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Minireview

The dioxin (aryl hydrocarbon) receptor as a model for adaptive responses of bHLH/PAS transcription factors

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Abstract This review examines the common theme of adaptive responses of bHLH/PAS proteins, using the dioxin receptor as a prototype. The bHLH/PAS family of transcriptional regulators are a group of key developmental and environmental stress sensing proteins. They employ a variety of post-translational control mechanisms to regulate their transcriptional output. Amongst this family, the dioxin receptor is best known for its ability to elicit toxic responses to dioxin and dioxin like chemicals even though it mediates more benign adaptive responses to non-toxic xenobiotics. We discuss what is known about dioxin receptor physiology, both adaptive and inherent, along with its molecular regulation and put this into the context of the wider bHLH/PAS family. We also raise the issue of its toxic responses, in particular the idea that it is the dysregulation of its poorly characterised housekeeping functions that leads to these outcomes.
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Keywords: Dioxin receptor; bHLH/PAS; HIF; NXF; ARNT; hsp90; AIP/Ara9/XAP2

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Abbreviations: bHLH, basic helix-loop-helix; PAS, PER ARNT Sim; DNA, deoxyribonucleic acid; DR, dioxin receptor; AhR, aryl hydrocarbon receptor; HIF, hypoxia inducible factor; ARNT, aryl hydrocarbon receptor nuclear translocator; HLF, HIF like factor; EPAS, endothelial PAS; PHD, prolyl hydroxylase; VHL, Von-Hippel Lindau; IPAS, inhibitory PAS; PER, period; BMAL, brain and muscle ARNT like protein; NXF, neuronal transcription factor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; NLS, nuclear localisation sequence; NES, nuclear export sequence; hsp90, heat shock protein 90; CoCoA, coiled-coil co-activator; GAC63, GRIP1-associated co-activator 63; CRM-1, chromosome region maintenance protein 1; XRE, xenobiotic response element; ER, estrogen receptor; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; AhRR, AhR repressor; XAP2, hepatitis virus B X associated protein 2

1. Introduction

1.1. The bHLH/PAS family of transcriptional regulators

The bHLH/PAS (*b*asic *h*elix-*l*oop-*h*elix/*P*ER *A*RNT *S*im) family of proteins are grouped due to their shared structural motifs and are characterised as transcriptional regulators. A number of the members of this family have important roles in adaptive responses to generalised and cellular stress. Those members involved in stress response also play key developmental roles.

The common domain organisation is of a basic region present within the N-terminal end of the first helix in the helix-loop-helix motif followed by two PAS domains and a poorly conserved C-terminus (Fig. 1) (although some exceptions to this arrangement arise from splice variants). The bHLH is a motif common to a wide range of transcription factors all of which bind deoxyribonucleic acid (DNA) as dimers. The basic region is responsible for direct contact with DNA and the HLH serves as the dimerisation interface. The PAS domain serves as a secondary dimerisation domain dictating partner specificity and increasing the strength of dimerisation (reviewed in [1]). The dioxin receptor (DR or AhR (aryl-hydrocarbon receptor)) PAS domain has also, recently, been demonstrated to exhibit intramolecular interaction with the bHLH. This interaction may underpin high affinity DNA binding and DNA bending observed for the DR [2], further differentiating the bHLH/PAS family from other bHLH transcription factors. bHLH/PAS proteins fall into two classes based on their dimerisation potential. Class I factors are only capable of hetero-dimerisation with class II factors, which are capable of both hetero- and homo-dimerisation and therefore form obligate partners for class I factors (Fig. 1). Class I members serve as the transcriptional regulatory unit (e.g. DR or HIFα (*h*ypoxia *i*nducible *f*actor's)) which senses stimulatory cues and transmits these signals to the nucleus. Class II factors are constitutively nuclear and are absolutely required to achieve a DNA binding form and hence transcriptional competency. Current evidence suggests that class II factors may be capable of some signal sensing (eg, protein kinase A and C modulation of ARNT (*a*ryl-hydrocarbon *r*eceptor *n*uclear *t*ranslocator) activity [3]), but it is generally accepted that these factors simply serve as partner factors for the signal sensing class I factors.

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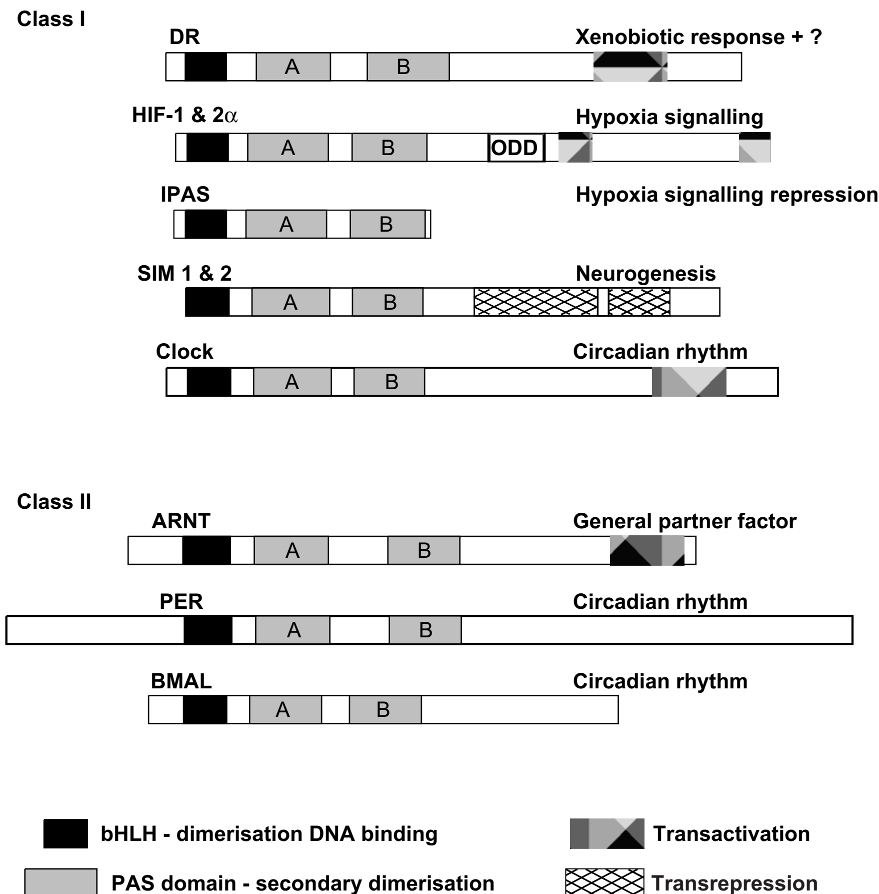

Fig. 1. The bHLH/PAS family of transcriptional regulators. Presented is a schematic representation of some of the bHLH/PAS family. PAS family members have in common an N-terminal bHLH DNA binding/dimerisation domain (with the exception of PER which lacks the basic DNA binding domain). The centralised PAS domain functions as a secondary dimerisation domain to enhance interactions between family members in addition to providing specificity between family members, such that members in the Class I signal sensing/responsive unit and are obligated to heterodimerise with a Class II family member. The PAS domain consists of two hydrophobic repeat regions A and B and in the case of the DR, PAS-B functions as a ligand binding domain.

