2. MECHANISM(S) OF ACTION*

2.1. INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) is the prototype for a class of halogenated aromatic hydrocarbons (HAHs) that produce similar patterns of toxicity and appear to have a common mechanism of action, although they differ in potency (Poland and Knutson, 1982; Safe, 1986). Because it is the most potent, TCDD has been studied much more extensively than other structurally related compounds (Figure 2-1). TCDD achieved notoriety in the 1970s, when it was discovered to be a contaminant in the herbicide Agent Orange and was shown to produce birth defects in rodents. Subsequently, dioxin has continued to generate concern because of its widespread distribution, its persistence as an environmental contaminant, its accumulation within the food chain, and its toxic potency. In animals, TCDD elicits a wide range of biological effects, including alterations in metabolic pathways, immunological changes, reproductive and developmental abnormalities, and neoplasia (Poland and Knutson, 1982; Safe, 1986; Birnbaum, 1994b). In humans, dioxin and related compounds can produce the skin condition known as chloracne. Increased cancer rates have also been associated with exposures to dioxin-like chemicals (International Agency for Research on Cancer, 1997). The possibility that dioxins also produce birth defects and developmental abnormalities is a particular public health concern. Many individuals have been exposed to TCDD, primarily from dietary sources, although occupational and accidental exposures have also occurred. TCDD is a poor substrate for detoxification systems such as the microsomal cytochrome P450 enzymes, which oxygenate other lipophilic compounds to inactive derivatives during their metabolic processing. Because of its relative resistance to metabolism, TCDD persists in the body, with a half-life in humans on the order of 7 to 10 years (Pirkle et al., 1989; Michalek et al., 1996; Michalek and Tripathi, 1999). Therefore, dioxin tends to accumulate in human tissues over time, raising concern that repeated exposures, even to "low" concentrations, may evoke adverse health effects. Epidemiological studies performed to date have not produced well-defined estimates of the health risk that dioxin poses to humans. There has been hope that knowledge of the mechanism of dioxin action will bring about a greater understanding of these issues (Andersen et al., 1994; Denes et al., 1996).

Mechanistic studies can reveal the biochemical pathways and types of biological and molecular events that contribute to dioxin's adverse effects. For example, much evidence indicates that TCDD acts via an intracellular protein (the aryl hydrocarbon receptor; Ah receptor), which functions as a ligand-dependent transcription factor in partnership with a second protein (known as the Ah receptor nuclear translocator; Arnt). Therefore, from a mechanistic standpoint, TCDD's adverse effects appear likely to reflect alterations in gene expression that occur at an inappropriate time and/or for an inappropriately long time. Mechanistic studies also indicate that several other proteins contribute to TCDD's gene regulatory effects and that the response to TCDD probably involves a relatively complex interplay between multiple genetic and environmental factors. If TCDD operates through such a mechanism, as all evidence indicates, then there are certain constraints on the possible models that can plausibly account for TCDD's biological effects and, therefore, on the assumptions used during the risk assessment process (e.g. Poland, 1996; Limbird and Taylor, 1998). Mechanistic knowledge of dioxin action may also be useful in other ways. For example, a further understanding of the ligand specificity and structure of the Ah receptor will likely assist in the identification of other chemicals to which humans are exposed that may either add to, synergize, or block the toxicity of TCDD. Knowledge of genetic polymorphisms that influence TCDD responsiveness may also allow the identification of individuals at greater risk from exposure to dioxin. In addition, knowledge of the biochemical pathways that are altered by TCDD may help identify novel targets for the development of drugs that can antagonize dioxin's adverse effects.

As described below, biochemical and genetic analyses of the mechanisms by which dioxin may modulate particular genes have revealed the outline of a novel regulatory system whereby a chemical signal can alter cellular regulatory processes. Future studies of dioxin action have the potential to provide additional insights into mechanisms of mammalian gene regulation that are of a broader interest. Additional perspectives on dioxin action can be found in several recent reviews (Birnbaum, 1994a,b; Schecter, 1994; Hankinson, 1995; Schmidt and Bradfield, 1996; Gasiewicz, 1997; Rowlands and Gustafsson, 1997; Denison et al., 1998; Hahn, 1998; Wilson and Safe, 1998; Gu et al., 2000).

2.2. THE "RECEPTOR" CONCEPT

The idea that a drug, hormone, neurotransmitter, or other chemical produces a physiological response by interacting with a specific cellular target molecule, i.e., a "receptor," evolved from several observations. First, many chemicals elicit responses that are restricted to specific tissues. This observation implies that the responsive tissue (e.g., the adrenal cortex) contained a "receptive" component whose presence is required for the physiologic effect (e.g., cortisol secretion). Second, many chemicals are quite potent. For example, picomolar to

nanomolar concentrations of numerous hormones and growth factors elicit biological effects. This observation suggests that the target cell contains a site(s) to which the particular chemical binds with high affinity. Third, stereoisomers of some chemicals (e.g., catecholamines, opioids) differ by orders of magnitude in their ability to produce the same biological response. This observation indicates that the molecular shape of the chemical strongly influences its biological activity. This, in turn, implies that the binding site on or in the target cell also has a specific, three-dimensional configuration. Together, these types of observations predict that the biological responses to some chemicals involve stereospecific, high-affinity binding of the chemicals to specific receptor sites located on or in the target cell.

The availability of compounds of high specific radioactivity has permitted quantitative analyses of their binding to cellular components in vitro. To qualify as a potential "receptor," a binding site for a given chemical must satisfy several criteria: (1) the binding site must be saturable, i.e., the number of binding sites per cell should be limited; (2) the binding should be reversible; (3) the binding affinity measured *in vitro* should be consistent with the potency of the chemical observed *in vivo*; (4) if the biological response exhibits stereospecificity, so should the *in vitro* binding; (5) for a series of structurally related chemicals, the rank order for binding affinity should correlate with the rank order for biological potency; and (6) tissues that respond to the chemical should contain binding sites with the appropriate properties.

The binding of a chemical ("ligand") to its cognate receptor is assumed to obey the law of mass action; that is, it is a bimolecular, reversible interaction. The concentration of the liganded, or occupied, receptor [RL] is a function of both the ligand concentration [L] and the receptor concentration [R]:

$$[L] + [R] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [RL]$$

Inherent in this relationship is that the fractional occupancy (i.e. $[RL] / [R_t]$) is a function of ligand concentration [L] and the apparent equilibrium dissociation constant K_D, which is a measure of the binding affinity of the ligand for the receptor, that is, $[RL] / [R_t] = [L] / (K_D + [L])$, where $K_D = [L] [R_t] / [LR] = k_2 / k_1$. Therefore, the relationship between receptor occupancy and ligand concentration is hyperbolic. At low ligand concentrations (where $[L] << K_D$), a small increase in [L] produces an approximately linear increase in fractional receptor occupancy. At high ligand concentration (where $[L] >> K_D$), the fractional occupancy of the receptor is already very close to 1, that is, almost all receptor sites are occupied. Therefore, a small increase in [L] is likely to produce only a slight increase in receptor occupancy.

Ligand binding constitutes only one aspect of the receptor concept. By definition, a receptor mediates a response, and the functional consequences of the ligand-receptor binding represent an essential aspect of the receptor concept. Receptor theory attempts to quantitatively relate ligand binding to biological responses. The classical "occupancy" model of Clark (1933) postulated that (1) the magnitude of the biological response is directly proportional to the fraction of receptors occupied and (2) the response is maximal when all receptors are occupied. However, analyses of numerous receptor-mediated effects indicate that the relationship between receptor occupancy and biological effect is not as straightforward as Clark envisioned. In certain cases, no response occurs even when there is some receptor occupancy. This suggests that there may be a threshold phenomenon that reflects the biological "inertia" of the response (Ariens et al., 1960). In other cases, a maximal response occurs well before all receptors are occupied, a phenomenon that reflects receptor "reserve" (Stephenson, 1956). Therefore, one cannot simply assume that the relationship between fractional receptor occupancy and biological response is linear. Furthermore, for a ligand (such as TCDD) that elicits multiple receptor-mediated effects, one cannot assume that the binding-response relationship for a simple effect (such as enzyme induction) will necessarily be identical to that for a different and more complex effect (such as cancer). The cascades of events leading to different complex responses (e.g., altered immune response to pathogens or development of cancer) are likely to be different, and other rate-limiting events likely influence the final biological outcome resulting in different dose-response curves. Thus, even though ligand binding to the same receptor is the initial event leading to a spectrum of biological responses, ligand-binding data may not always mimic the dose-effect relationship observed for particular responses.

Another level of complexity is added when one considers different chemical ligands that bind to the same receptor. Relative potencies are determined by two properties of the ligand: affinity for the receptor, and capacity to confer a particular response in the receptor (e.g., a particular conformational change), also called efficacy (Stephenson, 1956). Ligands with different affinities and the same degree of efficacy would be expected to produce parallel dose-response curves with the same maximal response within a particular model system. However, ligands of the same affinity with different efficacies may result in dose-response curves that are not parallel or that differ in maximal response.

2.3. THE Ah (DIOXIN) RECEPTOR

The unusual toxic potency of TCDD suggested the possible existence of a receptor for dioxin. Poland and coworkers, using radiolabeled TCDD as a ligand, demonstrated that the cytosolic fraction of C57BL/6J mouse liver contained a protein that bound the dioxin saturably (i.e., ~10^5 binding sites per cell), reversibly, and with high affinity (i.e., in the nanomolar range,

consistent with TCDD's biological potency in vivo). Competition binding studies with congeners of TCDD revealed that ligands with the highest binding affinity were planar and contained halogen atoms in at least three of the four lateral positions; thus, ligand binding exhibited stereospecificity. In addition, the ligand-receptor binding relationship resembled a rectangular hyperbola and, therefore, appeared to obey the law of mass action. Together, these findings demonstrated that the intracellular TCDD-binding protein had the ligand binding properties expected for a "dioxin receptor." The protein has been designated as the "Ah receptor" because it binds and mediates the response to other aromatic hydrocarbons (such as 3-methylcholanthrene) in addition to TCDD (Poland and Knutson, 1982).

Inbred mouse strains differ quantitatively in their responsiveness to TCDD and other aromatic hydrocarbons. For example, TCDD elicits its effects at about 10-fold lower concentrations in the more responsive mouse strains (typified by C57BL/6) than in the less responsive strains (typified by DBA/2). This polymorphism in responsiveness is genetic in origin, and, in crossbreeding studies, the more responsive phenotype segregates as an autosomal dominant trait. Numerous responses to TCDD (e.g., enzyme induction, thymic involution, cleft palate formation, hepatic porphyria) exhibit a segregation pattern identical to that for the binding of TCDD to the Ah receptor. Thus, the genetic locus (designated *Ah*) that governs this receptor polymorphism also governs the biological responses to TCDD. These findings implicate the Ah receptor in the mechanism of dioxin action (Poland and Knutson, 1982; Nebert et al., 1991). The resistance of recently developed Ah receptor null-allele ("knockout") mice to the enzyme inductive and toxic effects of very high doses of TCDD further corroborates the role of this protein in these responses (Fernandez-Salguero et al., 1996; Schmidt et al., 1996; Mimura et al., 1997; Hundeiker et al., 1999; Peters et al., 1999).

Studies of structure-activity relationships (SARs) reveal that, within groups of structurally-related compounds, a ligand's receptor-binding affinity often correlates with its potency in eliciting a biological response(s). Such SAR analyses are useful for assessing possible participation of the AhR in ligand-induced responses in experimental systems where genetic polymorphisms of the receptor are not available. Such SAR studies constitute biochemical evidence that implicates the Ah receptor in the mechanism of dioxin action. In general, the rank order binding affinity of TCDD and related chemicals to the Ah receptor has been demonstrated to be similar to their rank order of potency to elicit a broad spectrum of biochemical, morphologic, immunologic, neoplastic, developmental, and reproductive effects (Poland and Knutson, 1982; Safe, 1986, 1990). This rank order appears largely dependent on several structural constraints. For example, relatively planar aromatic compounds with approximate van der Waals dimensions of 14 x 12 x 5 Å with few bulky substituent groups have, in general, the highest ligand binding affinity and are the most potent for eliciting biological

effects (Poland and Knutson, 1982; Gillner et al., 1993; Waller and McKinney, 1995). However, it is becoming increasingly recognized that some Ah receptor ligands have both agonist and antagonist activity (Harris et al., 1989b; Kurl et al., 1993; Lu et al., 1996; Henry et al., 1999), suggesting that not all Ah receptor ligands have the same degree of efficacy (Hestermann et al., 2000).

The cloning of receptor cDNA (Burbach et al., 1992; Ema et al., 1992) has added important new insights into Ah receptor structure and function (reviewed in Hankinson, 1995; Rowlands and Gustafsson, 1997). The deduced amino acid sequence reveals that the Ah receptor has several features in common with a class of transcription factors known as basic helix-loop-helix (bHLH) proteins. The Ah receptor contains a bHLH domain, located towards the N-terminal end of the receptor. An analysis of this and other bHLH proteins indicates that the basic region mediates DNA binding, whereas the helix-loop-helix domain is necessary for dimerization with other proteins. Two other receptor regions function in dimerization. These are designated as "PAS" regions due to their sequence homology with Per (a *Drosophila* circadian rhythm protein), Arnt (another protein that contributes to dioxin responsiveness, described below), and Sim (a regulatory protein that participates in *Drosophila* central nervous system development) (Huang et al., 1993). In the AhR, the PAS domain consists of approximately 300 amino acid residues containing two copies of a repeat of about 50 amino acids, referred to as the PAS-A and PAS-B repeats. In the absence of an agonist, the PAS-B region associates with one heat shock protein 90 (hsp90) molecule, permitting binding of a second hsp90 to the HLH region (Whitelaw et al., 1993; Antonsson et al., 1995; Coumailleau et al., 1995; Whitelaw et al., 1995; Fukunaga et al., 1995). TCDD has been shown to interact with a ligand-binding pocket near the PAS-B region, the conformation of which is maintained by hsp90. Dimerization between AhR and Arnt is mediated through their HLH regions, but is further stabilized by PAS-PAS interactions (Reisz-Porszasz et al., 1994; Fukunaga et al., 1995). Immunohistochemical studies, using anti-receptor antibodies, reveal that the unliganded receptor resides in the cytoplasm; exposure of cells to (under in vitro conditions) TCDD leads to the accumulation of the receptor within the nucleus (Pollenz et al., 1994). Association with hsp90 is also thought to limit nuclear uptake of the receptor by blocking a N-terminal nuclear localization sequence (Pongratz et al., 1992; Ikuta et al., 1998). The carboxyl end of the protein contains a glutamine-rich region, which resembles certain "activation domains" present in some other transcription factors; by analogy, this region could interact with coactivator proteins that have yet to be characterized. Thus, like many proteins, the Ah receptor appears to be composed of several different functional domains. It is notable that the TCDD-bound Ah receptor does not, by itself, bind strongly to DNA; acquisition of DNA-binding capability appears to require that the receptor interact with another factor (such as the Arnt protein). Thus, the active form of the

receptor is heteromeric. The bHLH proteins identified to date are involved in transcriptional regulation and have a variety of roles in tissue growth and differentiation processes (Murre et al., 1994; Schmidt and Bradfield, 1996).

