDC16, CDC23, CDC27, SSN6, and SK13 [45]. Domains A and B of the third TPR domain of XAP2 are shown as α-helices, the amino acid residues represent the consensus residues found in the TPR of XAP2 (Panel B). The arginine and histidine residues in helix A are not conserved compared with the consensus sequence, these amino acid residues are isoleucine and tyrosine in FKBP52.

of the endogenous AhR of Hepa-1 cells, as well as transiently expressed AhR in COS-1 cells. In these cells, co-expression of the AhR and XAP2 resulted in the presence of AhR existing only with XAP2 bound, and correlates with a predominantly cytoplasmic subcellular localization of AhR–YFP in the presence of XAP2. Interestingly, the AhR from *Caenorhabditis elegans* and *Drosophilia* do not bind either XAP2 or TCDD, suggesting that the ability of the AhR to bind XAP2 and TCDD may occur only in vertebrates [47].

The study of truncation mutants of XAP2, used to localize domains required for AhR core complex assembly, has demonstrated that the C-terminal half (amino acids 154–330) of XAP2 is required for binding to both the AhR and hsp90 [36]. The functional significance of the N-terminal half of XAP2 remains to be determined. Point mutations in the conserved TPR motif have revealed that this region is important for binding to both the AhR and hsp90. Transient expression of XAP2 constructs containing point mutations in the conserved TPR-motif (Y268A, G272D, G272E, A284T, and F288A) has revealed that none were able to co-immunoprecipitate hsp90 [43]. Bell and Poland also described a TPR mutant of XAP2, K266A, which displayed reduced AhR binding and no observed binding to hsp90 [47]. In reticulocyte lysate, in the absence of hsp90, mutants Y268A, and A284T were capable of interacting with the AhR to a similar extent as wild-type XAP2, while mutants G272D, G272E, and F288A, were not. When these mutants were co-expressed in COS-1 cells with the AhR, each was able to co-immunoprecipitate both the AhR and hsp90, with the exception of G272D, and G272E, which were unable to co-immunoprecipitate either protein. These studies suggest that the conserved TPR motif is involved in binding to both the AhR and hsp90, with the hsp90 interaction being very sensitive to mutation in this region (e.g. no mutant tested bound hsp90 in the absence of AhR). While the ability of XAP2 to bind to the AhR appeared less sensitive to TPR mutations with only residue G272 being critical for assembly. These results also suggest that interaction of XAP2 with hsp90 is not critical for assembly of XAP2 into the AhR complex.

In addition to the TPR motif, the extreme C-terminus of XAP2 is also required for association with both the AhR and hsp90. Deletion of 32 amino acids from the C-terminus of XAP2 resulted in loss of hsp90 binding, while deletion of the last 17 amino acids of XAP2 appeared to result in rapid turnover in COS-1 cells, as expression of the mutant was undetectable [17]. Bell and Poland further examined the role of the C-terminus of XAP2 in binding to the AhR and hsp90 [47]. In reticulocyte lysate, they found that the five C-terminal amino acids of XAP2 are critical for interaction with hsp90, and alanine screening mutagenesis revealed that replacement of any of the last four amino acids with alanine results in a loss of binding to the AhR.

Hsp90 can be divided into three domains, N-terminal (residues 9–236), middle (residues 272–617), and C-terminal (residues 629–732). GST-fusions of these domains were used to determine the regions of hsp90 that are involved in binding to both the AhR and XAP2 [17]. Both the AhR and the estrogen receptor were found to bind to the middle region of hsp90, while XAP2 bound to hsp90's C-terminus, as did FKBP52 which interacts with hsp90 through a TPR region that

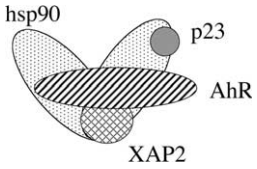

Fig. 4. Model for the arrangement of proteins found in the unliganded AhR complex.

is homologous to that of XAP2. Thus, the co-chaperone protein binds to the C-terminal domain of hsp90, which is also involved in hsp90 dimer formation [51].