Members of the bHLH/PAS family have been identified in a variety of multicellular animals including molluscs, arthropods, nematodes (see [4] and references therein) and a variety of vertebrates including mammals (for example see review [5]). So far none have been identified in prokaryotes, yeast, plants, protozoa or diploblasts suggesting this family of proteins arose during the appearance of the first triploblasts. The key structural elements of this family are ancient in origin, bHLH-containing proteins being found in plants and yeast and proteins containing PAS domains identified in all kingdoms. This suggests that these transcription factors may have arisen as a result of, or to accommodate, increased complexity of body plan.

The following is a brief description of several of the bHLH/PAS family members, focussing on those members that display a high level of regulation to control target gene output. The control mechanisms employed to modulate the activity of members of this protein family include novel forms of post translational modification, regulated nuclear import mechanisms, regulation of the ability of these factors to interact with transcriptional machinery and upregulation of target genes which act in negative feedback loops. Such a high level of control underpins the importance of these factors in adaptive regulation of physiological processes.

1.2. Hypoxia inducible factors

Among the bHLH/PAS family members, the hypoxia inducible factors (HIF's) provide some well understood regulation mechanisms. Oxygen delivery to cells is essential for survival of multicellular organisms and exquisite systems have evolved to sense and adapt to changes in both global and local-cellular oxygen concentration. One of these systems involves the HIF proteins, whose oxygen tension regulated activity is required as an essential part of development. Mice with targeted disruption of either HIF-1α or HIF-2α die of blood vessel malformation and for HIF-2α, potentially disrupted neurotransmitter signalling and lung function (reviewed in [6]). In normoxic conditions HIF-1α and HIF-2α (also known as HIF like factor (HLF) or endothelial PAS factor (EPAS)) protein levels are rapidly turned over via the ubiquitin/proteasome pathway (reviewed in [6,7]). Hydroxylation at critical proline residues by a family of prolyl hydroxylases (PHD 1, 2 and 3) enhances recruitment of the von Hippel Lindau/E3 ubiquitin ligase complex (VHL/E3 ligase) to affect degradation by the proteasome (reviewed in [6,7]). During periods of low oxygen tension, the oxygen dependent PHD enzymes are unable to function, recruitment of the VHL/E3 ligase is less efficient, the HIF-α subunits are more stable and translocate to the nucleus. In the

nucleus they dimerise with ARNT to form DNA binding complexes that interact with transcriptional co-activators such as CREB binding protein/p300 to activate target genes (reviewed in [7]). Interaction with co-activator proteins is also regulated by oxygen tension; in normoxia, hydroxylation of an asparagine residue within the C-terminal transactivation domain, by a separate enzyme (factor inhibiting HIF-1), inhibits co-activator recruitment (for review see [7]). Another layer of regulation exists whereby a novel bHLH/PAS factor termed inhibitory PAS (IPAS) (inhibitory PAS, a splice variant of HIF-3α) is upregulated in specific tissues in response to transient hypoxic conditions (reviewed in see [7]). For example, it is proposed that during sleep the cornea is exposed to hypoxia, triggering HIF-1 activation and upregulation of target genes including IPAS. IPAS lacks a C-terminal transactivation domain common to the HIF-1 factors so far identified, and thus acts as a dominant negative PAS factor, preventing upregulation of HIF-1 target genes during periods of sleep and hence preventing aberrant processes such as angiogenesis occurring in the eye.

1.3. Circadian rhythm proteins

A high proportion of the proteins involved in circadian rhythm are PAS family members, including one of the founding members of the PAS family: Period (PER). PER was isolated from a mutant locus that altered the light/dark cycle (circadian rhythm) of flies (reviewed in [8]). Biological rhythms are crucial elements in vertebrate biology and include the menstrual cycle, hormone levels and circadian rhythm. Since the initial cloning of PER, several PAS containing circadian factors have been cloned, which combine in a complex system of positive and negative regulation to co-ordinate adaptive molecular responses to external cues; a process known as entrainment (reviewed in [8]). There exists a remarkable conservation between components of the systems in simple eukaryotes, flies and mammals (reviewed in [8]). Central to the entrainment process are the PER proteins (PER 1-3), Clock and BMAL (*b*rain and *m*uscle *A*RNT *l*ike protein) (reviewed in [8]). As PER proteins lack a DNA binding domain, they are proposed to act as repressive proteins in this process (reviewed in [8]). The circadian rhythm proteins perform gene regulation via a combination of degradation of the signal sensing units, regulated nuclear/cytoplasmic shuttling and a complex interplay with negative acting factors, such as cryptochromes and timeless. This generates a competition effect between various clock components, for the E-box regulatory elements located in the control regions of target genes (reviewed in [8]). Two studies using high density and oligonucleotide arrays have shown that there are approximately 600 genes regulated in a rhythmic manner in the circadian pacemaker centre, the suprachiasmatic nucleus. Additionally, an equally high number of genes appear to be regulated in peripheral tissue such as the heart and liver (for example see [9]).

1.4. ARNT

A valuable tool in the analysis of DR signalling has been the murine hepatocellular carcinoma cell line Hepa1c1c7. Use of mutant variants of these cells with an elegant series of complementation experiments led to the cloning of the Class II partner factor, ARNT (reviewed in [1]). Subsequently, other ARNT like factors have been identified including ARNT2 and BMAL/ARNT3 (for review see [1]). ARNT or ARNT like factors are obligate partner factors for members of the bHLH/PAS family. *In vitro* gel shift experiments and reporter gene assays demonstrate that ARNT also has the ability to function as a homodimer by recognising the E-box element CACGTG (reviewed in [1]). Immunohistochemistry has demonstrated ARNT to be nuclear localised in both cell culture systems and in tissue sections [10,11]. However, an *in vivo* role for ARNT independent of a Class I PAS family member remains to be demonstrated. ARNT is ubiquitously expressed throughout development in all tissues ([12] and references therein) and is more ubiquitous in its expression than the other ARNT like proteins. The circadian rhythm factor BMAL is expressed primarily in the brain, heart and muscle [13] whilst ARNT2 is expressed predominantly in the brain and kidney [14]. Mice which have a targeted disruption in the ARNT gene fail to progress past embryonic day 10.5 due to a defect in vascularisation [15], coincident with the phenotype of the HIF-1α knockout (reviewed in [6]), demonstrating a crucial role for ARNT in HIF-1α activity and indicating a lack of functional redundancy between the ARNT proteins at this stage of development. Recently, it has been demonstrated that both ARNT and ARNT2 can act as partners for HIF-1α, but only ARNT can serve as a partner for DR [16]. This raises the possibility that adaptive responses might be further modulated through differential partner selection.

1.5. Neuronal transcription factor (NXF)

NXF (also Npas4 – neuronal PAS domain protein 4) is a recently identified brain specific member of the bHLH/PAS family [17] that is immediately and potently upregulated in ischaemia and seizure models ([18] and references therein). NXF appears to regulate its own expression in a feed forward mechanism, as well as activating Drebrin [17], a protein involved in dendritic function, and may be important in response to ischaemic stress.