Human cells also contain an intracellular protein whose ligand-binding and hydrodynamic properties resemble those of the Ah receptor identified in other species (see Cook and Greenlee, 1989; Harris et al., 1989a; Roberts et al., 1990; Lorenzen and Okey, 1991; Harper et al., 1991; Ema et al., 1994). Furthermore, the sequence of this protein shows homology with that of other mammalian species (Dolwick et al., 1993; Ema et al., 1994; Hahn, 1998). Compared with the rat or mouse Ah receptor, however, the human Ah receptor appears, at least under cell-free conditions, to have a several-fold lower affinity for TCDD (Manchester et al., 1987; Ema et al., 1994). Some data also suggest that the human Ah receptor may be many times less sensitive in terms of eliciting a response. For example, cultured human embryonic palatal cells were approximately 200 times less sensitive than mouse palatal cells with respect to the inducibility of *CYP1A1* by TCDD (Abbott et al., 1999a). Although these data might imply that human tissues would be less sensitive to the toxic effects of TCDD, the Ah receptor in human cells has also been shown to exist in more than one form (Perdew and Hollenbeck, 1995). The relative sensitivity and function of these different forms have not been evaluated. Furthermore, these observed differences may be related to the greater lability of the human receptor during tissue preparation and cell fractionation procedures (Manchester et al., 1987). Data also suggests considerable heterogeneity of Ah receptor concentrations and characteristics in the human population (e.g., Roberts et al., 1986, 1990, 1991). However, a limited analysis for human AhR polymorphisms identified only one amino acid exchanging polymorphism, and this is thought to have little or no functional significance (Wanner et al., 1999). Additional studies of the human receptor should increase our knowledge of its functional properties and role in mediating altered cell- and tissue-specific responses elicited by TCDD and related chemicals.

Evidence indicates that the Ah receptor evolved prior to the introduction of HAHs into the environment (Czuczwa et al., 1984; Hahn et al., 1997). Furthermore, what is known about the structure, regulation, and expression of the Ah receptor in different tissues indicates a purposeful regulation for some normal function. The ontogenically related and tissue-specific expression of the receptor (e.g., Abbott et al., 1995; Jain et al., 1998), as well as the conservation of its presence and protein sequence in diverse groups of vertebrates, implies an essential function (Hahn, 1998). Furthermore, the level and activity of the Ah receptor appear to be regulated by changes in cell differentiation stages, growth factors, cell activation, diurnal cycle, and prior exposure to receptor agonists (Vaziri et al., 1996; Crawford et al., 1997; Shimba et al., 1998; Hayashi et al., 1995; Wanner et al., 1995; Liu et al., 1996; Pollenz, 1996; Richardson et al., 1998). These aspects might, at least in part, contribute to the ability of TCDD to cause

tissue- and developmental stage-specific effects. The best evidence for a normal physiological function of the Ah receptor comes from studies with Ah receptor-deficient animals. These mice have altered hepatic growth and development, immune system abnormalities, development of vasculature, adverse reproductive outcomes and reproductive tissue development, and abnormal processes in a variety of tissues (Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Fernandez-Salguero et al., 1997; Abbott et al., 1999b; Hushka et al., 1998; Thurmond et al., 2000; Robles et al., 2000; Benedict et al., 2000; Lahvis et al., 2000).

Several reports have indicated stimulation of Ah receptor-dependent responses under certain conditions in the absence of added ligand (Sadek and Allen-Hoffman, 1994; Ma and Whitlock, 1996; Weiss et al., 1996; Crawford et al., 1997; Chang and Puga, 1998). However, it is not yet clear whether this occurs via an endogenously present ligand or some other process. Nevertheless, it has been postulated that some other compound(s) must represent the "natural" ligand(s) for the receptor (Poellinger et al., 1992). Naturally occurring high-affinity ligands for the receptor exist in the environment, particularly in plants (Gillner et al., 1985, 1989; Rannung et al., 1987; Bjeldanes et al., 1991). Other candidates for endogenous ligands include tryptophan derivatives (Helferich and Denison, 1991), carotinoids (Gradelet et al., 1996), arachidonic acid metabolites (Schaldach et al., 1999), and tetrapyrroles or their derivatives (Sinal and Bend, 1997). Thus, it is possible that the Ah receptor might have evolved as part of a substrate-inducible system designed to metabolize and/or activate dietary lipophilic substances, and TCDD may mimic the binding of such substances to the receptor. TCDD has been observed to produce changes in the proliferative/differentiated phenotype of a variety of cell types (Knutson and Poland, 1980; Blankenship et al., 1993; Gaido and Maness, 1994; Gierthy et al., 1994; Brodie et al., 1996; Yang et al., 1999). Thus, an additional possibility is that TCDD mimics an endogenous Ah receptor ligand involved in the regulation of such tissue-specific phenotypes. Several reports have also suggested a role of the Ah receptor in cell cycle control (Ma and Whitlock, 1996; Weiss et al., 1996; Kolluri et al., 1999), possibly through regulation of tissue growth factors such as transforming growth factor-β (Zaher et al., 1998).

2.4. THE ARNT PROTEIN

Biochemical and hydrodynamic findings suggest that more than one protein participates in the response to TCDD. In particular, overwhelming evidence indicates that the Ah receptor interacts with the Arnt protein to form a heteromeric, DNA-binding protein complex that can activate gene transcription (reviewed in Hankinson, 1995; Schmidt and Bradfield, 1996; Rowlands and Gustafsson, 1997; Denison et al., 1998; Whitlock, 1999).

The human Arnt cDNA encodes a protein of about 86 kDa, which has several features in common with the Ah receptor. Like the Ah receptor, it contains a bHLH domain, which

contributes both to DNA binding and to protein-protein interactions (Hoffman et al., 1991; Li et al., 1994; Reisz-Porszasz et al., 1994). Furthermore, the Arnt protein contains PAS domains homologous to Per and Sim, and C-terminal domains functional in transcriptional activation (Huang et al., 1993; Reisz-Porszasz et al., 1994; Lindebro et al., 1995) . The Arnt protein does not bind TCDD. Furthermore, it does not bind to Ah receptor recognition sites on DNA (termed dioxin or xenobiotic response elements; DREs or XREs) on DNA in the absence of the liganded Ah receptor protein (Whitelaw et al., 1993). Immunohistochemical studies, using anti-Arnt antibodies, reveal that the Arnt protein resides in the nucleus in uninduced mouse hepatoma cells and that TCDD exposure of cells produces no change in its intracellular distribution (Pollenz et al., 1994). Thus, Arnt appears to be a nuclear protein. Furthermore, the nuclear accumulation of the Ah receptor occurs in Arnt-defective cells. Together, these findings argue against a primary role for Arnt in the translocation per se of the receptor from cytoplasm to nucleus. Instead, it has been suggested that Arnt interacts with the liganded Ah receptor to form a heteromeric, DNA-binding protein complex that can activate gene transcription. Experiments in vitro support this idea. For example, immunoprecipitation experiments reveal that the liganded Ah receptor and the Arnt protein can interact in solution. The liganded receptor alone does not exhibit substantial DNA-binding activity in the absence of Arnt; rather, the presence of both proteins is required to generate a specific DNA-binding species and to activate the expression of a dioxin-responsive reporter gene. Furthermore, deletion of the bHLH domain of Arnt abrogates its functional interaction with the liganded Ah receptor (Whitelaw et al., 1993). Together, these findings imply that the transcriptionally active component of the dioxin-responsive system is a protein heteromer consisting of (at least) the liganded Ah receptor and Arnt. Although both proteins contain a C-terminal transactivation domain, the Ah receptor appears to provide the dominant activation function, and the relative contribution of the Arnt domain largely depends on the availability of other cell-specific factors (Corton et al., 1996; Ko et al., 1996).

Multiple forms of Arnt have been detected in several species (Drutel et al., 1996; Hirose et al., 1996; Pollenz et al., 1996). In mice and rats, Arnt1 and Arnt2 are 83% identical in amino acid sequence, and have the ability to dimerize with the AhR and bind to specific DNA elements in vitro (Hirose et al., 1996). However, the expression patterns for these two isoforms are quite different. Arnt1 is widely expressed in a variety of tissues, whereas Arnt2 is detected primarily in adult brain and kidney (Drutel et al., 1996; Hirose et al., 1996). In addition, each protein has a distinct expression pattern in the developing mouse (Jain et al., 1998). The respective roles of these proteins in contributing to the variety of TCDD-elicited responses are not yet clear.

Both the Ah receptor and Arnt protein belong to the bHLH class of transcription factors, which function as heterodimers and contribute to the control of numerous genes (Kadesch, 1993). The dimerization capabilities of bHLH proteins provides a potential mechanism for

generating regulatory diversity. For example, different heterodimers may exhibit different stabilities, may have different DNA-binding affinities, or may recognize different DNA sequences. The bHLH structure of the Ah receptor raises the possibility that it might form heterodimeric complexes with proteins other than Arnt, generating regulatory molecules with potentially novel properties. By analogy with other bHLH systems, both the absolute amount of each partner and their relative ratios could influence the extent and type of response to TCDD. Thus, diversity in heteromer formation might contribute to the diversity of responses that typifies dioxin action. Arnt1 has been shown to be a dimerization partner for several proteins including the Ah receptor, single-minded (Sim), hypoxia inducible factor-1α, and endothelial PAS protein 1, and several other as yet uncharacterized proteins (Ema et al., 1997; Hogenesch et al., 1997; Probst et al., 1997). In addition, Arnt1 has been shown to have an essential role in development (Kozak et al., 1997; Maltepe et al., 1997). However, Arnt is the only protein that has been demonstrated to be a functional partner for the Ah receptor in terms of conferring specificity of DNA binding and transactivation. Some HLH proteins, which lack a basic region (typified by Id), may act as dominant negative regulators of transcription by dimerizing with bHLH proteins and inhibiting their DNA binding ability (Kadesch, 1993). There is evidence for an inducible dominant negative regulator of the Ah receptor, termed the Ah receptor repressor, that interacts with Arnt (Mimura et al., 1999). It is possible that the tissue-specific presence or absence of this factor regulates Ah receptor function and thus its response to xenobiotics by competing with the receptor for Arnt. In any case, the bHLH structure of the Ah receptor and the Arnt protein suggests that some of the diversity in TCDD's biological effects might reflect differential gene regulation by a mechanism involving formation of different protein heterodimers. Future mechanistic studies should aid in elucidating how such processes regulate dioxin action.

2.5. OTHER PROTEINS THAT PARTICIPATE IN THE RESPONSE TO DIOXIN

Attempts to purify the unliganded Ah receptor under nondenaturing conditions revealed that it tends to associate with other proteins in vitro, in particular the 90-kDa heat shock protein (hsp90). The hsp90 protein is an abundant factor that can interact with numerous other proteins and that may have multiple functions (Buchner, 1999). Immunoprecipitation studies and immunosedimentation experiments using anti-hsp90 antibodies reveal that the unliganded Ah receptor associates with hsp90 in vitro (Denis et al., 1988; Perdew, 1988). In view of previous findings implicating hsp90 in glucocorticoid receptor function (Picard et al., 1990), the association between the Ah receptor and hsp90 in vitro may be more than fortuitous. Hsp90 may be needed to maintain the unliganded receptor in a configuration that facilitates ligand binding. It might have a role in regulating nuclear translocation and DNA binding of the Ah receptor (Pongratz et al., 1992; Antonsson et al., 1995; Coumailleau et al., 1995; Phelan et al., 1998).

Studies in yeast have demonstrated the requirement for hsp90 in the formation of a functional Ah receptor (Carver et al., 1994). However, since complete hsp90 dissociation does not appear to be essential for nuclear localization (Heid et al., 2000), the dissociation of the two hsp90 molecules may occur during separate events in the cytosol and the nucleus, initiated by ligand binding and Arnt association, respectively. An additional protein, ARA9/AIP/XAP2, has been shown to enhance the transcriptional activity of the AhR-Arnt complex (Ma and Whitlock, 1997; Carver et al., 1998; Meyer et al., 1998). Although this protein does not appear to be required for AhR-hsp90 interaction, it apparently stabilizes this interaction and has some function in regulating the rate of AhR turnover in the cytosol or intracellular localization (Meyer and Perdew, 1999; LaPres et al., 2000; Petrulis et al., 2000; Bell and Poland, 2000). Recently, a 23-kDa protein has been shown to associate with the ligand-binding form of the Ah receptor. This protein is thought to play a role in stabilizing the complex containing receptor and hsp90 (Kazlauskas et al., 1999). Given the important functions of these proteins in stabilizing an Ah receptor form that can bind ligand and transduce the ligand-initiated signal to the nuclear compartment, the relative presence of these proteins might play an important role in the tissue-specific sensitivity to Ah receptor ligands like TCDD.