A number of reports have described the regions of the AhR that are important for binding to XAP2. Deletion of the N-terminal 130 amino acids of the AhR did not disrupt binding to XAP2, while deletion of the N-terminal 287 amino acids abolished XAP2 binding [41]. From the C-terminus of the AhR, deletion of 313 amino acids did not reduce association with XAP2, while deletion of 425 amino acids from the C-terminus disrupted all binding to XAP2 [17,41]. The sequence of the Ah receptor that is required for XAP2 binding has been mapped to between amino acid residue 380–419, which is just C-terminal to the PAS domain [17]. Additionally, XAP2 has been shown to bind directly to the AhR isolated from cell extracts and stripped of hsp90, further suggesting that the presence of hsp90 is not required for XAP2 interaction [17]. Although after initial synthesis, hsp90 may well be required for the AhR to fold into the proper conformation to allow subsequent binding of XAP2. We have also found that the interaction of XAP2 with the AhR core complex is hydrophobic in nature. Co-immunoprecipitates of XAP2 with the mAhR were washed with increasing concentrations of either NaCl (50–1000 mM) or sodium deoxycholate (0.05–0.4% w/v) in the wash buffer. Washes with up to 1 M NaCl had little effect on XAP2 co-immunoprecipitating with the AhR, while co-immunoprecipitated XAP2 was reduced by $>60\%$ by inclusion of 0.15% w/v deoxycholate in the wash buffer (unpublished results, Perdew).

A proposed model showing the arrangement of the proteins in the unliganded cytosolic AhR complex is shown in Fig. 4. XAP2 appears to interact with both the AhR and hsp90 through its highly conserved TPR domain and C-terminus. Hsp90 interacts with the AhR through its central region, and XAP2 binds to hsp90 at the C-terminus, which appears to be the site of TPR-containing co-chaperone binding. The AhR interacts with XAP2 through its central region, which is also important for ligand binding and interaction with hsp90. Whether the AhR contacts both hsp90 molecules is difficult to assess, but appears to be likely. Considering that both a region within the PAS domain and the HLH region of the AhR are important for hsp90 binding, there may be two distinct contact sites.

6. Role of XAP2 in the AhR core complex

The discovery of XAP2 as a core component of the AhR complex has led several research groups to carefully examine its ability to influence AhR activity. One of

the first findings upon examination of the functional role of XAP2 in the AhR complex was that it appeared to enhance the transcriptional activity of the AhR. This was observed in Hepa 1c1c7 cells over-expressing XAP2, where TCDD treatment resulted in a 2–3-fold increase in CYP 1A1 mRNA levels relative to wild-type Hepa 1c1c7 cells [35]. Both Hepa 1c1c7 and HeLa cells transiently transfected with a DRE-driven luciferase reporter construct showed a twofold increase in reporter activity in cells co-transfected with XAP2 and followed by exposure to TCDD [10]. It is important to realize that this increase was obtained in cells that already express significant endogenous levels of XAP2. In a yeast AhR signaling system, the addition of XAP2 led to a fivefold decrease in the observed EC_{50} and a 2.5-fold increase in the maximal response following treatment with the AhR agonist β-naphthoflavone (βNF). This suggests that XAP2 is important in the initial folding and/or stabilization of receptor conformation. The mechanism underlying the enhancement of AhR transcriptional activity in mammalian cells appears to arise from the ability of XAP2 to stabilize the unliganded cytoplasmic AhR, leading to increased receptor levels, and hence an increase in signal transduction [17,42,52]. Meyer and Perdew reported that COS-1 cells transiently expressing the murine AhR and XAP2 had twofold higher levels of AhR relative to cells transiently expressing the AhR alone, as determined by western blot analysis of cytosolic extracts [17]. In a detailed examination of this phenomenon, LaPres et al. used both yeast and mammalian cell co-expression systems to examine XAP2's stabilizing effects on the AhR complex [52]. XAP2 was found to increase the levels of cytoplasmic AhR, and to increase the stability of the receptor under heat stress. The stabilizing effect of XAP2 on the AhR has been shown to be specific, and mediated by direct assembly of XAP2 into the core complex. Neither the immunophilin FKBP52, which shares significant homology with XAP2, and assembles into GR– and PR–hsp90 complexes, nor the TPR-containing protein PP5 (protein phosphatase 5) had an effect on receptor levels. Additionally, XAP2 constructs containing point mutations in the TPR-domains that are deficient in assembling into the complex had no effect on receptor levels [42]. A recent report has provided evidence that increased AhR levels result from the ability of XAP2 to protect the unliganded AhR from ubiquitination, thus limiting proteosomal degradation of the AhR [53]. One intriguing area of investigation is the determination of the reason that some steroid receptors (e.g. GR, PR) have evolved to contain FKBP52 as a co-chaperone, certain kinases (e.g. c-raf, cdk4) have the co-chaperone Cdc37, and the AhR contains XAP2 as a hsp90 co-chaperone. Perhaps each hsp90 co-chaperone has evolved to impart a distinct type of activity within these complexes. A comparison of the proteins found in steroid receptors, a protein kinase and in the AhR complex is shown in Table 1.