2. The dioxin receptor – a prototype bHLH/PAS factor: stress and the rest

2.1. DR in the context of the organism

The DR is the only vertebrate member of the bHLH/PAS family known to bind and be activated by small chemical ligands. Many synthetic and naturally occurring planar polyaromatic xenobiotics can act as ligands, with the prototypical agonist for the DR being 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) (for review see [19]). Most naturally occurring ligands are of plant origin and are susceptible to metabolism and excretion, although metabolism of some ligands (e.g. the combustion derived benzo[a]pyrene) can produce toxic compounds. Amongst the large numbers of known and potential ligands, none are unequivocally endogenous, physiological ligands. Much evidence, however, indicates endogenous ligand(s) and/or activation mechanisms exist and this is discussed elsewhere in this issue [20].

Toxicity of dioxin in humans remains controversial, however both acute and chronic exposure leads to a range of toxic responses in animal models. These include severe wasting syndrome, chloracne, thymic involution, severe immune suppression, reduced fertility, hepatotoxicity, teratogenicity, tumour promotion and death [21]. Many xenobiotic meta-

bolising enzymes are upregulated in response to dioxin (and other ligand agonists) (reviewed in [19]), although dioxin itself is not a substrate and is not metabolised or readily cleared from the body (age and exposure dependent half-life in humans is up to 7 years (reviewed in [22]). Studies in DR null mice ([23] and references therein), mice with targeted replacement of DR with a nuclear localisation deficient DR [24] and the strong correlation between dioxin toxicity and intraspecies differences in DR affinity provide compelling evidence that the majority, if not all, of the toxic effects of dioxin are mediated by the DR ([23] and references therein).

Much evidence suggest that the DR has a role beyond adaptive response to xenobiotics. Evidence of developmental and immune function, together with data suggesting that activation can be independent of exogenous ligand, are discussed elsewhere in this issue [20]. A number of genuine target genes that do not fit into a xenobiotic metabolising role have been identified, including Ecto-ATPase (an extracellular enzyme with a possible role in the cell adhesion process), Adservin (a calcium dependent actin binding protein), *N*-Myristoyltransferase 2 (responsible for addition of myristoyl groups and implicated in regulating intracellular signalling) and Interleukin 2 (IL-2, a cytokine critical for T-cell proliferation and survival) (reviewed in [22]). This has been expanded using gene expression studies in which mRNA profiles were compared from livers of DR+/+ and DR−/− mice [25]. This analysis provides a list of 392 candidate DR dependent genes, whose expression is independent of exogenous ligand [25]. These data support a model in which the DR has roles, and cognate target genes, that might be described as housekeeping and developmental as well as an adaptive role in regulating the xenobiotic metabolising gene battery. Housekeeping/developmental roles are proposed to be independent of exogenous ligand, while adaptive roles are exogenous ligand dependent integrating the idea that aspects of TCDD toxicities arise from aberrant, sustained activation of target genes outside the adaptive repertoire.

Microarray studies investigating the effects of TCDD have given different data sets depending on tissue or cell type. These studies have shown that while prototypical genes for xenobiotic metabolism are globally induced, regulation of genes unrelated to xenobiotic metabolism vary widely. Evidence from rodents and zebrafish suggests that toxicity does not result from persistent upregulation of CYP1A1 levels (reviewed in [22]). Thus, the complex range of toxicities might be explained by multifactorial aberrations, varying widely across different tissues.

Much has been published on cross talk between DR and ER (estrogen receptor) work by Ohtake et al. [26,27] has demonstrated DR modulation of ER target gene activity by functioning as a transcriptional cofactor [26] and a component of a ubiquitin ligase complex [27]. As a co-activator, DR forms transcriptionally active complexes with unliganded ER on ER target gene promoters [26]. This activity is modulated through the DR's ability to act as a adaptor in an E3 ligase complex, an activity that is dependent on DR ligand [27]. It has also been demonstrated that unliganded ER can function as a co-activator for the DR [28,29]. These studies differ in the reported effect that ER and estrogen have on the activity of DR. It is clear that the ER can operate as a transcriptional cofactor on the *CYP1A1* promoter but it may depend on cell line, and hence other factors, as to whether estrogen stimulated ER enhances or represses transcription. These studies provide insight as to how the pro- and anti-estrogenic effects of dioxin may occur, but raises the question of what the normal biological function of this interaction is.

2.2. Regulation of DR activity

DR activation is regulated at several different levels, the best characterised of this regulation centring on the interaction of the ligand binding domain of the DR with the molecular chaperone heat shock protein 90 (hsp90). Initial models proposed that the major mode of DR regulation was cytoplasmic localisation and hence compartmental isolation from the partner factor ARNT [11] (and target gene enhancers). Ligand activation invokes a nuclear translocation event, inferred to be the result of a conformational change in the DR, exposing a nuclear localisation sequence located within the bHLH region (bipartite nuclear localisation sequence (NLS) aa13–17 and 37–42 of mDR (Figs. 2 and 3)) [30]. Upon entry into the nucleus, hsp90 is shed and the DR heterodimerises with ARNT (Fig. 2). Subsequent to ARNT dimerisation, DNA binding to XREs (Xenobiotic Response Elements (minimal consensus TNGCGTG [31])) enables the DR/ARNT complex to recruit basal transcription machinery, transcriptional coactivators and chromatin remodelling enzymes, upregulating transcription of target genes (Fig. 2). Coactivators and chromatin remodelling enzymes recruited by DR include transcription initiation factor IIB, steroid receptor coactivator 1, receptor interacting protein 140, brahma-related protein 1, thyroid receptor interacting protein 230 (reviewed in [32]), which bind defined regions within the DR C-terminal region and coiled-coil co-activator (CoCoA) and GRIP1-associated co-activator 63 (GAC63) ([33] and references therein) within the N-terminal region.

Several negative feedback systems exist to terminate DR signalling. In the case of metabolisable ligands, increased expression of xenobiotic metabolising enzymes eradicates the activating ligand and thus removes the initiating stimulus. Ligand activation also leads to targeting of the DR for ubiquitin-mediated degradation (see [34] for review) however the sequences mediating this degradation remain to be determined. The DR also contains two nuclear export sequences (NES) that promote cytoplasmic localisation of the DR (Figs. 2 and 3). One present at the end of PAS-A (aa216–224 of mDR) mediates nuclear export of unliganded receptor in a chromosome region maintenance protein 1 (CRM-1) dependent manner (reviewed in [34]). The second, N-terminal, NES is present in the bHLH (aa49–53 of mDR) mediates export of the ligand activated receptor and thus acts to dampen DR signalling (reviewed in [34]). This NES conforms to a consensus CRM-1 recognition site, however, both CRM-1 dependent (reviewed in [34]) and independent export [35] have been reported. Whether these NES motifs regulate the degradation of the DR by exporting it to the cytosol or whether the DR can be degraded in the nucleus remain to be elucidated. In either case the DR is rapidly depleted upon treatment with ligand [36] providing additional damping of signalling. It is expected that the DR will be subjected to post translational modifications to control the various stages of its pathway, but these events are, at this stage, ill defined.