Several lines of evidence suggest that phosphorylation/dephosphorylation of the Ah receptor and the Arnt protein may contribute to the function of the dioxin-responsive system. Treatment of nuclear extracts with potato acid phosphatase, which dephosphorylates proteins, inhibits the binding of the liganded receptor heteromer to its DNA recognition sequence in vitro (Pongratz et al., 1991). Modulation of protein kinase C (PKC) activity is also associated with a reduction in DNA-binding capability of the receptor heteromer in vitro and alteration of Ah receptor-dependent transcriptional function in vivo (Carrier et al., 1992; Okino et al., 1992; Berghard et al., 1993; Chen and Tukey, 1996; Long et al., 1998). Furthermore, immunoprecipitation experiments using anti-receptor antibodies reveal that the receptor can undergo phosphorylation in vivo (Berghard et al., 1993; Mahon and Gasiewicz, 1995). Additional experiments in vitro suggest that phosphorylation of the Arnt protein is required for its heterodimerization with the Ah receptor; however, phosphorylation of the receptor is not required (Berghard et al., 1993). Together, these findings suggest that PKC and other protein kinases might influence heterodimerization, binding of the receptor-Arnt heteromer to DNA, or transcriptional activation of the Ah receptor. Although tyrosine phosphorylation in particular appears to be important for regulation of DNA binding of the AhR-Arnt complex (Park et al., 2000), the exact amino acid residue, the mechanism of regulation, and if this, or other, phosphorylation may be partially responsible for tissue- and developmental stage-specific regulation of AhR activity, have yet to be determined. It is known that many mammalian transcription factors undergo cycles of phosphorylation and dephosphorylation; however, in

most cases, the physiological significance of the modification is unknown (Hunter and Karin, 1992). Additional research is necessary to fully delineate the role of protein phosphorylation in the biological response to TCDD, and to identify the particular protein kinases and phosphatases that participate in the regulation of Ah receptor and Arnt protein.

The Ah receptor has been shown to physically interact with both retinoblastoma and NF-kB proteins (Ge and Elferink, 1998; Tian et al., 1999; Puga et al., 2000a). Both proteins are known to function in the regulation of cell cycle and cell differentiation in response to a variety of cytokines and growth factors (e.g., Beg and Baltimore, 1996; Van Antwerp et al., 1996; Zacksenhaus et al., 1996). It is reasonable to speculate that these interactions contribute to the noted ability of TCDD and related xenobiotics to affect cell cycle and cellular differentiation states, but the exact pathways that lead to these cell- and tissue-specific responses have yet to be determined. Nevertheless, this certainly represents a promising area of future research.

It appears likely that additional proteins, which remain to be identified and characterized, also contribute to the dioxin-response system. Both the Ah receptor and Arnt activate gene expression, at least in part, through direct interaction with basal transcription factors (Rowlands et al., 1996). Furthermore, there appears to be considerable cross-regulation of the Ah receptor with other signaling proteins including hypoxia inducible factor, estrogen receptors, and the retinoic acid and thyroid hormone receptors (e.g., Caruso et al., 1999; Chan et al., 1999; Duan et al., 1999; Kumar et al., 1999; Nguyen et al., 1999). There is evidence to indicate that protein-protein interactions have an important part in regulating the transcriptional activity of several nuclear receptors (e.g., Chen and Evans, 1995). Likewise, Ah receptor-Arnt complex-mediated transcriptional activities may be modulated by coactivator/corepressor proteins in a cell- and gene-specific manner (Watson and Hankinson, 1988; Watson et al., 1992; Ma et al., 1995; Kobayashi et al., 1997; Kress and Greenlee, 1997; Gradin et al., 1999; Kumar et al., 1999; Nguyen et al., 1999; Eltom et al., 1999). The relative importance of these interactions in the dose-related and tissue-specific responses elicited by TCDD are unknown.

2.6. ACTIVATION OF GENE TRANSCRIPTION BY DIOXIN

2.6.1. In Vitro Studies

Much of our current understanding of the mechanism of dioxin action is based on analyses of the induction of particular enzyme activities by TCDD. Aryl hydrocarbon hydroxylase activity reflects the action of the cytochrome P4501A1 (*CYP1A1*) enzyme, which catalyzes oxygenation of polycyclic aromatic substrates as the initial step in their metabolic processing to water-soluble derivatives (Conney, 1982). TCDD induces *CYP1A1* activity in many tissues. In particular, the relatively strong induction of *CYP1A1* activity by TCDD in cultured cells has facilitated the application of molecular genetic techniques to the analysis of the

induction mechanism (Whitlock, 1999). Table 2-1 and Figure 2-2 summarize some of the molecular events involved in this induction response.

In mouse hepatoma cells, nuclear transcription experiments reveal that TCDD induces hydroxylase activity by stimulating transcription of the corresponding *CYP1A1* gene. The response to TCDD occurs within a few minutes and is direct in that it does not require ongoing protein synthesis. Thus, the regulatory components required for the activation of *CYP1A1* transcription are present constitutively within the cell. TCDD fails to activate *CYP1A1* transcription in Ah receptor-defective cells and in Arnt-defective cells, indicating that the response requires both the Ah receptor and Arnt.

Observations that TCDD activates transcription and the liganded Ah receptor binds to DNA led to the discovery of a dioxin-responsive regulatory DNA sequence upstream of the *CYP1A1* gene. Recombinant DNA methods were used to construct chimeric genes in which potential regulatory DNA sequences from the *CYP1A1* gene were ligated to a heterologous "reporter" gene. After transfection of the recombinant genes into mouse hepatoma cells, TCDD was observed to activate the expression of the reporter gene. Additional transfection experiments defined the size of the dioxin-responsive domain and revealed that it had the properties of a transcriptional enhancer (Jones et al., 1986; Neuhold et al., 1986; Fujisawa-Sehara et al., 1987; Fisher et al., 1990). Furthermore, the recombinant gene responded poorly when transfected into Ah receptor-defective cells or Arnt-defective cells. Thus, both the receptor protein and the Arnt protein are required for enhancer function. Analyses of stable transfectants revealed that the dioxin-responsive enhancer can function in a chromosomal location distinct from that of the *CYP1A1* gene (Fisher et al., 1989). Therefore, in principle, an analogous enhancer element could mediate the transcriptional response of other genes to TCDD.

The DNA upstream region of the *CYP1A1* gene contains a second control element, a transcriptional promoter, that functions to ensure that transcription is initiated at the correct site. The promoter binds proteins that are expressed constitutively by the cell; however, the promoter contains no binding sites for the liganded Ah receptor heteromer. Transfection experiments indicate that neither the enhancer nor the promoter functions in the absence of the other (Jones and Whitlock, 1990). On might question how the enhancer and promoter, which are separated by hundreds of nucleotides, function in concert. TCDD-induced alterations in the chromatin structure of the *CYP1A1* gene play an important part in this process, as described later.

As indicated above, enhancer function requires both Ah receptor and Arnt proteins. Furthermore, the liganded, heteromeric form of the receptor exhibits an increased affinity for DNA. Together these data indicate that activation of *CYP1A1* transcription involves binding of the receptor heteromer to the enhancer. Analyses of protein-DNA interactions in vitro by gel retardation were done using enhancer DNA sequences and nuclear extracts from uninduced and

TCDD-induced cells. These studies revealed the existence of an inducible, receptor-dependent, and Arnt-dependent protein-DNA interaction, whose characteristics were those expected for the binding of the receptor heteromer to DNA (Denison et al., 1989; Hapgood et al., 1989; Saatcioglu et al., 1990a,b). The receptor heteromer recognizes the specific core nucleotide sequence

5'-NTGCGTG-3'
3'-NACGCAC-5'

present in multiple copies within the enhancer. These are often referred to as a DRE or XRE. Studies with a [^{125}I]-labeled dioxin indicate that the receptor heteromer binds in a 1:1 ratio to its DNA recognition sequence (Denison et al., 1989). Methylation protection and interference experiments in vitro reveal that the receptor heteromer lies within the major DNA groove and contacts the four guanines of the recognition sequence (Neuhold et al., 1989; Shen and Whitlock, 1989; Saatcioglu et al., 1990a, b). Transfection analyses of the six enhancer binding sites (in the mouse) for the receptor heteromer, as well as several mutated sites synthesized in vitro, reveal that nucleotides adjacent to the core recognition sequence contribute to enhancer function because the core nucleotides alone fail to exhibit enhancer activity (Denison et al., 1988). Studies of protein-DNA interactions reveal that there is no strict relationship between the affinity of the receptor heteromer for DNA and the extent of enhancer activation (Shen and Whitlock, 1992; Neuhold et al., 1989). These latter observations suggest that the protein-DNA interaction per se does not suffice to activate transcription and that an additional event (such as DNA bending--see below) is necessary. Mutational analyses reveal that the four base-pair sequence

5'-CGTG-3'
3'-GCAC-5'

is required for the receptor heteromer to bind to DNA in vitro (Shen and Whitlock, 1992; Yao and Denison, 1992; Lusska et al., 1993). Six distinct DRE sites have been identified within the mouse *CYP1A1* promoter (Lusska et al., 1993), whereas the rat and human *CYP1A1* gene promoters contain two and three copies of the core DRE sequence, respectively (Swanson and Bradfield, 1993). Thus, the homologous gene from different species may have different regulatory units. It is inappropriate to assume that the relative sensitivity of these genes to modulation by TCDD may be increased or decreased based on the number of DREs or their relative position in the upstream regulatory region, because this is also influenced by a variety of other regulatory factors. It is also possible that additional transcription factors, activators or

repressors, may have overlapping DNA binding specificities with that of the Ah receptor-Arnt complex (e.g., Duan et al., 1999; Klinge et al., 1999).

The DNA recognition sequence for the receptor heteromer contains two CpG dinucleotides. Studies in other systems have revealed that cytosine methylation at CpG is associated with decreased gene expression, often in tissue-specific fashion. Cytosine methylation of the CpG dinucleotides within the recognition sequence diminishes both the binding of the receptor heteromer to the enhancer (as measured by gel retardation) and the functional activity of the enhancer element (as determined by transfection experiments). Therefore, given that the TCDD-responsive receptor/enhancer system can regulate the transcription of genes other than *CYP1A1*, methylation of the enhancer may constitute one mechanism for controlling expression of such genes in a tissue-specific fashion (Shen and Whitlock, 1989).

An extensive analysis of the manner in which other bHLH proteins dimerize and bind to their respective DNA recognition sequences has been performed (reviewed in Swanson et al., 1995; Schmidt and Bradfield, 1996; Wilson and Safe, 1998). The bHLH/PAS family can be subdivided based on the basic region amino acid sequence and the DNA recognition sequence of its members. Arnt-like proteins recognize a 3'-half-site GTG, whereas heterodimer partners for Arnt, such as the Ah receptor, are more selective with unique basic domains. This suggests that although the Arnt-like component may function as a generic coregulator, specificity may be designated by Arnt's dimerization partner. These data are consistent with the finding that Arnt can dimerize with several other bHLH proteins; to date, the Ah receptor has been found to dimerize only with Arnt. Thus the family of bHLH/PAS proteins may exhibit a multitude of possible dimerizations, each complex recognizing a unique DNA sequence. The relative presence and state of activation of these proteins represent a novel mechanism to determine diversity of tissue-, cell-, and gene-specific regulation.

Promoter regions of TCDD-responsive genes have also been found to contain negative regulatory elements (Hines et al., 1988; Walsh et al., 1996; Piechocki and Hines, 1998). Superinducibility of *CYP1A1* by TCDD has been observed following the treatment of cells with cycloheximide, a protein synthesis inhibitor, suggesting that constitutively bound proteins are involved in negative regulation of *CYP1A1* (Lusska et al., 1992). The function and regulation of these negative elements are not completely understood and are likely to be gene-, cell-, and species-specific.

Gel retardation analyses also reveal that binding of the receptor heteromer to its recognition sequence bends the DNA in vitro. The site of the bend is at, or very near, the site of the protein-DNA interaction (Elferink and Whitlock, 1990). These findings suggest that binding

of the receptor heteromer to chromatin might also alter the configuration of the enhancer DNA in vivo.

2.6.2. In Vivo Studies

Studies that use transfection and in vitro techniques for analyzing protein-DNA interactions provide important clues about the functional components of the TCDD-responsive system. However, such experimental approaches necessitate removing the DNA regulatory elements from their native context within the chromosome and, therefore, have the potential to generate misleading results. For example, in the intact cell, nuclear DNA is complexed with histones and other chromosomal proteins, and the structure of the nucleoprotein complex (chromatin) makes important contributions to the control of gene transcription (Grunstein, 1990; Felsenfeld, 1992; Kornberg and Lorch, 1992). Transfection experiments and studies of protein-DNA interactions in vitro do not adequately control for this variable. For this reason, the protein-DNA interaction at the dioxin-responsive enhancer was analyzed in intact cells (Wu and Whitlock, 1993). These experiments revealed that the inactive (i.e., uninduced) enhancer binds few, if any, proteins within the major DNA groove. This finding implies that the inactive enhancer is relatively inaccessible to DNA-binding proteins in vivo. In addition, from a mechanistic standpoint, the absence of protein-enhancer interactions in uninduced cells argues against the idea that, at least for the *CYP1A1* gene, a specific repressor protein maintains the enhancer in an inactive configuration. Exposure of cells to TCDD leads to rapid binding of receptor heteromers, and a few other proteins, to the enhancer in the regulatory region of the *CYP1A1* gene. Therefore, in isolated hepatoma cells the liganded receptor heteromer appears to activate transcription of *CYP1A1* by a mechanism that does not require other enhancer-binding proteins (Wu and Whitlock, 1993).

Proteins that bind to the *CYP1A1* promoter are expressed constitutively, and TCDD has no effect on their interactions with promoter DNA in vitro, as measured by DNase footprinting (Jones and Whitlock, 1990). However, in intact cells, these proteins fail to bind to the inactive (i.e., uninduced) promoter. Thus, the promoter, like the enhancer, is inaccessible in uninduced cells. Exposure of cells to TCDD induces a rapid conformational change at the promoter region, such that it becomes accessible to the constitutively expressed proteins. This change represents a primary effect of TCDD that does not require transcription since it is insensitive to actinomycin D. In addition, it is receptor dependent and Arnt dependent, because it does not occur in Ah receptor- and Arnt-deficient cells. These observations indicate that the receptor-enhancer interaction increases accessibility of the downstream promoter to transcription factors (Durrin and Whitlock, 1989; Wu and Whitlock, 1992).