An interesting effect of XAP2, first reported by LaPres et al. is the ability to modulate the subcellular localization of the AhR [52]. Although the AhR is generally considered to be a cytoplasmic protein, transient expression results in distinct nuclear localization of the unliganded receptor. Indirect immunofluorescence microscopy of transiently expressed AhR in COS-1 cells revealed clear nuclear localization in the absence of ligand, while co-expression of XAP2 resulted

in a distinctly cytoplasmic receptor, which underwent nuclear translocation upon ligand treatment [52]. Examination of this phenomena in more detail with the use of an AhR–yellow fluorescent protein (AhR–YFP) fusion, in which YFP was linked to the C-terminus of the AhR [50]. The fusion protein was found to localize primarily to the nuclei of COS-1 and NIH 3T3 cells in the absence of ligand. Co-expression of XAP2 resulted in a clearly cytoplasmic receptor that was able to undergo ligand-dependent nuclear translocation. Neither XAP2 point mutants incapable of assembling into the AhR complex nor the immunophilin FKBP52 elicited any effect on the subcellular localization of AhR–YFP, demonstrating a specific requirement for assembly of XAP2 into the complex in order to obtain the modulation of subcellular localization. Hepa-1 cells were also examined by immunofluorescence microscopy for AhR localization. The endogenous AhR was found to localize to both cytoplasm and nuclei. The ratio of XAP2 to AhR in immunoprecipitated AhR complexes was examined and it was found that, in all cases where nuclear localization was observed in the absence of ligand, there existed a significant population of AhR complexes that lacked XAP2. This result strongly suggests that including XAP2 in the complex is required for cytoplasmic localization. In addition, the data indicates the existence of two populations of AhR, one including and one lacking XAP2, suggesting that each complex may be functionally distinct. This might help explain tissue specific differences in AhR activity due to the ubiquitous, but highly variable expression of XAP2 between tissues. The relatively high concentration of AhR in the liver coupled with the very low level of XAP2 expression would appear to indicate a low ratio of XAP2 in hepatic AhR complexes. Similar yet somewhat unique observations have been reported using a construct produced by fusing green fluorescent protein (GFP) to the N-terminus of the AhR (GFP–AhR), which was redistributed to the cytoplasm by XAP2 in HeLa cells [53]. However, in this system XAP2 also caused a marked delay in ligand-dependent nuclear translocation of the chimeric receptor, a result that has not been reported in other systems.