Charge heterogeneity of the DR has been known for a long time and it has also been shown that this changes upon activation ([37] and references therein). *In vitro* dephosphorylation of the DR/ARNT heterodimer completely abolishes DNA

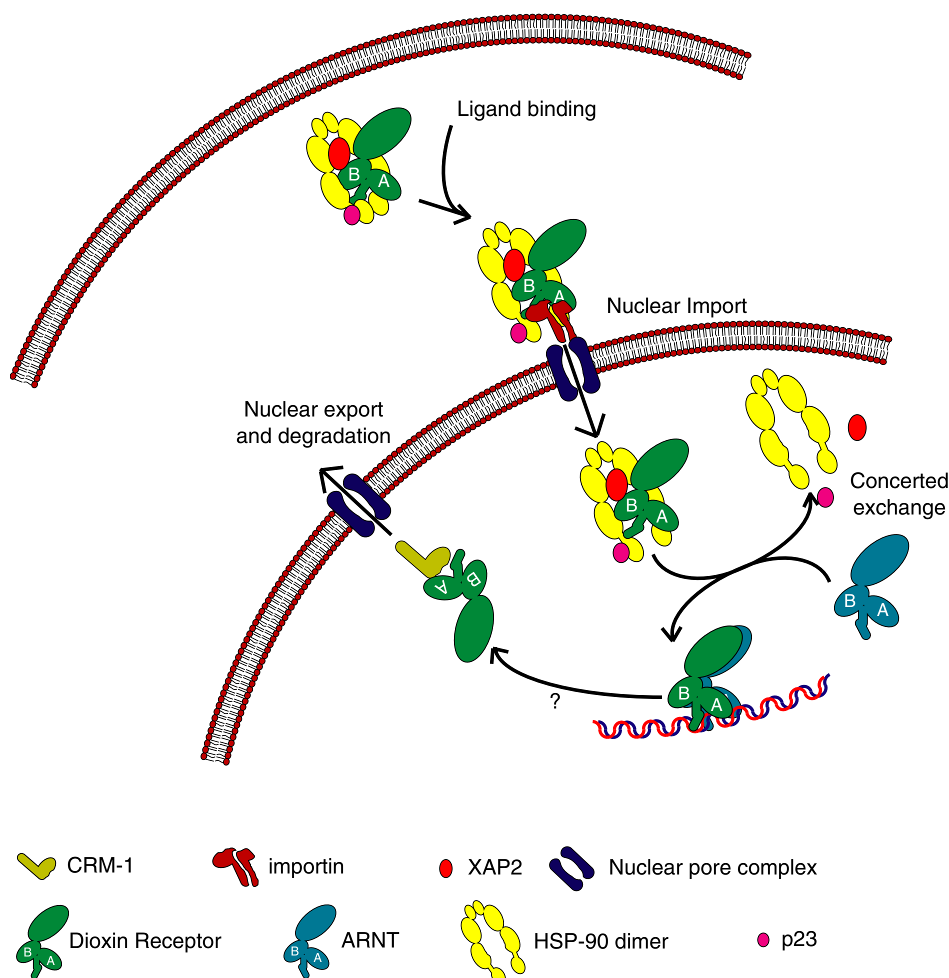

Fig. 2. The DR pathway. The DR resides in the cytosolic compartment of the cell, bound to the molecular chaperones hsp90, XAP2 and p23. Ligand activation of the DR invokes nuclear translocation via an N-terminal nuclear localisation sequence, transporting the DR into the nucleus by interaction with importin α and moves through the nuclear pore complex (NPC). Once in the nucleus, the DR heterodimerises with the nuclear partner factor ARNT to upregulate expression of xenobiotic metabolising enzymes in addition to the AhR repressor protein. During the activation process, hsp90 is shed from the DR in a poorly understood mechanism. Following ligand activation the DR is targeted for degradation via the addition of ubiquitin (Ub) and 26S proteasome mediated degradation. Shuttling between compartments also occurs.

binding [38]. Mutation of a tyrosine residue near the very N-terminus (aa9 of mDR) of the DR to phenylalanine almost completely abolishes DNA binding. This residue does not appear to be subject to phosphorylation ([39] and references therein), but its mutation does affect overall levels of DR phosphorylation. The tyrosine kinase inhibitor genistein completely blocks transformation of DR to the DNA binding form, as does *in vitro* treatment with tyrosine specific phosphatases ([40] and references therein). The tyrosine kinase c-src has been implicated in regulation of DR activity [41], although there is no evidence that DR is, in fact, phosphorylated by c-src. These data are further confounded by the fact that bacterially expressed, and therefore non-phosphorylated, N-terminal fragments of the DR and ARNT (corresponding to the bHLH plus PAS-A) bind the XRE DNA response element with high affinity (0.4 nM) [42]. The serine/threonine kinase inhibitors staurosporine [38] and LY294002 [43] have been reported to block TCDD induced DNA binding and target gene activation, although others have reported varied effects from staurosporine [44,45]. Induction of PKC (protein kinase C) activity by PMA (phorbol 12-myristate 13-acetate) results in superinduction of a DR responsive reporter ([46] and references therein). Some of this effect may be due to an increase in the level or activity of ARNT [3] and some may be due to stabilisation of DR via PKC activation of Erk [47]. There is evidence for regulation of nuclear import and export by phosphorylation, with PKC phosphorylation of residues adjacent the NLS (serines 12 and 36) inhibiting nuclear import and phosphorylation of serine 68 (possibly by the MAP kinase p38), adjacent the N-terminal NES inhibiting nuclear export ([48] and references therein). Phosphorylation of serines 36 and 68 have been confirmed using sequence specific anti-phosphoserine antibodies but no other post-translational modifications have been positively identified, although it seems likely that many exist. Interpretation of these data is made more complex due to the fact that the unliganded DR exists in a complex of several proteins (see later). In some instances these chaperone proteins may be the target of regulation, emphasising the need to establish what residues of the DR are subject to signal regulated post-translational modification.

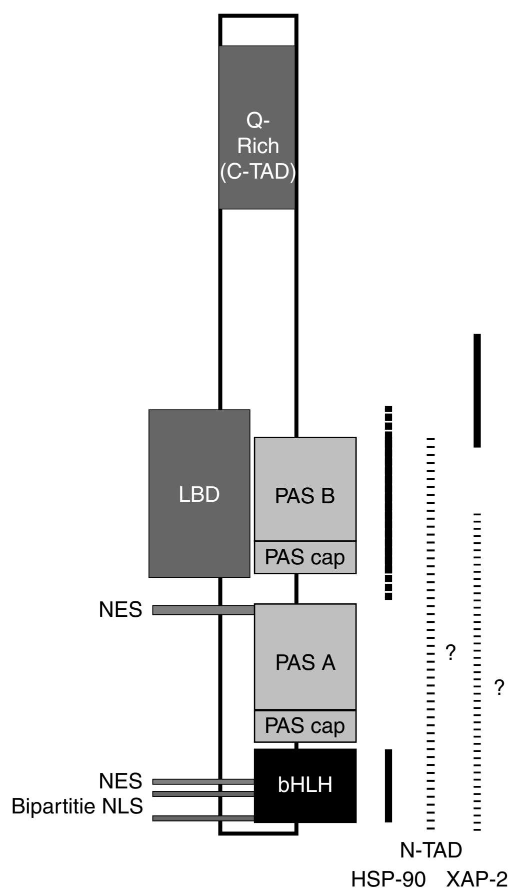

Fig. 3. Location of key domains and major protein interactions of the DR. The size of domains and interactions is drawn to linear scale proportional to amino acid number. The PAS domain and the PAS cap structure, necessary for correct folding of this domain are shown. The LBD illustrated corresponds to the consensus minimal LBD. The DR interacts with a variety of regulatory proteins, of which the interaction with hsp90 is the predominant interaction. Hsp90 binding co-localises with the ligand binding PAS-B region of the DR. A range of co-activators interact with the glutamine rich C-terminal activation domain (Q-Rich (C-TAD) and only rudimentary data is available for interaction of CoCoA and GAC63 in the N-terminal half of the DR (N-TAD). Poorly characterised or questionable interactions are shown with dashed lines, solid lines are well characterised interactions. See text for references to the relevant interactions. NLS, nuclear localisation signal; NES, nuclear export signal; bHLH, basic-helix-loop-helix; PAS, PER ARNT Sim homology domain.