Studies of *CYP1A1* gene chromatin structure reveal that, in the transcriptionally inactive state, the enhancer/promoter region assumes a nucleosomal structure that is specifically positioned at the promoter (Morgan and Whitlock, 1992). Its organization into nucleosomes plausibly accounts for the inaccessibility of the enhancer/promoter region to DNA-binding proteins in uninduced cells. Exposure to TCDD produces a rapid and actinomycin D-insensitive loss of the positioned nucleosomes at the promoter; this change in chromatin structure accounts for the TCDD-induced increase in promoter accessibility and increased *CYP1A1* transcription in vivo (Morgan and Whitlock, 1992). However, studies indicate that Ah receptor-Arnt binding to DNA and increased chromatin accessibility at the enhancer are not sufficient to induce *CYP1A1* gene expression, but that enhancer-promoter communication is also required. This is postulated to be mediated, at least in part, by the ability of the C-terminal region of the Ah receptor to interact with other proteins (Okino and Whitlock, 1995; Ko et al., 1996).

The mechanism by which binding of liganded receptor heteromers to the enhancer alters chromatin structure is unknown. One possibility is that the DNA-bound receptor complex affects histones (e.g., by recruiting other proteins possessing and/or activating histone acetylase activity), thereby weakening histone-DNA interactions and destabilizing nucleosomes. Studies using trichostatin A, a histone deacetylase inhibitor, show that histone acetylation plays an important role in Ah receptor-elicited activation of the *CYP1A1* and *1A2* genes (Xu et al., 1997). The Ah receptor-Arnt complex has been shown to interact with several coactivator proteins that may enhance transcription under some contexts (Kobayashi et al., 1996; Kumar et al., 1999; Nguyen et al., 1999). Furthermore, several coactivators have been identified that contain regions homologous to the PAS domain of the Ah receptor, Arnt and other bHLH proteins (Kamei et al., 1996; Voegel et al., 1996). Under in vitro conditions, AhR and Arnt have been found to directly interact with several proteins that make up the basal transcriptional machinery, such as TBP (TATA-box binding protein), TFIIF, and TFIID, which has acetyltransferase activity (Rowlands et al., 1996; Swanson and Yang, 1998). A second possibility is that the receptor-enhancer interaction alters the DNA structure of the enhancer/promoter region, thereby stabilizing it in a non-nucleosomal configuration. This idea is consistent with the observation that the receptor heteromer bends DNA in vitro (Elferink and Whitlock, 1990).

The six binding sites for the receptor heteromer on the *CYP1A1* enhancer in mice are arranged in an irregular pattern. The absence of regular spacing between sites suggests that enhancer activation does not require protein-protein interactions between adjacent DNA-bound receptor heteromers. Instead, irregular spacing of binding sites may reflect constraints imposed by chromatin structure because the receptor heteromer must bind to nucleosomes. For example, as the DNA helix wraps around the histone core of the nucleosome, the major groove (which contains the binding sites for the receptor heteromer) is periodically accessible. Increasing the

number of binding sites at irregular intervals increases the probability that at least one site will be accessible even when the DNA is nucleosomal. In addition, the receptor heteromer contacts a relatively short (six base-pair) DNA segment, increasing the probability that the entire binding site in the nucleosome will be accessible. Thus, the multiplicity, irregular distribution, and small size of the binding sites may have evolved as a mechanism for overcoming the steric constraint imposed by the nucleosomal organization of the inactive enhancer in vivo.

2.7. EVIDENCE FOR DIFFERENT MECHANISMS OF TOXICITY.

As indicated above, much of our understanding of the possible mechanisms by which TCDD and related chemicals may act has been elucidated through analyses of how they induce *CYP1A1* gene expression. Based on this model, it is logical to hypothesize that the binding of TCDD to the Ah receptor, receptor-Arnt dimerization, binding of this complex to DREs present in 5' promoter regions of responsive genes, and inappropriate modulation of gene expression, represent the initial steps in a series of biochemical, cellular, and tissue changes that result in the toxicity observed. This hypothesis is further supported by numerous studies evaluating structure-activity relationships of various Ah receptor ligands, the genetics of mutant Ah receptor genes, receptor-deficient mice, and the molecular events contributing to and regulating expression of the Ah receptor and its activity. For example, it is striking that dosages of TCDD, that produce a variety of toxic effects in normal mice, produce no effects in Ah receptor knockout mice. Although the promoter regions of many genes, including *CYP1A1*, contain DREs (Lai et al., 1996), only a few of these are known to be directly regulated by the Ah receptor-Arnt complex (reviewed in Denison et al., 1998). However, the modulated expression of these genes does not completely explain (at least not as yet) the diversity of toxic effects elicited by TCDD in numerous animal species. By analogy, it is predicted that other genes have inappropriate expression (or repression) directly related to particular toxic events. The findings that many Ah receptor-modulated genes are regulated in a species-, cell-, and developmental stage-specific manner suggest that molecular and cellular pathways leading to any particular toxic event are extremely complex. Recent work demonstrated the modulation of at least 310 known genes in human hepatoma cells exposed to TCDD (Puga et al., 2000b). Indeed, precise dissection of these events represents a considerable challenge, especially in that a toxic response may depend on timely modulation of several genes rather than of just one particular gene, and possibly modulation of these genes in several rather than just one cell type.

As our understanding of the receptor and the molecular events that regulate its activity has progressed, it has become apparent that biochemical and biological outcomes of TCDD exposure can be modulated by numerous other proteins with which the Ah receptor interacts. Thus, it is possible that dioxin could modulate gene expression by pathways that do not involve

interaction of the receptor with either Arnt or DREs. Although conditional disruption of the Arnt gene results in the inability of TCDD to induce several responsive genes including CYP1A1 (Tomita et al., 2000), there are, in fact, no data proving that Arnt is required for any toxic effects elicited by dioxin. No other functional heterodimer partner for the Ah receptor has been identified. Thus, it is conceivable that, through interaction with other proteins, the receptor could bind to DNA elements that are uniquely different from the consensus DRE identified. This possibility seems even more plausible given the multiple dimerization partners identified for Arnt and other bHLH-PAS proteins.

It is also reasonable to hypothesize that the Ah receptor might modulate gene expression by a mechanism that does not require its direct interaction with DNA. It is plausible, for example, that the Ah receptor and the Ah receptor-Arnt complex could divert other proteins and transcription factors from other signaling pathways. Several studies provide evidence to support this possibility. For example, direct interaction between the Ah receptor and retinoblastoma protein has been shown (Ge and Elferink, 1998; Puga et al., 2000a). Progression of the cell cycle through G1 phase is regulated, in part, by the retinoblastoma protein (Weinberg, 1995). TCDD exposure has been shown to arrest cells in G1 (Weiss et al., 1996; Kolluri et al., 1999), and the Ah receptor appears to be necessary for progression of mouse hepatoma cells through G1 (Ma and Whitlock, 1996). These observations are consistent with a hypothesis that the dioxin-activated Ah receptor may disrupt normal retinoblastoma protein-mediated differentiation processes. Notably, another protein, p27Kip1, which is important for regulating the progression of cells from G1 to S phase, has been shown to be induced by TCDD (Kolluri et al., 1999). Furthermore, the Ah receptor is expressed in cells in a cycle-dependent manner, with expression peaking in the late S phase (Vaziri et al., 1996). Similarly, recently described interactions between the Ah receptor and the transcription factor NF-kB could potentially explain several biological effects associated with TCDD exposure (Tian et al., 1999). Data suggests that activation of the AhR may increase the activity of specific NF-kB subunits (Kim et al., 2000; Schlezinger et al., 2000). For example, the AhR has been shown to associate with the RelA subunit of NF-kB to activate the C-myc gene promoter in human breast cancer cells (Kim et al., 2000). Likewise, dioxin treatment has been shown to inhibit progesterone receptor signaling (Kuil et al., 1998). This might be due to the sequestration of common accessory factors or coactivator proteins used by other transcription factors. It has been demonstrated that overexpression of one steroid hormone receptor can decrease transcriptional activity of another, presumably through this mechanism (Meyer et al., 1989). It is also possible that because Arnt (or other as yet unidentified partners for the Ah receptor) can dimerize with other proteins, some of the biological effects of the dioxins could be related to recruitment of Arnt by the Ah receptor away from these other pathways. Thus, toxicity could result from loss of Arnt function. At this

point, there is no evidence for this mechanism. In fact, recent data suggest that the noted functional interference between hypoxia-induced pathways and Ah receptor-mediated signaling (Gradin et al., 1996) does not occur through competition between the Ah receptor and HIF-1α for Arnt (Pollenz et al., 1999). Data also suggest that ligand binding to the Ah receptor initiates a phosphorylation/dephosphorylation cascade resulting in modulated activity of other transcription factors (Enan and Matsumura, 1996; Blankenship and Matsumura, 1997). Precise components and events of these modulated pathways have yet to be delineated. How or whether any of these interactions in fact lead to any or all of the toxic effects elicited by dioxin exposure has yet to be established. Nevertheless, it is clear that inappropriate activation of the Ah receptor could have profound effects on regulation of a variety of signal transduction pathways; disruption of these could have serious consequences on a number of cell and tissue processes.

Despite the wealth of evidence to indicate a role of the Ah receptor in the toxicity elicited by the dioxins, it also seems possible that these chemicals may cause toxic effects through mechanisms involving their interaction with biological effector molecules other than the receptor. Clearly, a causal link between Ah receptor-regulated gene expression (regardless of the mechanism of gene modulation) and any of the demonstrated toxic effects has not been established. Furthermore, even though data from structure activity relationship and genetic studies are consistent with the notion that the Ah receptor mediates some toxic effects, not all effects have been thoroughly examined by these parameters. Similarly, Ah receptor knockout mice have not been thoroughly examined for the ability of dioxin to elicit all of its known toxic effects. Finally, although the Ah receptor is present in human cells and tissues, and studies using human cells are consistent with the hypothesis that the Ah receptor mediates effects of the dioxins, it is not known whether these same relationships would exist following exposure of intact humans. Thus, it seems possible that dioxin might cause effects in people and animals by a mechanism involving macromolecules other than the Ah receptor. Furthermore, within the same organism some effects may involve the receptor and others may not. To date, there is, however, no relevant evidence to support these possibilities.

It has been demonstrated that Ah receptor-deficient mice show no signs of toxicity at doses of TCDD (200 µg/kg) approximating the LD50 dose in mice containing the Ah receptor (Fernandez-Salguero et al., 1996). However, a single exposure of 2000 µg/kg to Ah receptor-deficient animals produced several minor lesions including scattered necrosis and vasculitis in the liver and lungs. These data suggest that a pathway leading to toxicity exists, albeit at very high doses, that is independent of the Ah receptor. However, these data also clearly indicate that the major in vivo effects of TCDD are mediated, at least in mice, through the Ah receptors. Notably, the level of TCDD exposure in these Ah receptor-deficient animals is well beyond that known to occur in any human population.

There is much evidence to indicate that the immunotoxic effects of TCDD are mediated by the Ah receptor (Luster et al., 1985; Silkworth and Antrim, 1985; Davis and Safe, 1988, 1989, 1990; Safe, 1990; Ackerman et al., 1989; Pavylak et al., 1989; Kerkvliet et al., 1990; Silkworth et al., 1993). These studies primarily investigate structure-activity relationships following a single dose, and compare mouse strains that are sensitive or less sensitive to TCDD because of alterations in the amino acid sequence of the Ah receptor. However, the results of other investigations using repeated dosing schedules could be interpreted to indicate that the Ah receptor is not involved in some immunotoxic responses. When TCDD was administered to mice daily for 2 weeks, no differences in the inhibition effects of TCDD on antibody-forming cells in the spleen were observed between sensitive and less sensitive strains of mice (Morris et al., 1992). A simple interpretation of these results might be that molecules other than the Ah receptor may mediate the effects of TCDD following multiple or chronic exposures. However, the authors did observe differences in sensitivity between different control groups, and when the data were expressed as a percentage of the appropriate control, strain differences in response to TCDD were observed. It also seems likely that relative sensitivity to TCDD contributes to strain differences in the pharmacokinetics of this chemical. TCDD and related chemicals have been shown to induce expression of cytochrome P4501A2 (CYP1A2) in the liver, and the presence or absence of this protein has been shown to influence tissue distribution of TCDD (Poland et al., 1989; Kedderis et al., 1993; Diliberto et al., 1997). Thus, it is likely that, because of the induction of CYP1A2 and greater sequestration of dioxin in the livers of responsive animals, less is available to other tissues compared with less responsive animals. Data have been presented that indicate this (Diliberto et al., 1999).

Several other studies performed in isolated cells might also be interpreted to indicate that some of the immunotoxic effects of the dioxins are Ah receptor independent (Tucker et al., 1986; Davis and Safe, 1991; Morris et al., 1991; Morris and Holsapple, 1991). However, these data appear to conflict with results obtained in vivo and have been shown to be dependent on culture conditions (Morris and Holsapple, 1991). Furthermore, actual cellular concentrations of chemicals used in several of these in vitro studies were much higher than those known to occur in vivo where immunotoxic effects are observed (Neumann et al., 1992).

Several biochemical responses have been shown to occur rapidly in isolated cells following exposure to TCDD and related dioxins. These include increases in protein kinase and phospholipase C activities, and affects on plasma membranes (Bombick et al., 1984; Bombick et al., 1985; Beebe et al., 1990; Ma et al., 1992; Puga et al., 1992; Ashida et al., 2000b). Some of these responses occur in cells lacking Arnt and in cells with highly reduced levels of Ah receptor (Puga et al., 1992), implying either a non nuclear role of the Ah receptor in mediating these events or an Ah receptor-independent process. Blankenship and Matsumura (1997) have shown

that TCDD, directly or indirectly, activates protein tyrosine kinase activity in murine hepatic cytosol, also suggesting non-nuclear activity of this chemical. In another recent study, TCDD was shown to stimulate c-fos activity in CV-1 cells in which no Ah receptor could be detected by immunoblot analysis or by the induction of *CYP1A1* activity (Hoffer et al., 1996). However, this study failed to completely rule out the presence of very low levels of receptor. Notably, certain bone marrow stromal cell lines lack immunodetectable Ah receptor and fail to show induction of *CYP1A1* following treatment with TCDD. However, in these same cell lines, Ah receptor mRNA transcripts could be detected and *CYP1B1* mRNA was clearly inducible by TCDD (Lavin et al., 1998).