XAP2 is capable of enhancing the stability of the AhR as well as retaining the AhR in the cytoplasm [42,50,52,53]. Treatment of cells with leptomycin has revealed that the AhR rapidly accumulates in the nucleus, indicating that the AhR undergoes nucleocytoplasmic shuttling in the absence of ligand [54–56]. Lepto-

Table 1
Composition of select receptor/kinase complexes

	AhR	GR	c-Raf	PR
Hsp90	+	+	+	+
*Cdc*37	–	–	+	–
PKBP51/52	–	+	–	+
CYP40	–	+	–	–
XAP2	+	–	–	–
p23	+	+	?	+
14-3-3	?	+	+	?

mycin is an inhibitor of CRM1, a protein key to nuclear export of protein that recognizes nuclear export sequences. The presence of XAP2 prevents the AhR from undergoing nucleocytoplasmic shuttling. Recently the mechanism for this effect has been determined to be due to XAP2 altering the ability of the AhR to be recognized by importin β. This effect does not appear to be due to XAP2 physically blocking the nuclear localization sequence (unpublished data, Perdew), which suggests that XAP2 is released prior to nuclear transport of the unliganded AhR complex during nucleocytoplasmic shuttling. In Fig. 2 we also suggest that, upon ligand binding, the AhR–hsp90–XAP2 complex translocates into the nucleus. Ligand mediated nuclear uptake of the AhR complex is mediated by a conformational change that leads to enhanced importin recognition. The concept that XAP2 may alter the conformation of the AhR, coupled with the fact that XAP2 binds to the AhR near its ligand binding domain, may lead to alteration in ligand affinity or selectivity, but this possibility remains to be explored.

7. Presence of other proteins in the AhR core complex

The hsp90 accessory protein p23 has also been implicated in the function of the AhR. The unliganded AhR complex has been demonstrated to contain p23 in reticulocyte lysate and appears to play a role in the ligand mediated release of the AhR from hsp90 [38]. However, whether p23 plays a role in regulating AhR function within the cell remains to be established. The presence of p23 bound to hsp90 acts to stabilize its ATP-bound form and appears to stabilize mature steroid receptor complexes. Interestingly, p23's effect on steroid receptor activity varies, depending on the receptor examined. The glucocorticoid receptor is enhanced by co-expression of p23 in HeLa cells, while in contrast, the androgen and thyroid hormone are repressed by p23 [57]. Studies in reticulocyte lysate suggest that the AhR complex can associate with p60 and Hip, suggesting that the initial assembly of the AhR/hsp90 complex is similar to what has been described for the progesterone receptor [39].

8. Other roles for XAP2

As mentioned earlier, XAP2 was discovered due to its ability to bind to the X-protein of the HBV. XAP2 has been found to abolish transactivation by the X-protein, a promiscuous transcriptional activator whose function is unclear. The X-protein appears to be required for viral replication in vivo, and may play a role in HBV-induced hepatocarcinogenesis [37]. More recently, XAP2 has been reported to associate with another virally expressed protein, EBNA-3 (Epstein–Barr virus nuclear antigen 3), whose functional role in viral pathogenesis is currently unclear [58]. Considering that XAP2 is expressed in most tissue and cell types, this would suggest that XAP2 is involved in a number of physiological functions that remain to be discovered.

9. Conclusions

The AhR was been shown to assemble into a large oligomeric complex similar to the glucocorticoid and progesterone receptors. However, these complexes differ in terms of the hsp90 co-chaperone in the complex, with the AhR binding to XAP2, and GR and PR containing FKBP51/52 or CYP40. XAP2 binds to both hsp90 and the AhR and appears to stabilize the complex. In addition, XAP2 presence in the AhR complex appears to block nucleocytoplasmic shuttling through decreased importin binding. Whether or not XAP2 modulates ligand-mediated activity of the AhR remains to be established. For example, whether XAP2 alters the AhR's affinity for ligand or its relative affinities for various ligands needs to be explored. Considering that expression of XAP2 varies considerably from one tissue to another the effects of XAP2 on AhR activity could result in tissue specific effects. Clearly further studies are needed to unravel the role of XAP2 in modulating AhR function.

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