A separate negative feedback loop system is proposed to regulate DR transcriptional output. One of the target genes upregulated by the DR encodes a protein that displays homology to the N-terminal region of the DR but diverges after the PAS-A domain. This protein has the ability to heterodimerise with ARNT and bind the XRE (*in vitro*) and has been termed the AhR repressor (AhRR, [49]) as overexpression of the AhRR in cell culture systems ablates dioxin mediated reporter gene induction [49]. The AhRR has been shown to be upregulated both *in vitro* and *in vivo* upon exposure to the DR ligand 3-methylcholanthrene [49,50]. The AhRR promoter and adjacent regulatory sequence possess several XRE like sequences, which, when mutated in combination, diminish the inducibility of a reporter gene in transient transfection assays, implying that the repressor is indeed a *bona fide* target gene of the DR [50].

2.3. Repression of DR activity through the LBD

The ligand binding domain of the DR sets this protein apart from the other vertebrate members of the bHLH/PAS family. It is a key region of regulation for the DR and understanding this regulation is of particular interest.

The DR exists in the cytosol as a complex of several proteins (reviewed in [51]). This complex includes the molecular chaperone hsp90, whose interaction maps to two distinct regions of the DR (Fig. 3), a major binding region co-localising with the ligand binding domain and a minor interaction mapping to the N-terminal region which likely masks the bipartite NLS ([30] and references therein), retaining the DR in the cytoplasm. Aside from masking a nuclear localisation sequence, chaperone association is also thought to maintain the DR in a form competent to bind ligand. This is validated by the fact that the major hsp90 binding region of the DR co-localises with the ligand binding region ([52] and references therein), and *in vitro* translation of the DR in wheat germ lysate (a system lacking a functional hsp90 homologue) produces a form of the DR with poor affinity for ligand ([53] and references therein). Additionally, yeast strains with regulable levels of hsp82 (the yeast homologue of hsp90) have been shown to require hsp82 to restore DR signalling ([53] and references therein). These studies demonstrate an absolute requirement for hsp90 in DR signalling.

Upon ligand binding, the DR translocates from the cytoplasm to the nucleus and heterodimerises with ARNT (Fig. 2). During this process, shedding of the molecular chaperone complex occurs, however, the sequence of events leading to this final outcome are poorly defined. Several experimental approaches have demonstrated that ligand binding is inadequate for hsp90 removal from the DR [54,55]. Studies demonstrate that addition of ligand to *in vitro* translated DR is insufficient to release hsp90 from the DR but that ARNT is required in the transformation mixture to induce DR/hsp90 release [54]. Further, evidence strongly suggests that the DR/chaperone complex translocates to the nucleus [55], where hsp90 release is concomitant with ARNT heterodimerisation (Fig. 2). This latter suggestion is based on the rapid lability of the DR following treatment with geldanamycin, an ansamyocin antibiotic that inhibits the ATPase activity of hsp90 and dissociates DR/hsp90 complexes [55], making it unlikely that unchaperoned DR exists within the cell. Additionally, nuclear localisation of the DR independent of ligand is insufficient to shed the molecular chaperones and invoke ARNT hetero-dimerisation [55]. Also, the studies using geldanamycin infer that a correctly structured LBD must be maintained and appropriately derepressed in order to generate the structural integrity necessary for a functional transcription factor. If the chaperones are removed from the DR artificially then the DR is functionally inactive despite being able to heterodimerise with ARNT and bind DNA [55]. The LBD appears to more broadly regulate DR activity because, whilst dioxin can fully invoke the DR pathway through cytosolic-nuclear translocation to transcriptional activation, there exist other ligands for the DR that bind the DR but exhibit mixed abilities to progress the DR along the activation pathway. For example 3-methoxy-4-nitroflavone binds the DR but maintains the DR in a chaperone

bound complex in the cytosol [56]. Resveratrol, a wine constituent, has the ability to transform the DR to an ARNT bound DNA binding complex but remains unable to activate transcription [57]. This is not to say that the LBD domain *per se* is essential for DR mediated transcription, as a deletion mutant of the DR lacking the LBD (amino acids 287–421 of mDR) is capable of ligand independent activation of target genes and generates tumours in transgenic mice ([58] and references therein). The LBD, therefore, acts to repress DR function at a number of levels and can be derepressed in at least three distinct manners: derepression resulting in nuclear localisation without exchange of hsp90 for ARNT, derepression resulting in nuclear localisation, exchange of hsp90 for ARNT, DNA binding without apparent transactivation and, lastly, full derepression resulting in nuclear localisation, exchange of hsp90 for ARNT followed by DNA binding and transactivation of target genes. Given there appear to be distinct developmental/housekeeping and adaptive sets of target genes it is possible that alternative mechanisms for LBD derepression conform the DR/ARNT heterodimer for activation of different genes.

2.4. The role of co-chaperone proteins in DR signalling

In addition to hsp90, the DR has been shown by yeast two hybrid and biochemical analysis to interact with an immunophilin like protein termed AIP (aryl hydrocarbon receptor interacting protein; murine clone)/Ara9 (aryl hydrocarbon receptor associated protein 9; human clone)/XAP2 (hepatitis virus B X associated protein 2; simian clone) (reviewed in [34]). Sequence alignment shows a loose homology between XAP2 and FKBP52, the immunophilin associated with several steroid hormone receptor complexes. Transient transfection studies demonstrate an approximate twofold increase in XRE driven reporter gene activity upon XAP2 coexpression with the DR, correlating to an approximate twofold increase in DR protein levels (reviewed in [34]). To this end XAP2 decreases ubiquitinated forms of the DR in transient overexpression experiments [59]. Furthermore DR chimeric proteins fused to a fluorescent tag suggest a role for XAP2 in cytosolic retention for this protein (reviewed in [34]) Treatment of the DR/chaperone complex *in vitro* with geldanamycin destabilizes this complex such that p23 (see below) and XAP2 are lost from the complex [60]. This supports the idea that XAP2 stabilizes the DR/hsp90/p23 complex, preventing transient unmasking of the N-terminal nuclear localisation sequence within the DR and diminishing ligand independent nuclear accumulation of the DR. Additionally, the presence of XAP2 in the latent chaperone complex protects the DR from proteasome-mediated degradation [59], an observation indicative of a stable DR–chaperone complex. Evidence points to the DR undergoing a process of chaperone loading similar to hormone receptor systems, requiring a progression through several intermediate (or immature) steps which are characterised by the association of the DR with the molecular chaperones hip, hop, hsp70, hsp90, p23 and XAP2 (reviewed in [34]). As XAP2 interacts with both hsp90 and the DR (reviewed in [34]) it is likely that XAP2 acts as a stabilising factor and the presence of XAP2 in this complex may denote a subset of mature DR–chaperone complexes. Recent evidence from Hepa-1 cells confirms that XAP2 functions to prevent nucleocytoplasmic shuttling, but that its depletion has no affect on ligand induced signalling in these cells [61]. Further, Hollingshead et al. [62] have addressed the apparent deficit of XAP2 in hepatocytes using a transgenic model and demonstrate that, in these cells, it is not limiting in terms of ligand induced DR activity. This recent evidence supports a model in which XAP2 is dispensable, however these studies have only addressed the role of XAP2 in the context of DR's response to xenobiotics within hepatocytes leaving open its role in housekeeping and developmental DR activities.