Thus, at present the wealth of evidence available indicates that most, if not all, of the biological and toxic effects of dioxins are mediated by the Ah receptor. Although the receptor may be necessary for the occurrence of these events, clearly it is not sufficient because other proteins and conditions are known to affect activity of the receptor and its ability to alter gene expression. There is some evidence to support mechanisms involving pathways for Ah receptor action that do not involve Arnt, although the exact steps involved in these pathways have yet to be fully detailed. Certain studies could be interpreted to indicate Ah receptor-independent mechanisms, although these have not clearly ruled out involvement of the Ah receptor. The only consistent, but limited, evidence for dioxin-elicited effects that do not involve the Ah receptor comes from studies using Ah receptor-deficient animals. Here however, observed minor effects occurred only following treatment with extremely high doses of TCDD. Notably, these doses are well beyond that which any humans are known to have been exposed.

2.8. FUTURE RESEARCH

The cloning of cDNAs encoding the Ah receptor and Arnt proteins, and the development of anti-receptor and anti-Arnt antibodies, open the way for additional mechanistic studies of dioxin action. It is now practical to analyze the structure and function of these proteins using mutagenesis techniques, to analyze the structure and regulation of the corresponding genes, to determine whether different forms of the receptor and Arnt exist, and to directly analyze the role of posttranslational modifications on receptor and Arnt function. In vitro transcription can be used to study the functional components of the dioxin-responsive pathway.

The observation that the Ah receptor and Arnt proteins heterodimerize via HLH motifs raises the likelihood that they may also dimerize with other partners (perhaps in a tissue-specific fashion) to generate different protein combinations that may have novel regulatory properties. The types of protein complexes formed may depend on the relative amount of each potential partner protein present in different cell types and at different times of tissue development/differentiation, as well as on the stability of each type of heterodimer. This

combinational mechanism for regulating dioxin responses may also allow for functional interactions with other signal transduction pathways, increasing the potential diversity of dioxin-induced responses even further (see, e.g., Pimental et al., 1993; Hogenesch et al., 1997; Caruso et al., 1999; Chan et al., 1999; Nebert et al., 2000). These are promising areas for future research, and are likely to provide novel insights into the mechanism of dioxin action, in particular, and the regulation of mammalian gene transcription (especially by bHLH proteins), in general. If such studies reveal new proteins (and corresponding genes) that influence rate-limiting steps in the response to dioxin, the findings might also prove useful from a risk assessment standpoint.

The teratogenic and tumor-promoting effects of TCDD, its effects on differentiation of a variety of cell types, and results from Ah receptor deficient animals suggest that the Ah receptor and other components of the dioxin-responsive system contribute to important developmental and proliferative pathways. Further study of transgenic animals, in which both alleles for the Ah receptor or the Arnt protein have been permanently or conditionally inactivated, will provide new insights into such pathways. In addition, the use of transgenic animals in toxicological studies might be helpful to assess what cellular components participate in the adverse responses to particular chemicals.

Chromatin structure and nucleosome positioning have important effects on mammalian gene expression (Grunstein, 1990; Felsenfeld, 1992; Kornberg and Lorch, 1992). The TCDD-responsive *CYP1A1* gene is an interesting model system that can be used to analyze the mechanism by which a protein complex, such as the liganded receptor heteromer, can trigger chromatin structural changes that increase DNA accessibility. Such studies may also provide novel insights into the function of the dioxin-responsive system.

Some dioxin-responsive genes (e.g., *CYP1A2*, *CYP1B1*, *glutathione S-transferase Ya*) exhibit substantial constitutive ("basal") transcription, which is increased further upon TCDD treatment. The constitutive expression of these genes implies that the promoter must be maintained in an accessible configuration even in the absence of dioxin; therefore, TCDD may induce the transcription of such genes by a mechanism that does not involve major changes in chromatin structure. As such, the liganded receptor heteromer may be able to increase gene transcription by both chromatin-dependent and chromatin-independent mechanisms. This may be an interesting area for future research.

The overall evidence indicates that the Ah receptor participates in every biological response to TCDD (Poland and Knutson, 1982; Safe, 1986; Birnbaum, 1994). Thus, through its interaction with Arnt and other transcription factors, TCDD likely activates transcription of other genes via a receptor- and enhancer-dependent mechanism analogous to that described for the *CYP1A1* gene. For example, TCDD induces the expression of genes encoding CYP1A2,

CYP1B1, glutathione S-transferase Ya subunit, aldehyde dehydrogenase, prostaglandin endoperoxide H synthase 2, and quinone reductase. In some cases, induction has been shown to occur at the transcriptional level, to be Ah receptor and Arnt dependent, and to involve a DNA recognition sequence analogous to that found upstream of the *CYP1A1* gene (Dunn et al., 1988; Quattrochi and Tukey, 1989; Favreau and Pickett, 1991; Takimoto et al., 1992; Pimental et al, 1993; Rushmore and Pickett, 1993; Sutter et al., 1994; Kraemer et al., 1996). Other observations reveal that TCDD activates the transcription of several other genes by unknown mechanisms (see Denison et al., 1998). For dioxin-responsive genes other than *CYP1A1*, and especially for genes that respond in a tissue-specific fashion, the presence of the receptor/enhancer system may not be sufficient for dioxin action. Rather, other tissue-specific regulatory components may play a dominant role in governing the response to TCDD. For example, estrogens (presumably via estrogen receptors) influence TCDD-induced liver neoplasia in rats (Lucier et al., 1991). In addition, in vitro experiments suggest that, in some cell types, a repressor protein inhibits the response to TCDD by competing for the receptor binding site(s) on DNA (Gradin et al., 1993; Walsh et al., 1996; Piechocki and Hines, 1998). Future research may reveal the existence of additional stimulatory or inhibitory gene regulatory components that can modulate the activity of the dioxin-responsive receptor/enhancer system. Further research is also needed to determine whether Ah receptor-dependent modulation of gene expression is exclusively dependent on Arnt. Other heterodimeric partners for the Ah receptor may be identified and these complexes may recognize unique DNA response elements that are involved in the control of a variety of genes. In addition, it is also possible that the Ah receptor controls certain activities, such as phosphorylation/dephosphorylation, through pathways that do not directly involve nuclear localization and transcriptional activation.

The potential teratogenic, developmental, and neoplastic effects of TCDD have raised particular concerns about the human health effects of dioxin. In particular, experimental studies in animals suggest that developing tissues are especially sensitive. These effects have been characterized by altered cell proliferation, metaplasia, and modified terminal differentiation, and can occur at dosages that have no overt toxicity to the pregnant dam (see Theobald and Peterson, 1994; Gray and Ostby, 1995; Roman et al., 1995). Concentrations of TCDD as low as 0.8 ng/g in the murine embryonic palate have been shown to result in cleft palate (Abbott et al., 1996). These responses may reflect complicated cascades of biochemical changes that are difficult to analyze mechanistically; therefore, a major challenge for the future will be to establish experimental systems in which such complex phenomena are amenable to study at the molecular level.

The actual molecular mechanisms underlying differences in sensitivity between species, tissues, and periods of development have yet to be determined. Indeed, this is a central issue for

relating animal studies to human exposures and sensitivity. A major obstacle has been our lack of understanding of the direct tissue and cell targets, critical periods of increased sensitivity, and actual dose to the target cell type. Even with the same dosage per cell, it is not difficult to envision that given the multitude of regulatory controls for any particular gene at a particular period of cellular development, different dose-dependent response curves for these genes would be observed. For example, rat *UDP glucuronosyltransferase 1A6* gene is about 1000 times less sensitive than the *CYP1A1* gene to induction of transcription by dioxin (Vanden Heuvel et al., 1994). Furthermore, considering the likely multitude of additional molecular events that contribute to the ultimate biological/toxic response following initial gene modulation, it is also not difficult to envision that different endpoints might have very different sensitivities, even though binding to the same receptor is the common initial trigger. Considering this complexity, it remains a particular challenge to develop appropriate model systems that accurately mimic the in vivo effects associated with exposure. It is also important to consider that a particular response to TCDD may be mediated through effects on multiple cells. Evidence was recently presented to indicate that Ah receptor activation in both hepatic parenchymal and hematopoietic cells contributes to the hepatic lesions induced by TCDD in mice (Thurmond et al., 1999).

Clearly, most data are consistent with the belief that the Ah receptor has some normal cellular function. We can now appreciate that the Ah receptor is a member of a family of proteins that are conserved through evolution and involved in growth and differentiation processes. The expression and activity of the Ah receptor appear to be well regulated in a differentiation and cell cycle stage-specific manner. Furthermore, we now know that the genes regulated by the receptor are involved in not only xenobiotic metabolism, but also in cell growth and differentiation processes. Further identification of other Ah receptor-regulated genes and their function, as well as factors that may control the ability of the receptor to regulate these in a cell-specific manner, will undoubtedly assist in our understanding of its normal function and how aberrant function may lead to toxicity. For example, recognition that the Ah receptor influences cell cycle regulation, and a further dissection of the pathways involved, will likely help us understand how dioxins affect developmental and neoplastic processes. In addition, further research to characterize and identify possible endogenous ligands for the receptor, and its role in regulating cellular processes, will have a great impact in these areas. It seems plausible that the spectrum of genes regulated by the receptor may mediate both endogenous ligand function and prevention of its inappropriate action through metabolism (Nebert et al., 2000).

2.9. MECHANISTIC INFORMATION AND RISK ASSESSMENT

A substantial body of evidence from investigations using experimental animals indicates that the Ah receptor mediates the biological effects of TCDD. Although studies using human

tissues are much less extensive, it appears reasonable to assume that dioxin's effects in humans are also receptor mediated. Studies using human organs and cells in culture are consistent with this hypothesis. A receptor-based mechanism would predict that, except in cases where the concentration of TCDD is already high (i.e., [TCDD]~K_D), incremental exposure to TCDD will lead to some increase in the fraction of Ah receptors occupied. However, it cannot be assumed that an increase in receptor occupancy will necessarily elicit a proportional increase in all biological response(s), because numerous molecular events (e.g., cofactors, other transcription factors, genes) contributing to the biological endpoint are integrated into the overall response. That is, the final biological response should be considered as an integration of a series of dose-response curves with each curve dependent on the molecular dosimetry for each particular step. Dose-response relationships that will be specific for each endpoint must be considered when using mathematical models to estimate the risk associated with exposure to TCDD. It remains a challenge to develop models that incorporate all the complexities associated with each biological response. Furthermore, the parameters for each mathematical model may only apply to a single biological response within a given tissue and species.

Given TCDD's widespread distribution, its persistence, and its accumulation within the food chain, it is likely that most humans are exposed to some level of dioxin; thus, the population at potential risk is large and genetically heterogeneous. By analogy with the findings in inbred mice, polymorphisms in the Ah receptor probably exist in humans. Therefore, a concentration of TCDD that elicits a particular response in one individual may not do so in another. For example, studies of humans exposed to dioxin following an industrial accident at Seveso, Italy, fail to reveal a simple and direct relationship between blood TCDD levels and development of chloracne (Mocarelli et al., 1991). These differences in responsiveness to TCDD may reflect genetic variation either in the Ah receptor or in some other component of the dioxin-responsive pathway. Therefore, analyses of human polymorphisms in the Ah receptor and Arnt genes have the potential to identify genotypes associated with higher (or lower) sensitivities to dioxin-related effects. Such molecular genetic information may be useful in the future for accurately predicting the health risks dioxin poses to humans.

Complex responses (such as cancer) probably involve multiple events and multiple genes. For example, a homozygous recessive mutation at the *hr* (hairless) locus is required for TCDD's action as a tumor promoter in mouse skin (Poland et al., 1982). Thus, the *hr* locus influences the susceptibility of a particular tissue (in this case skin) to a specific effect of dioxin (tumor promotion). An analogous relationship may exist for the effects of TCDD in other tissues. For example, TCDD may produce porphyria cutanea tarda only in individuals with inherited uroporphyrinogen decarboxylase deficiency (Doss et al., 1984). Such findings suggest

that, for some adverse effects of TCDD, the population at risk may be limited to individuals with a particular genetic predisposition.

Other factors can influence an organism's susceptibility to TCDD. For example, female rats are more prone to TCDD-induced liver neoplasms than are males; this phenomenon is related to the hormonal status of the animals (Lucier et al., 1991). In addition, hydrocortisone and TCDD synergize in producing cleft palate in mice. Retinoic acid and TCDD produce a similar synergistic teratogenic effect (Couture et al., 1990). These findings indicate that, in some cases, TCDD acts in combination with hormones or other chemicals to produce adverse effects. Such phenomena might also occur in humans. If so, the difficulty in assessing risk is increased, given the diversity among humans in hormonal status, lifestyle (e.g., smoking, diet), and chemical exposure. For example, several compounds present in the diet have been found to inhibit activation of the AhR induced by TCDD (Ashida et al., 2000a).

Dioxin's action as a tumor promoter and developmental toxicant presumably reflects its ability to alter cell proliferation and differentiation processes. There are several plausible mechanisms by which this could occur. First, TCDD might activate a gene (or genes) that is directly involved in tissue proliferation. Second, TCDD-induced changes in hormone metabolism may lead to tissue proliferation (or lack thereof) and altered differentiation secondary to altered secretion of a trophic hormone. Third, TCDD-induced changes in the expression of growth factor or hormone receptors may alter the sensitivity of a tissue to proliferative stimuli. Fourth, TCDD-induced toxicity may lead to cell death, followed by regenerative proliferation. These mechanisms likely differ among tissues and periods of development, and might be modulated by different genetic and environmental factors. As such, this complexity increases the difficulty associated with assessing the human health risks form dioxin exposure.

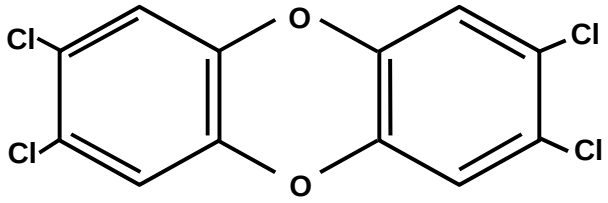
Under certain circumstances, exposure to TCDD may elicit beneficial effects. For example, TCDD protects against the carcinogenic effects of PAHs in mouse skin, possibly reflecting induction of detoxifying enzymes (Cohen et al., 1979; DiGiovanni et al., 1980). In other situations, TCDD-induced changes in estrogen metabolism may alter the growth of hormone-dependent tumor cells, producing a potential anticarcinogenic effect (Spink et al., 1990; Gierthy et al., 1993). However, several recent studies in mice indicate that the Ah receptor has an important role in the genetic damage and carcinogenesis caused by components in tobacco smoke such as benzo[a]pyrene through its ability to regulate *CYP1A1* gene induction (Dertinger et al., 1998, 2000; Shimizu et al., 2000). These issues complicate the risk assessment process for dioxin.

TCDD's biological effects likely reflect a complicated interplay between genetic and environmental factors. Thus, it may be overly simplistic to use mechanistic information derived

largely from the relatively simple responses described in this chapter (i.e., *CYP1A1* induction) as the basis for developing a quantitative approach to dioxin risk assessment in humans. While this approach represents a start toward incorporating mechanistic information into risk assessment, future biologically based dose-response models will require a better understanding not only of the TCDD-induced biochemical alterations that produce disease, but also of the relationships between genetic and environmental factors that influence an individual's susceptibility to TCDD. Molecular toxicology and mechanistic studies have great potential to provide new insights into such issues in the future.

Table 2-1. Events in the activation of *CYP1A1* gene transcription by dioxin

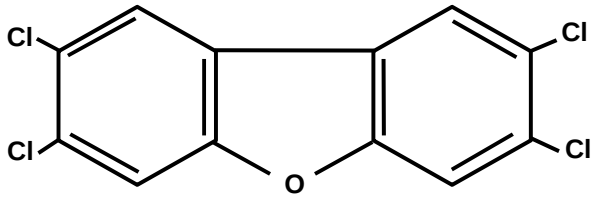
●	Diffusion into the cell
●	Binding to the Ah receptor protein
●	Conversion of liganded receptor to the DNA-binding form
●	Dissociation from hsp90
●	Active translocation from cytoplasm to nucleus
●	Association with Arnt protein
●	Binding of liganded receptor heteromer to enhancer DNA
●	Enhancer activation
●	Altered DNA configuration
●	Histone modification
●	Recruitment of additional proteins
●	Nucleosome disruption
●	Increased accessibility of transcriptional promoter
●	Binding of transcription factors to promoter
●	Enhanced mRNA and protein synthesis



2,3,7,8-Tetrachlorodibenzo-p-dioxin

Polychlorinated dibenzopara-dioxins

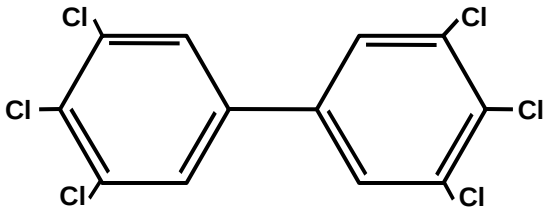
75 congeners



2,3,7,8-Tetrachlorodibenzofuran

Polychlorinated dibenzofurans

135 congeners



3,3',4,4',5,5'-Hexachlorobiphenyl

Figure 2-1. Chemical structure of dioxin and smiliar compounds.

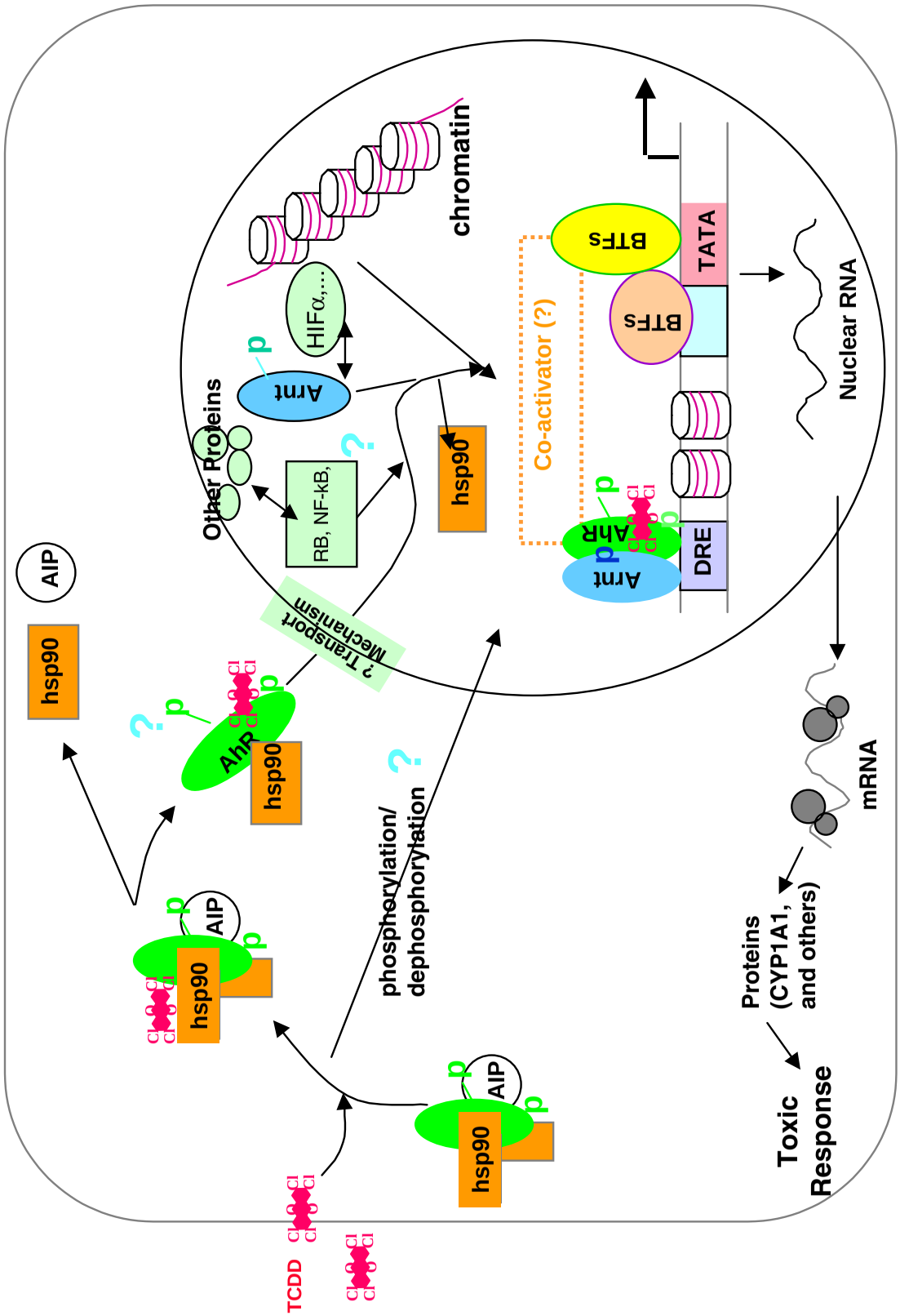


Figure 2-2. Cellular Mechanisms for Ah Receptor Action

REFERENCES FOR CHAPTER 2

Abbott, B.D.; Birnbaum, L.S.; Diliberto, J.J. (1996) Rapid distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to embryonic tissues in C57BL/6N mice and correlation with palatal uptake in vitro. Toxicol. Appl. Pharmacol. 141: 256-263.

Abbott, B.D.; Birnbaum, L.S.; Perdew, G.H. (1995) Developmental expression of two members of a new class of transcription factors. I. Expression of aryl hydrocarbon receptor in the C57BL/6N mouse embryo. Dev. Dyn. 204: 133-143.

Abbott, B.D.; Held, G.A.; Wood, A.R.; Buckalew, J.G.; Brown, J.G.; Schmid, J. (1999a) AhR, ARNT, and CYP1A1 mRNA quantitation in cultured human embryonic palates exposed to TCDD and comparison with mouse palate in vivo and in culture. Toxicol. Sci. 47: 62-75.

Abbott, B.D.; Schmid, J.E.; Pitt, J.A.; Buckalew, A.R.; Wood, C.R.; Held, G.A.; Diliberto, J.J. (1999b) Adverse reproductive outcomes in the transgenic Ah receptor-deficient mouse. Toxicol. Appl. Pharmacol. 155: 62-70.

Ackerman, M.F.; Gasiewicz, T.A.; Lamm, K.R.; Germolec, D.R.; Luster, M.I. (1989) Selective inhibition of polymorphonuclear neutrophil activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 101: 470-480.

Anderson, M.E.; Mills, J.J.; Fox, T.R.; Goldsworthy, T.L.; Conolly, R.B.; Birnbaum, L.S. (1994). Receptor-mediated toxicity and implications for risk assessment. In: Spitzer, HL; Slaga, TJ; Greenlee, WF; McClain, M. (eds.). Receptor-medicated biological processes. Implications for evaluating carcinogenesis. New York: Wiley-Liss, NY. pp. 295-310.

Antonsson, C.; Whitelaw, J.L.; McGuire, J.; Gustafsson, J-A.; Poellinger, L. (1995) Distinct roles of the molecular chaperone hsp90 in modulating dioxin receptor function via the basic helix-loop-helix and PAS domains. Mol. Cell. Biol. 15: 756-765.

Ariens, E.J.; Van Rossum, J.M.; Koopman, P.C. (1960) Receptor reserve and threshold phenomena. I. Theory and experiments with autonomic drugs tested on isolated organs. Arch. Int. Pharmacodyn. 127: 459-478.

Ashida, H.; Nagy, S.; Matsumura, F. (2000) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-induced changes in activities of nuclear protein kinases and phosphatases affecting DNA binding activity of c-Myc and AP-1 in the livers of guinea pigs. Biochem. Pharmacol. 59: 741-751.

Ashida, H.; Fukuda, I.; Yamashita, T.; Kanazawa, K. (2000a) Flavones and flavonols at dietary levels inhibit a transformation of aryl hydrocarbon receptor induced by dioxin. FEBS Lett. 476: 213-217.

Beebe, L.; Park, S.S.; Anderson, L.M. (1990) Differential enzyme induction of mouse liver and lung following a single low or high dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J. Biochem. Toxicol. 5: 211-219.

Beg, A.A.; Baltimore, D. (1996) An essential role for NF-kB in preventing TNF-α-induced cell death. Science 274: 782-784.

Bell, D.R.; Poland, A. (2000). Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein. J. Biol. Chem. 275: 36407-36414.

Benedict, J.C.; Lin, T-M.; Loeffler, I.K.; Peterson, R.E.; Flaws, J.A. (2000) Physiological role of the aryl hydrocarbon in mouse ovary development. Toxicol. Sci. 56: 382-388.

Berghard, A.; Gradin, K.; Pongratz, I.; Whitelaw, M.; Poellinger, L. (1993) Cross-coupling of signal transduction pathways: the dioxin receptor mediates induction of cytochrome P4501A1 expression via a protein kinase C mechanism. Mol. Cell. Biol. 13: 677-689.

Birnbaum, LS. (1994a) Evidence for the role of the Ah receptor in responses to dioxin. In: Spitzer, H.L.; Slaga, T.J.; Greenlee, W.F.; McClain, M., eds. Receptor-mediated biological processes: implications for evaluating carcinogenesis. Progress in Clinical and Biological Research, vol. 387. New York: Wiley-Liss, pp. 139-154.

Birnbaum, LS. (1994b) Mechanism of dioxin toxicity: relationship to risk assessment. Environ. Health Perspect. 102 (Suppl. 9): 157-167.

Bjeldanes, L.F.; Kim, J.Y.; Grose, K.R.; Bartholomew, J.S.; Bradfield, C.A. (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Proc. Natl. Acad. Sci. U.S.A. 88: 9543-9547.

Blankenship, A.; Matsumura, F. (1997) 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced activation of a protein tyrosine kinase pp60src in murine hepatic cytosol using a cell-free system. Mol. Pharmacol. 52: 667-675.

Blankenship, A.L.; Suffia, M.C.; Matsumura, F.; Walsh, K.J.; Wiley, L.M. (1993) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) accelerates differentiation of murine preimplantation embryos in vitro. Reprod. Toxicol. 7: 255-261.

Bombick, D.W.; Madhukar, B.V.; Brewster, D.W.; Matsumura, F. (1985) TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes increases in protein kinases particularly protein kinase C in the hepatic plasma membrane of the rat and the guinea pig. Biochem. Biophys. Res. Commun. 127: 296-302.

Bombick, D.W.; Matsumura, F.; Madhakar, B.V. (1984) TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes reduction in the low density lipoprotein (LDL) receptor activities in the hepatic plasma membrane of the guinea pig and rat. Biochem. Biophys. Res. Commun. 118: 548-554.

Brodie, A.E.; Azarenko, V.A.; Hu, C.Y. (1996) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) inhibition of fat cell differentiation. Toxicol. Lett. 84: 55-59.

Buchner, J. (1999) Hsp90 & Co. – a holding for folding. Trends Biochem. Sci. 24: 136-141.

Burbach, K.M.; Poland, A.; Bradfield, C.A. (1992) Cloning of the Ah-receptor cDNA reveals a novel ligand-activated transcription factor. Proc. Natl. Acad. Sci. U.S.A. 89: 8185-8189.

Carrier, F.; Owens, R.A.; Nebert, D.W.; Puga, A. (1992) Dioxin-dependent activation of murine Cyp1a1 gene transcription requires protein kinase C-dependent phosphorylation. Mol. Cell. Biol. 12: 1856-1863.

Caruso, J.A.; Laird, D.W.; Batist, G. (1999) Role of HSP90 in mediating cross-talk between the estrogen receptor and the Ah receptor signal transduction pathways. Biochem. Pharmacol. 58: 1395-1403.

Carver, L.A.; Bradfield, C.A. (1997) Ligand-dependent interaction of the aryl hydrocarbon receptor with a novel immunophilin homolog in vivo. J. Biol. Chem. 272: 11452-11456.