An additional factor in chaperone systems is a smaller co-chaperone protein p23. This protein is an auxiliary factor for hsp90 mediated chaperoning of steroid hormone receptor substrates including the estrogen, androgen, glucocorticoid, thyroid and progesterone receptors ([63] and references therein). p23 is expressed highly in the majority of murine tissues [63], however p23 and a related protein (transcript similar p23) are alternately expressed in different tissues [63]. In both mammalian cell culture systems and a yeast reconstitution systems the two forms of p23 can act in either identical or opposing fashions, depending on the steroid receptor substrate [63]. The role of p23 in hsp90 mediated chaperone processes is poorly understood but is proposed to facilitate the ATPase activity of hsp90, however this is not due to an intrinsic increase in ATPase activity of hsp90, rather p23 is proposed to transmit the ATPase induced conformational change within hsp90 more efficiently [64]. It appears that for several steroid hormone signalling systems p23 is actually dispensable, as has been demonstrated *in vivo* for the estrogen receptor in a p23 depleted yeast strain [65].

Reconstitution studies in yeast utilising a p23 deletion strain demonstrate that p23 augments the DR response but is not essential for DR signalling [66]. It is not yet clear what role p23 plays in DR signalling in mammalian cells. Studies focussing on the DR have shown that p23 is associated with the DR both *in vitro* and *in vivo* as the DR is immunoprecipitated with antibodies directed against p23 using either *in vitro* translated DR or using cell extracts ([60] and references therein).

2.5. The DR: regulation at multiple levels but to what cause?

Even though poorly understood, it appears that the DR is subject to a high degree of regulation to control transcriptional activity. Given the importance of the other PAS family members mentioned one could hypothesise that this reflects the physiological importance of this protein. As mentioned above, developmental expression studies, targeted disruption experiments, recent advances in understanding the interactions with the ER and the identification of target genes outside the xenobiotic metabolism strongly support a role for the DR outside xenobiotic metabolism.

Regulation of the DR hinges on its LBD. As yet, no crystal structure for the LBD of the DR exists. This is presumably due to the requirement of molecular chaperones for correct folding of the LBD. In the absence of functional homologues in prokaryotes, large-scale preparation of this part of the protein has not been possible. Until recently, a similar imposition existed for the GR. However, mutation of a single amino acid within the ligand binding domain of the GR led to solubilization of this region in a bacterial system and enabled the derivation of a crystal structure [67]. Recently there have been significant advances in understanding the structure of PAS domains [68] and of hsp90 and its client protein binding (for review see [69]). We also have to revise our model of DR/ARNT

interaction with DNA to take into account the interaction of PAS-A with the bHLH and possibly DNA itself [2]. These new data suggest the DR adopts a compact structure with both bHLH and PAS-A in close proximity with DNA (Fig. 2). The structure and client binding of hsp90, along with our knowledge of interaction sites between hsp90 and DR suggest that the bHLH and PAS-B/LBD are in close proximity (Fig. 2). If this were a direct interaction, this would provide a direct means for ligand binding to affect exposure of the NLS. It may also explain the data on mutation of tyrosine 9, as this residue might be involved in bHLH – PAS-B interaction, rather than in DNA binding. Moreover, a compact structure in which the LBD is forming an intramolecular interaction with the bHLH and, perhaps, the transactivation domain would allow different modes of derepression to be transmitted to the different parts of the molecule. This sort of compact arrangement accords with evidence about DR's E3 ligase activity, in which interaction of the transactivation domain with cullin4B is not only ligand dependent, but dependent on ligand with full agonist activity [27]. This model has analogy in the conformations the ER adopts depending on whether it is agonist or antagonist bound. In the case of the ER, different structures can recruit activator or co-repressor complexes [70]. Identification of post-translational modifications of the DR, particularly any that are signal regulated may also advance the field. Given evidence from other bHLH/PAS factors, nuclear hormone receptors and from within the DR field, it is likely that interaction with various components in the activation pathway will turn out to be regulated via post-translational modification. It is also possible that regulated post-translational modifications may be involved in ligand independent derepression of the LBD.

3. Future directions

Whilst significant advancements in terms of documenting all of the proteins that interact with and regulate the DR have been made over the last several years, in reality our overall understanding of how this regulation pieces together to coordinate DR signalling and how this impacts on whole organism physiology remains rudimentary. Early work has proposed that these proteins act in a concerted effort to control cellular localisation, and maintenance of a form that is unable to heterodimerise with the partner factor in the absence of ligand. Extension of studies on the physiological role of DR, in particular within the immune system where a wide range elegant tools are available, are likely to provide significant advances in understanding both stress and housekeeping roles. Furthermore, it will be fascinating to identify the endogenous ligand or activation mechanism. The biggest advances in the field will come when a link is made between the apparent alternate biological function of the DR during development and the mechanism(s) involved in its developmental activation as this will close the loop on why the dysregulation of the adaptive response results in such toxic responses.

References

[1] Kewley, R.J., Whitelaw, M.L. and Chapman-Smith, A. (2004) The mammalian basic helix-loop-helix/PAS family of transcriptional regulators. Int. J. Biochem. Cell Biol. 36, 189–204.

[2] Chapman-Smith, A. and Whitelaw, M.L. (2006) Novel DNA binding by a basic helix-loop-helix protein. The role of the dioxin receptor PAS domain. J. Biol. Chem. 281, 12535–12545.

[3] Long, W.P., Chen, X. and Perdew, G.H. (1999) Protein kinase C modulates aryl hydrocarbon receptor nuclear translocator protein-mediated transactivation potential in a dimer context. J. Biol. Chem. 274, 12391–12400.

[4] Butler, R.A., Kelley, M.L., Powell, W.H., Hahn, M.E. and Van Beneden, R.J. (2001) An aryl hydrocarbon receptor (AHR) homologue from the soft-shell clam, Mya arenaria: evidence that invertebrate AHR homologues lack 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and beta-naphthoflavone binding. Gene 278, 223–234.

[5] Crews, S.T. (1998) Control of cell lineage-specific development and transcription by bHLH-PAS proteins. Genes Dev. 12, 607–620.

[6] Ke, Q. and Costa, M. (2006) Hypoxia-inducible factor-1 (HIF-1). Mol. Pharmacol. 70, 1469–1480.

[7] Hirota, K. and Semenza, G.L. (2005) Regulation of hypoxia-inducible factor 1 by prolyl and asparaginyl hydroxylases. Biochem. Biophys. Res. Commun. 338, 610–616.