Carver, L.A.; Jackiw, V.; Bradfield, C.A. (1994) The 90-kDa heat shock protein is essential for Ah receptor signaling in a yeast expression system. J. Biol. Chem. 269: 1-4.

Carver, L.A.; LaPres, J.J.; Jain, S.; Dunham, E.E.; Bradfield, C.A. (1998) Characterization of the Ah receptor-associated protein, ARA9. J. Biol. Chem. 273: 33580-33587.

Chan, W.K.; Yao, G.; Gu, Y-Z.; Bradfield, C.A. (1999) Cross-talk between the aryl hydrocarbon receptor and hypoxia inducible factor signaling pathways. J. Biol. Chem. 274: 12115-12123.

Chang, C-Y.; Puga, A. (1998) Constitutive activation of the aromatic hydrocarbon receptor. Mol. Cell. Biol. 18: 525-535.

Chen, J.D.; Evans, R.M. (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377: 454-457.

Chen, Y.H.; Tukey, R.H. (1996) Protein kinase C modulates regulation of the CYP1A1 gene by the aryl hydrocarbon receptor. J. Biol. Chem. 271: 26261-26266.

Clark, A.J. (1993) The mode of action of drugs on cells. Baltimore, MD: Williams and Wilkins.

Cohen, G.M.; Bracken, W.M.; Iyer, R.P.; Berry, D.L.; Selkirk, J.K.; Slaga, T.J. (1979) Anticarcinogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene tumor initiation and its relationship to DNA binding. Cancer Res. 39: 4027-4033.

Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons. Cancer Res. 42: 4875-4917.

Cook, J.C.; Greenlee, W.F. (1989) Characterization of a specific binding protein for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human thymic epithelial cells. Mol. Pharmacol. 35: 713-719.

Corton, J.C.; Moreno, E.S.; Hovis, S.M.; Leonard, L.S.; Gaido, K.W.; Joyce, M.M.; Kennett, S.B. (1996) Identification of a cell-specific transcription activation domain within the human Ah receptor nuclear translocator. Toxicol. Appl. Pharmacol. 139: 272-280.

Coumailleau, P.; Poellinger, L.; Gustaffson, J-A.; 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.

Couture, L.A.; Abbott, B.D.; Birnbaum, L.S. (1990) A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: recent advances toward understanding the mechanism. Teratology 42: 619-627.

Crawford, R.B.; Holsapple, M.P.; Kaminski, N.E. (1997) Leukocyte activation induces aryl hydrocarbon receptor upregulation, DNA binding, and increased CYP1a1 expression in the absence of ligand. Mol. Pharmacol. 52: 921-927.

Czuczwa, J.M.; McVeety, B.D.; Hites, R.A. (1984) Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediments from Siskicoit Lake, Isle Royale. Science 226: 568-569.

Davis, D.; Safe, S. (1988) Immunosuppressive activities of polychlorinated dibenzofuran congeners: quantitative structure-activity relationships and interactive effects. Toxicol. Appl. Pharmacol. 94: 141-149.

Davis, D.; Safe, S. (1989) Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interactions with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Lett. 48: 35-43.

Davis, D.; Safe, S. (1990) Immunosuppressive activities of polychlorinated biphenyls in C57BL/6N mice: structure-activity relationships as Ah receptor agonists and partial antagonists. Toxicology 63: 97-111.

Davis, D.; Safe, S. (1991) Halogenated aryl hydrocarbon-induced suppression of the in vitro plaque-forming cell response to sheep blood cells is not dependent on the Ah receptor. Immunopharmacol. 21: 183-190.

Denes, J.; Blakey, D.; Krewski, D.; Withey, J.R. (1996) Applications of receptor-binding models in toxicology. In: Fan, AM; Chang, LW. (eds.). Toxicology and Risk Assessment. Principles, Methods, and Applications. New York: Marcel Dekker, NY. pp. 447-472.

Denis, M.; Cuthill, S.; Wikstrom, A.C.; Poellinger, L.; Gustafsson, J.-A. (1988) Association of the dioxin receptor with the M_r 90,000 heat shock protein. Biochem. Biophys. Res. Commun. 155: 801-807.

Denison, M.S.; Fisher, J.M.; Whitlock, J.P., Jr. (1988) The DNA recognition site for the dioxin-Ah receptor complex: nucleotide sequence and functional analysis. J. Biol. Chem. 263: 17721-17724.

Denison, M.S.; Fisher, J.M.; Whitlock, J.P., Jr. (1989) Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. J. Biol. Chem. 264: 16478-16482.

Denison, M.S.; Phelan, D.; Elferink, C.J. (1998) The Ah receptor signal transduction pathway. In: Denison, MS; Helferich, WG. (eds). Toxicant-Receptor Interactions. Bristol. PA: Taylor & Francis, pp. 3-33.

Dertinger, SD; Silverstone, AE; Gasiewicz, TA. (1998) Influence of aromatic hydrocarbon receptor-mediated events on the genotoxicity of cigarette smoke condensate. Carcinogenesis 19: 2037-2042.

Dertinger, S.D.; Nazarenko, D.A.; Silverstone, A.E.; Gasiewicz, T.A. (2000) Aryl hydrocarbon receptor signaling plays a significant role in mediating benzo[a]pyrene and cigarette smoke condensate-induced cytogenetic damage in vivo. Carcinogenesis 22: 171-177.

DiGiovanni, J.; Berry, D.L.; Gleason, G.L.; Kishore, G.S.; Slaga, T.J. (1980) Time-dependent inhibition by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of skin tumorigenesis with polycyclic hydrocarbons. Cancer Res. 40: 1580-1587.

Diliberto, J.J.; Burgin, D.; Birnbaum, L.S. (1997) Role of CYP1A2 in hepatic sequestration of dioxin: studies using CYP1A2 knock-out mice. Biochem. Biophys. Res. Commun. 236: 431-433.

Diliberto, J.J.; Burgin, D.E.; Birnbaum, L.S. (1999) Effects of CYP1A2 on disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 23,4,7,8-pentachlorodibenzofuran, and 2,2',4,4',5,5'-hexachlorobiphenyl in CYP1A2 knockout and parental (C57BL/6N and 129/Sv) strains of mice. Toxicol. Appl. Pharmacol. 159: 52-64.

Dolwick, K.M.; Swanson, H.I.; Bradfield, C.A. (1993) Cloning and expression of a human Ah receptor cDNA. Mol. Pharmacol. 44: 911-917.

Doss, M.; Saver, H.; von Tiepermann, R.; Colombi, A.M. (1984) Development of chronic hepatic porphyria (porphyria cutanea tarda) with inherited uroporphyrinogen decarboxylase deficiency under exposure to dioxin. J. Biochem. 16: 369-373.

Drutel, G.; Kathmann, M.; Heron, A.; Schwartz, J.-C.; 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.

Duan, R.; Porter, W.; Samudio, I.; Vyhlidal, C.; Kladde, M.; Safe, S. (1999) Transcriptional activation of c-fos protooncogene by 17b-estradiol: mechanism of aryl hydrocarbon receptor-mediated inhibition. Mol. Endocrinol. 13: 1511-1521.

Dunn, T.J.; Lindahl, R.; Pitot, H.C. (1988) Differential gene expression in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). J. Biol. Chem. 263: 10878-10886.

Durrin, L.K.; Whitlock, J.P., Jr. (1989) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Ah receptor-mediated change in cytochrome $P_1$450 chromatin structure occurs independent of transcription. Mol. Cell. Biol. 9: 5733-5737.

Elferink, C.J.; Whitlock, J.P., Jr. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inducible, Ah receptor-mediated bending of enhancer DNA. J. Biol. Chem. 265: 5718-5721.

Eltom, S.E.; Zhang, L.; Jefcoate, C.R. (1999) Regulation of cytochrome P-450 (CYP) 1B1 in mouse Hepa-1 variant cell lines: a possible role for aryl hydrocarbon receptor nuclear translocator (ARNT) as a suppressor of CYP1B1 gene expression. Mol. Pharmacol. 55: 594-604.

Ema, M.; Ohe, N.; Suzuki, M.; Mimura, J.; Sogawa, K.; Ikawa, S.; Fujii-Kuriyama, Y. (1994) Dioxin binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors. J. Biol. Chem. 269: 27337-27343.

Ema, M.; Sogawa, K.; Wanatabe, N.; Chujoh, Y.; Matsushita, N.; Gotoh, O.; Funae, Y.; Fujii-Kuriyama, Y. (1992) cDNA cloning and structure of mouse putative Ah receptor. Biochem. Biophys. Res. Commun. 184: 246-253.

Ema, M.; Taya, S.; Yokotani, N.; Sowaga, K.; Matusda, Y.; Fujii-Kuriyama, Y. (1997) A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1a regulates the VEGF expression and is potentially involved in lung and vascular development. Proc. Natl. Acad. Sci. USA 94: 4273-4278.

Enan, E.; Matsumura, F. (1996) Identification of c-Src as the integral component of the cytosolic Ah receptor complex transducing the signal of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) through the protein phosphorylation pathway. Biochem. Pharmacol. 52: 1599-1612.

Favreau, L.V.; Pickett, C.B. (1991) Transcriptional regulation of the rat NAD(P)H; quinone reductase gene. J. Biol. Chem. 266: 4556-4561.

Felsenfeld, G. (1992) Chromatin as an essential part of the transcriptional mechanism. Nature 355: 219-224.

Fernandez-Salguero, P.M.; Hilbert, D.M.; Rudikoff, S.; Ward, J.M.; Gonzalez, F.J. (1996) Aryl-hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced toxicity. Toxicol. Appl. Pharmacol. 140: 173-179.

Fernandez-Salguero, P.; Pineau, T.; Hilbert, D.M.; McPhail, T.; Lee, S.S.T.; Kimura, S.; Nebert, D.W.; Rudikoff, S.; Ward, J.M.; Gonzalez, F.J. (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science 268: 722-726.

Fernandez-Salguero, P.M.; Ward, J.M.; Sundberg, J.P.; Gonzalez, F.J. (1997) Lesions of aryl-hydrocarbon receptor-deficient mice. Vet. Pathol. 34: 605-614.

Fisher, J.M.; Jones, K.W.; Whitlock, J.P., Jr. (1989) Activation of transcription as a general mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. Mol. Carcinog. 1: 216-221.

Fisher, J.M.; Wu, L.; Denison, M.S.; Whitlock, J.P., Jr. (1990) Organization and function of a dioxin-responsive enhancer. J. Biol. Chem. 265: 9676-9681.

Fujisawa-Sehara, A.; Sogawa, K.; Yamane, M.; Fujii-Kuriyama, Y. (1987) Characterization of xenobiotic responsive elements upstream from the drug-metabolizing cytochrome P450c gene: a similarity to glucocorticoid regulatory elements. Nucleic Acids Res. 15: 4179-4191.

Fukunaga, B.N.; Probst, M.R.; Reisz-Porszasz, S.; Hankinson, O. (1995) Identification of functional domains of the aryl hydrocarbon receptor. J. Biol. Chem. 270: 29270-29278.

Gaido, K.W.; Maness, S.C. (1994) Regulation of gene expression and acceleration of differentiation in human keratinocytes by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 127: 199-208.

Gasiewicz, T.A. (1997) Dioxins and the Ah receptor: probes to uncover processes in neuroendocrine development. Neurotoxicology 18: 393-414.

Ge, N-L.; Elferink, C.J. (1998) A direct interaction between the aryl hydrocarbon receptor and retinoblastoma protein. Linking dioxin signaling to the cell cycle. J. Biol. Chem. 273: 22708-22713.

Gierthy, JF; Bennett, JA; Bradley, LM; Cutler, DS. (1993) Corrleation of in vitro and in vivo growth suppression of MCF-7 human breast cancer by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Canc. Res. 53: 3149-3153.

Gierthy, J.F.; Silkworth, J.B.; Tassinari, M.; Stein, G.S.; Lian, J.B. (1994) 2,3,7,8-Tetrachlorodibenzo-p-dioxin inhibits differentiation of normal diploid rat osteoblasts in vitro. J. Cell. Biochem. 54: 231-238.

Gillner, M.; Bergman, J.; Cambillau, C.; Alexandersson, M.; Fernstrom, A.; Gustaffson, J-A. (1993) Interactions of indolo[3,2-b]carbazoles and related polycyclic aromatic hydrocarbons with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver. Mol. Pharmacol. 44: 336-345.

Gillner, M.; Bergman, J.; Cambillau, C.; Fernstrom, B.; Gustafsson, J.A. (1985) Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. Mol. Pharmacol. 28: 357-363.

Gillner, M.; Bergman, J.; Cambillau, C.; Gustafsson, J.A. (1989) Interactions of rutaecarpine alkaloids with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. Carcinogenesis 10: 651-654.

Gradin, K.; McGuire, J.; Wenger, R.H.; Kvietikova, I.; Whitelaw, M.L.; Toftgard, R.; Tore, L.; Gassmann, M.; Poellinger, L. (1996) Functional interference between hypoxia and dioxin signal transduction pathways: competition for recruitment of the Arnt transcription factor. Mol. Cell. Biol. 16: 5221-5231.

Gradelet, S.; Leclerc, J.; Siess, M-H.; Astorg, P.O. (1996) β-Apo-8'-carotenal, but not β-carotene, is a strong inducer of liver cytochromes P4501A1 and 1A2 in rat. Xenobiotica 26: 909-919.

Gradin, K.; Toftgard, R.; Poellinger, L.; Berghard, A. (1999) Repression of dioxin signal transduction in fibroblasts. identification of a putative repressor associated with Arnt. J. Biol. Chem. 274: 13511-13518.

Gradin, K.; Wilhelmsson, A.; Poellinger, L.; Berghard, A. (1993) Nonresponsiveness of normal human fibroblasts to dioxin correlates with the presence of constitutive xenobiotic response element-binding factor. J. Biol. Chem. 268: 4061-4068.

Gray, L.E.; Ostby, J.S. (1995) In utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. Toxicol. Appl. Pharmacol. 133: 285-294.

Grunstein, M. (1990) Histone function in transcription. Annu. Rev. Cell Biol. 6: 643-678.