[8] Hendricks, J.C. (2003) Invited review: sleeping flies don't lie: the use of *Drosophila melanogaster* to study sleep and circadian rhythms. J. Appl. Physiol. 94, 1660–1672, discussion 1673.

[9] Storch, K.F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F.C., Wong, W.H. and Weitz, C.J. (2002) Extensive and divergent circadian gene expression in liver and heart. Nature 417, 78–83.

[10] Hord, N.G. and Perdew, G.H. (1994) Physicochemical and immunocytochemical analysis of the aryl hydrocarbon receptor nuclear translocator: characterization of two monoclonal antibodies to the aryl hydrocarbon receptor nuclear translocator. Mol. Pharmacol. 46, 618–626.

[11] Pollenz, R.S., Sattler, C.A. and Poland, A. (1994) The aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator protein show distinct subcellular localizations in Hepa 1c1c7 cells by immunofluorescence microscopy. Mol. Pharmacol. 45, 428–438.

[12] Jain, S., Maltepe, E., Lu, M.M., Simon, C. and Bradfield, C.A. (1998) Expression of ARNT, ARNT2, HIF1 alpha, HIF2 alpha and Ah receptor mRNAs in the developing mouse. Mech. Dev. 73, 117–123.

[13] Ikeda, M. and Nomura, M. (1997) cDNA cloning and tissue-specific expression of a novel basic helix-loop-helix/PAS protein (BMAL1) and identification of alternatively spliced variants with alternative translation initiation site usage. Biochem. Biophys. Res. Commun. 233, 258–264.

[14] Drutel, G., Kathmann, M., Heron, A., Schwartz, J.C. and Arrang, J.M. (1996) Cloning and selective expression in brain and kidney of ARNT2 homologous to the Ah receptor nuclear translocator (ARNT). Biochem. Biophys. Res. Commun. 225, 333–339.

[15] Maltepe, E., Schmidt, J.V., Baunoch, D., Bradfield, C.A. and Simon, M.C. (1997) Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. Nature 386, 403–407.

[16] Sekine, H., Mimura, J., Yamamoto, M. and Fujii-Kuriyama, Y. (2006) Unique and overlapping transcriptional roles of arylhydrocarbon receptor nuclear translocator (Arnt) and Arnt2 in xenobiotic and hypoxic responses. J. Biol. Chem. 281, 37507–37516.

[17] Ooe, N., Saito, K., Mikami, N., Nakatuka, I. and Kaneko, H. (2004) Identification of a novel basic helix-loop-helix-PAS factor, NXF, reveals a Sim2 competitive, positive regulatory role in dendritic-cytoskeleton modulator drebrin gene expression. Mol. Cell Biol. 24, 608–616.

[18] Shamloo, M. et al. (2006) Npas4, a novel helix-loop-helix PAS domain protein, is regulated in response to cerebral ischemia. Eur. J. Neurosci. 24, 2705–2720.

[19] Denison, M.S. and Nagy, S.R. (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu. Rev. Pharmacol. Toxicol. 43, 309–334.

[20] Barouki, R., Coumoul, X. and Fernandez-Salguero, P.M. (in press) The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. FEBS Lett., doi:10.1016/j.febslet.2007.03.046.

[21] Poland, A. and Knutson, J.C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. 22, 517–554.

[22] Bock, K.W. and Kohle, C. (2006) Ah receptor: dioxin-mediated toxic responses as hints to deregulated physiologic functions. Biochem. Pharmacol.

[23] Shimizu, Y., Nakatsuru, Y., Ichinose, M., Takahashi, Y., Kume, H., Mimura, J., Fujii-Kuriyama, Y. and Ishikawa, T. (2000) Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc. Natl. Acad. Sci. USA 97, 779–782.

[24] Bunger, M.K., Moran, S.M., Glover, E., Thomae, T.L., Lahvis, G.P., Lin, B.C. and Bradfield, C.A. (2003) Resistance to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity and abnormal liver development in mice carrying a mutation in the nuclear localization sequence of the aryl hydrocarbon receptor. J. Biol. Chem. 5, 5.

[25] Tijet, N., Boutros, P.C., Moffat, I.D., Okey, A.B., Tuomisto, J. and Pohjanvirta, R. (2006) Aryl hydrocarbon receptor regulates distinct dioxin-dependent and dioxin-independent gene batteries. Mol. Pharmacol. 69, 140–153.

[26] Ohtake, F. et al. (2003) Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 423, 545–550.

[27] Ohtake, F. et al. (2007) Dioxin receptor is a ligand-dependent E3 ubiquitin ligase. Nature 446, 562–566.

[28] Beischlag, T.V. and Perdew, G.H. (2005) ER alpha-AHR-ARNT protein–protein interactions mediate estradiol-dependent transrepression of dioxin-inducible gene transcription. J. Biol. Chem. 280, 21607–21611.

[29] Matthews, J., Wihlen, B., Thomsen, J. and Gustafsson, J.A. (2005) Aryl hydrocarbon receptor-mediated transcription: ligand-dependent recruitment of estrogen receptor alpha to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-responsive promoters. Mol. Cell Biol. 25, 5317–5328.

[30] Ikuta, T., Eguchi, H., Tachibana, T., Yoneda, Y. and Kawajiri, K. (1998) Nuclear localization and export signals of the human aryl hydrocarbon receptor. J. Biol. Chem. 273, 2895–2904.

[31] Swanson, H.I., Chan, W.K. and Bradfield, C.A. (1995) DNA binding specificities and pairing rules of the Ah receptor, ARNT, and SIM proteins. J. Biol. Chem. 270, 26292–26302.

[32] Hankinson, O. (2005) Role of coactivators in transcriptional activation by the aryl hydrocarbon receptor. Arch. Biochem. Biophys. 433, 379–386.

[33] Chen, Y.H., Beischlag, T.V., Kim, J.H., Perdew, G.H. and Stallcup, M.R. (2006) Role of GAC63 in transcriptional activation mediated by the aryl hydrocarbon receptor. J. Biol. Chem. 281, 12242–12247.

[34] Pollenz, R.S. (2002) The mechanism of AH receptor protein down-regulation (degradation) and its impact on AH receptor-mediated gene regulation. Chem. Biol. Interact. 141, 41–61.

[35] Berg, P. and Pongratz, I. (2002) Two parallel pathways mediate cytoplasmic localization of the dioxin (aryl hydrocarbon) receptor. J. Biol. Chem. 277, 32310–32319.

[36] Pollenz, R.S. (1996) The aryl-hydrocarbon receptor, but not the aryl-hydrocarbon receptor nuclear translocator protein, is rapidly depleted in hepatic and nonhepatic culture cells exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Mol. Pharmacol. 49, 391–398.

[37] Perdew, G.H. (1991) Comparison of the nuclear and cytosolic forms of the Ah receptor from Hepa 1c1c7 cells: charge heterogeneity and ATP binding properties. Arch. Biochem. Biophys. 291, 284–290.

[38] Carrier, F., Owens, R.A., Nebert, D.W. and Puga, A. (1992) Dioxin-dependent activation of murine Cyp1a-1 gene transcription requires protein kinase C-dependent phosphorylation. Mol. Cell Biol. 12, 1856–1863.

[39] Minsavage, G.D., Park, S.K. and Gasiewicz, T.A. (2004) The aryl hydrocarbon receptor (AhR) tyrosine 9, a residue that is essential for AhR DNA binding activity, is not a phosphoresidue but augments AhR phosphorylation. J. Biol. Chem. 279, 20582–20593.

[40] Park, S., Henry, E.C. and Gasiewicz, T.A. (2000) Regulation of DNA binding activity of the ligand-activated aryl hydrocarbon receptor by tyrosine phosphorylation. Arch. Biochem. Biophys. 381, 302–312.

[41] Backlund, M. and Ingelman-Sundberg, M. (2005) Regulation of aryl hydrocarbon receptor signal transduction by protein tyrosine kinases. Cell Signal 17, 39–48.

[42] Chapman-Smith, A., Lutwyche, J.K. and Whitelaw, M.L. (2004) Contribution of the Per/Arnt/Sim (PAS) domains to DNA binding by the basic helix-loop-helix PAS transcriptional regulators. J. Biol. Chem. 279, 5353–5362.

[43] Guo, M., Joiakim, A. and Reiners Jr., J.J. (2000) Suppression of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-mediated aryl hydrocarbon receptor transformation and CYP1A1 induction by the phosphatidylinositol 3-kinase inhibitor 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). Biochem. Pharmacol. 60, 635–642.

[44] Schafer, M.W., Madhukar, B.V., Swanson, H.I., Tullis, K. and Denison, M.S. (1993) Protein kinase C is not involved in Ah receptor transformation and DNA binding. Arch. Biochem. Biophys. 307, 267–271.

[45] Chen, Y.H. and Tukey, R.H. (1996) Protein kinase C modulates regulation of the CYP1A1 gene by the aryl hydrocarbon receptor. J. Biol. Chem. 271, 26261–26266.

[46] Long, W.P. and Perdew, G.H. (1999) Lack of an absolute requirement for the native aryl hydrocarbon receptor (AhR) and AhR nuclear translocator transactivation domains in protein kinase C-mediated modulation of the AhR pathway. Arch. Biochem. Biophys. 371, 246–259.

[47] Chen, S., Operana, T., Bonzo, J., Nguyen, N. and Tukey, R.H. (2005) ERK kinase inhibition stabilizes the aryl hydrocarbon receptor: implications for transcriptional activation and protein degradation. J. Biol. Chem. 280, 4350–4359.

[48] Ikuta, T., Kobayashi, Y. and Kawajiri, K. (2004) Cell density regulates intracellular localization of aryl hydrocarbon receptor. J. Biol. Chem. 279, 19209–19216.

[49] Mimura, J., Ema, M., Sogawa, K. and Fujii-Kuriyama, Y. (1999) Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev. 13, 20–25.

[50] Baba, T. et al. (2001) Structure and expression of the Ah receptor repressor gene. J. Biol. Chem. 276, 33101–33110.

[51] Petrulis, J.R. and Perdew, G.H. (2002) The role of chaperone proteins in the aryl hydrocarbon receptor core complex. Chem. Biol. Interact. 141, 25–40.

[52] Coumailleau, P., Poellinger, L., Gustafsson, J.A. and Whitelaw, M.L. (1995) Definition of a minimal domain of the dioxin receptor that is associated with Hsp90 and maintains wild type ligand binding affinity and specificity. J. Biol. Chem. 270, 25291–25300.

[53] Whitelaw, M.L., McGuire, J., Picard, D., Gustafsson, J.A. and Poellinger, L. (1995) Heat shock protein hsp90 regulates dioxin receptor function *in vivo*. Proc. Natl. Acad. Sci. USA 92, 4437–4441.

[54] McGuire, J., Whitelaw, M.L., Pongratz, I., Gustafsson, J.A. and Poellinger, L. (1994) A cellular factor stimulates ligand-dependent release of hsp90 from the basic helix-loop-helix dioxin receptor. Mol. Cell Biol. 14, 2438–2446.

[55] Lees, M.J. and Whitelaw, M.L. (1999) Multiple roles of ligand in transforming the dioxin receptor to an active basic helix-loop-helix/PAS transcription factor complex with the nuclear protein Arnt. Mol. Cell Biol. 19, 5811–5822.

[56] Lee, J.E. and Safe, S. (2000) 3′,4′-Dimethoxyflavone as an aryl hydrocarbon receptor antagonist in human breast cancer cells. Toxicol. Sci. 58, 235–242.

[57] Casper, R.F., Quesne, M., Rogers, I.M., Shirota, T., Jolivet, A., Milgrom, E. and Savouret, J.F. (1999) Resveratrol has antagonist activity on the aryl hydrocarbon receptor: implications for prevention of dioxin toxicity. Mol. Pharmacol. 56, 784–790.

[58] Andersson, P., McGuire, J., Rubio, C., Gradin, K., Whitelaw, M.L., Pettersson, S., Hanberg, A. and Poellinger, L. (2002) A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors. Proc. Natl. Acad. Sci. USA 99, 9990–9995.

[59] Kazlauskas, A., Poellinger, L. and Pongratz, I. (2000) The immunophilin-like protein XAP2 regulates ubiquitination and subcellular localization of the dioxin receptor. J. Biol. Chem. 275, 41317–41324.

[60] Kazlauskas, A., Sundstrom, S., Poellinger, L. and Pongratz, I. (2001) The hsp90 chaperone complex regulates intracellular localization of the dioxin receptor. Mol. Cell Biol. 21, 2594–2607.

[61] Pollenz, R.S., Wilson, S.E. and Dougherty, E.J. (2006) Role of endogenous XAP2 protein on the localization and nucleocytoplasmic shuttling of the endogenous mouse Ahb-1 receptor in the presence and absence of ligand. Mol. Pharmacol. 70, 1369–1379.

[62] Hollingshead, B.D., Patel, R.D. and Perdew, G.H. (2006) Endogenous hepatic expression of the hepatitis B virus X-associated protein 2 is adequate for maximal association with aryl hydrocarbon receptor-90-kDa heat shock protein complexes. Mol. Pharmacol. 70, 2096–2107.

[63] Freeman, B.C., Felts, S.J., Toft, D.O. and Yamamoto, K.R. (2000) The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies. Genes Dev. 14, 422–434.

[64] Young, J.C. and Hartl, F.U. (2000) Polypeptide release by Hsp90 involves ATP hydrolysis and is enhanced by the co-chaperone p23. Embo J. 19, 5930–5940.

[65] Knoblauch, R. and Garabedian, M.J. (1999) Role for Hsp90-associated cochaperone p23 in estrogen receptor signal transduction. Mol. Cell Biol. 19, 3748–3759.

[66] Cox, M.B. and Miller 3rd., C.A. (2002) The p23 co-chaperone facilitates dioxin receptor signaling in a yeast model system. Toxicol. Lett. 129, 13–21.

[67] Bledsoe, R.K. et al. (2002) Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell 110, 93–105.

[68] Yildiz, O. et al. (2005) Crystal structure and interactions of the PAS repeat region of the Drosophila clock protein PERIOD. Mol. Cell 17, 69–82.

[69] Pearl, L.H. and Prodromou, C. (2006) Structure and mechanism of the hsp90 molecular chaperone machinery. Annu. Rev. Biochem. 75, 271–294.

[70] Brzozowski, A.M. et al. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389, 753–758.