Gu, Y-Z; Hogenesch, J.B.; Bradfield, C.A. (2000) The PAS superfamily: Sensors of environmental and developmental signals. Ann. Rev. Pharmacol. Toxicol. 40: 519-561

Hahn, M.E. (1998) The aryl hydrocarbon receptor: a comparative perspective. Comp. Biochem. Physiol. 121: 23-53.

Hahn, M.E.; Karchner, S.I; Shapiro, M.A.; Perera, S.A. (1997) Molecular evolution of two vertebrate aryl hydrocarbon (dioxin) receptors (AHR1 and AHR2) and the PAS family. Proc. Natl. Acad. Sci. USA 94: 13743-13748.

Hankinson, O. (1995) The aryl hydrocarbon receptor complex. Annu. Rev. Pharmacol. Toxicol. 35: 307-340.

Hapgood, J.; Cuthill, S.; Denis, M.; Poellinger, L.; Gustafsson, J.A. (1989) Specific protein-DNA interactions at a xenobiotic-responsive element: copurification of dioxin receptor and DNA-binding activity. Proc. Natl. Acad. Sci. U.S.A. 86: 60-64.

Harper, P.A.; Prokipcak, R.D.; Bush, L.E.; Golas, C.L.; Okey, A.B. (1991) Detection and characterization of the Ah receptor in the human colon adenocarcinoma cell line LS 180. Arch. Biochem. Biophys. 290: 27-36.

Harris, M.; Piskorska-Pliszczynska, J.; Zacharewski, T.; Romkes, M.; Safe, S. (1989a) Structure-dependent induction of aryl hydrocarbon hydroxylase in human breast cancer cell lines and characterization of the Ah receptor. Cancer Res. 49: 4531-4545.

Harris, M.; Zacharewski, T.; Astroff, B.; Safe, S. (1989b) Partial antagonism of 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated induction of aryl hydrocarbon hydroxylase by 6-methyl-1,3,8-trichlorodibenzofuran: mechanistic studies. Mol. Pharmacol. 35: 729-735.

Hayashi, S.; Okabe-Kado, J.; Honma, Y.; Kawajiri, K. (1995) Expression of the Ah receptor (TCDD receptor) during human monocyte differentiation. Carcinogenesis 16: 1403-1409.

Heid, S.E.; Pollenz, R.S.; Swanson, H.I. (2000). Role of heat shock protein 90 dissociation in mediating agonist-induced activation of the aryl hydrocarbon receptor. Mol. Pharmacol. 57: 82-92.

Helferich, W.; Denison, M.S. (1991) Photooxidized products of tryptophan can act as dioxin agonists. Mol. Pharmacol. 40: 674-678.

Henry, E.C.; Kende, A.S.; Rucci, G.; Totleben, M.J.; Willey, J.J.; Dertinger, S.D.; Pollenz, R.S.; Jones, J.P.; Gasiewicz, T.A. (1999) Flavone antagonists bind competitively with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the aryl hydrocarbon receptor but inhibit nuclear uptake and transformation. Mol. Pharmacol. 55: 716-725.

Hestermann, E.V.; Stegeman, J.J.; Hahn, M.E. (2000) Relative contributions of affinity and intrinsic efficacy to aryl hydrocarbon receptor ligand potency. Toxicol. Appl. Pharmacol. 168: 160-172.

Hines, R.N.; Mathis, J.M.; Jacob, C.S. (1988) Identification of multiple regulatory elements on the human cytochrome P4501a1 gene. Carcinogenesis 9: 1599-1605.

Hirose, K.; Morita, M.; Ema, M.; Mimura, J.; Hamada, H.; Fujii, H.; Saijo, Y.; Gotoh, O.; Sogawa, K.; Fujii-Kuriyama, Y. (1996) cDNA cloning and tissue-specific expression of a novel basic helix-loop-helix/PAS factor (Arnt2) with close sequence similarity to the aryl hydrocarbon receptor nuclear translocator (Arnt). Mol. Cell. Biol. 16: 1706-1713.

Hoffer, A.; Chang, C-Y.; Puga, A. (1996) Dioxin induces transcription of fos and jun genes by Ah receptor-dependent and –independent pathways. Toxicol. Appl. Pharmacol. 141: 238-247.

Hoffman, E.C.; Reyes, H.; Chu, F.-F.; Sander, F.; Conley, L.H.; Brooks, B.A.; Hankinson, O. (1991) Cloning of a factor required for activity of the Ah (dioxin) receptor. Science 252: 954-958.

Hogenesch, J.B.; Chan, W.K.; Jackiw, V.H.; Brown, R.C.; Gu, Y.Z.; Pray-Grant, M.; Perdew, G.H.; Bradfield, C.A. (1997) Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. J. Biol. Chem. 272: 8581-8593.

Huang, Z.J.; Edery, I.; Rosbash, M. (1993) PAS is a dimerization domain common to Drosophila period and several transcription factors. Nature 364: 259-262.

Hundeiker, C.; Pineau, T.; Cassar, G.; Betensky, R.A.; Gleichmann, E.; Esser, C. (1999) Thymocyte development in Ah-receptor-deficient mice is refractory to TCDD-inducible changes. Int. J. Immunopharmacol. 21: 841-859.

Hunter, T.; Karin, M. (1992) The regulation of transcription by phosphorylation. Cell 70: 375-387.

Hushka, J.J.; Williams, J.S.; Greenlee, W.F. (1998) Characterization of 2,3,7,8-tetrachlorodibenzofuran-dependent suppression and AH receptor pathway gene expression in the developing mouse mammary gland. Toxicol. Appl. Pharmacol. 152: 200-210.

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

International Agency for Research on Cancer. (1997) Monographs on the evaluation of carcinogenic risks to humans. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. Vol. 69. Lyon, France: World Health Organization.

Jain, S.; Maltepe, E.; Lu, M.M.; Simon, C.; Bradfield, C.A. (1998) Expression of ARNT, ARNT2, HIF1a, HIF2a, and Ah receptor mRNAs in the developing mouse. Mech. Dev. 73: 117-123.

Jones, K.W.; Whitlock, J.P., Jr. (1990) Functional analysis of the transcriptional promoter for the CYP1A1 gene. Mol. Cell. Biol. 10: 5098-5105.

Jones, P.B.C.; Durrin, L.K.; Galeazzi, D.R.; Whitlock, J.P., Jr. (1986) Control of cytochrome P1-450 gene expression: analysis of a dioxin-responsive enhancer system. Proc. Natl. Acad. Sci. U.S.A. 83: 2802-2806.

Kadesch, T. (1993) Consequences of heteromeric interactions among helix-loop-helix proteins. Cell Growth Differ. 4: 49-55.

Kamei, Y.; Xu, L.; Heinzel, T.; Torchia, J.; Kurokawa, R.; Gloss, B.; Lin, S-C.; Heyman, R.A.; Rose, D.W.; Glass, C.K.; Rosenfeld, M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85: 403-414.

Kazlauskas, A.; Poellinger, L.; Pongratz, I. (1999) Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (aryl hydrocarbon) receptor. J. Biol. Chem. 274: 13519-13524.

Kedderis, L.B.; Mills, J.J.; Anderson, M.E.; Birnbaum, L.S. (1993) A physiologically based pharmacokinetic model for 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) in the rat: tissue distribution and CYP1A induction. Toxicol. Appl. Pharmacol. 121: 87-98.

Kerkvliet, N.I.; Baecher-Steppan, L.B.; Smith, J.A.; Youngberg, J.A.; Henderson, M.C.; Buhler, D.R. (1990) Role of the Ah locus in suppression of cytotoxic T lymphocyte (CTL) activity by halogenated aromatic hydrocarbons (PCBs and TCDD): structure-activity relationships and effects in C57BL/6 mice. Fund. Appl. Toxicol. 14: 532-541.

Kim, D.W.; Gazourian, L.; Quandri, S.A.; Romieu-Mourez, R.; Sherr, D.H. (2000) The RelA NF-kB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. Oncogene 19: 5498-5506.

Klinge, C.M.; Bowers, J.L.; Kulakosky, P.C.; Kamboj, K.K.; Swanson, H.I. (1999) The aryl hydrocarbon receptor (AHR)/AHR nuclear translocator (ARNT) heterodimer interacts with naturally occurring estrogen response elements. Mol. Cell. Endocrinol. 157: 105-119.

Knutson, J.C.; Poland, A. (1980) Keratinization of mouse teratoma cell line XB produced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: an in vitro model of toxicity. Cell 22: 27-36.

Ko, H.P.; Okino. S.T.; Ma, Q.; Whitlock, J.P., Jr. (1996) Dioxin-induced CYP1A1 transcription in vivo: the aromatic hydrocarbon receptor mediates transactivation, enhancer-promoter communication, and changes in chromatin structure. Mol. Cell. Biol. 16: 430-436.

Kobayashi, A.; Sogawa, K.; Fujii-Kuriyama, Y. (1996) Cooperative interaction between AhR-Arnt and Sp1 for the drug-inducible expression of CYP1A1 gene. J. Biol. Chem. 271: 12310-12316.

Kobayashi, A.; Numayama-Tsuruta, K,; Sogawa, K.; Fujii-Kuriyama, Y. (1997) CPB/p300 functions as a possible transcriptional coactivator of the Ah receptor nuclear translocator (Arnt). J. Biochem. (Tokyo) 122: 703-710.

Kolluri, S.K.; Weiss, C.; Koff, A.; Gottlicher, M. (1999) p27kip1 induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells. Genes Dev. 13: 1742-1753.

Kornberg, R.D.; Lorch, Y. (1992) Chromatin structure and transcription. Annu. Rev. Cell. Biol. 8: 563-587.

Kozak, K.R.; Abbott, B.; Hankinson, O. (1997) ARNT-deficient mice and placental differentiation. Dev. Biol. 191: 297-305.

Kraemer, S.; Arthur, K.; Denison, M.S.; Smith, W.L.; DeWitt, D.L. (1996) Regulation of prostaglandin endoperoxide H synthase-2 expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Biochem. Biophys. 330: 319-328.

Kress, S.; Greenlee, W.F. (1997) Cell-specific regulation of human CYP1A1 and CYP1A2 genes. Cancer Res. 57: 1264-1269.

Kuil, C.W.; Brouwer, A.; van der Saag, P.T.; van der Burg, B (1998) Interference between progesterone and dioxin signal transduction pathways. J. Biol. Chem. 273: 8829-8834.

Kumar, M.B.; Tarpey, R.W.; Perdew, G.H. (1999) Differential recruitment of coactivator RIP140 by Ah and estrogen receptors. J. Biol. Chem. 274: 22155-22164.

Kurl, R.N.; DePetrillo, P.B.; Olnes, M.J. (1993) Inhibition of Ah (dioxin) receptor transformation by 9-hydroxy ellipticine: involvement of protein kinase C? Biochem. Pharmacol. 46: 1425-1433.

Lahvis, G.P.; Lindell, S.L.; Thomas, R.S.; McCuskey, R.S.; Murphy, C.; Glover, E.; Bentz, M.; Southard, J.; Bradfield, C.A. (2000) Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 97: 10442-10447.

Lai, Z-W.; Pineau, T.; Esser, C. (1996) Identification of dioxin-responsive elements (DREs) in the 5' regions of putative dioxin-inducible genes. Chem. Biol. Interact. 100: 97-112.

LaPres, J.J.; Glover, E.; Dunham, E.E.; Bunger, M.K.; Bradfield, C.A. (2000). ARA9 modifies agonist signaling through an increase in cytosolic aryl hydrocarbon receptor. J. Biol. Chem. 275: 6153-6159.

Lavin, A.L.; Hahn, D.J.; Gasiewicz, T.A. (1998) Expression of functional aromatic hydrocarbon receptor and aromatic hydrocarbon nuclear translocator proteins in murine bone marrow stromal cells. Arch. Biochem. Biophys. 352: 9-18.

Li, H., Dong, L., Whitlock, J.P., Jr. (1994) Transcriptional activation function of the mouse Ah receptor nuclear translocator. J. Biol. Chem. 269: 28098-28105.

Limbird, LE; Taylor. P. (1998) Endocrine disruptors signal the need for receptor models and mechanisms to inform policy. Cell 93: 157-163.

Lindebro, M.C.; Poellinger, L.; Whitelaw, M.L. (1995) Protein-protein interaction via PAS domains; role of the PAS domain in positive and negative regulation of the bHLH/PAS dioxin receptor–Arnt transcription factor complex. EMBO J. 14: 3528-3539.

Liu, P.C.; Phillips, M.A.; Matsumura, F. (1996) Alteration by 2,3,7,8-tetrachlorodibenzo-p-dioxin of CCAAT/enhancer binding protein correlates with suppression of adipocyte differentiation in 3T3-L1 cells. Mol. Pharmacol. 49: 989-997.

Long, W.P.; Pray-Grant, M.; Tsai, J.C.; Perdew, G.H. (1998) Protein kinase C activity is required for aryl hydrocarbon receptor pathway-mediated signal transduction. Mol. Pharmacol. 53: 691-700.

Lorenzen, A.; Okey, A.B. (1991) Detection and characterization of Ah receptor in tissue and cells from human tonsils. Toxicol. Appl. Pharmacol. 107: 203-214.

Lu, Y-F.; Santostefano, M.; Cunningham, B.D.M.; Threadgill, M.D.; Safe, S. (1996) Substituted flavones as aryl hydrocarbon (Ah) receptor agonists and antagonists. Biochem. Pharmacol. 51: 1077-1087.

Lucier, G.W.; Tritscher, A.; Goldsworthy, T.; Foley, J.; Clark, G.; Goldstein, J.; Marenpot, R. (1991) Ovarian hormones enhance TCDD-mediated increases in cell proliferation and preneoplastic foci in a two stage model for rat hepatocarcinogenesis. Cancer Res. 51: 1391-1397.

Lusska, A.; Shen, E.; Whitlock, J.P., Jr. (1993) Protein-DNA interactions at a dioxin-responsive enhancer: analysis of six bona fide DNA-binding sites for the liganded Ah receptor. J. Biol. Chem. 268: 6575-6580.

Lusska, A.; Wu, L.; Whitlock, J.P., Jr. (1992) Superinduction of CYP1A1 transcription by cycloheximide: role of the DNA binding site for the liganded Ah receptor. J. Biol. Chem. 267: 15146-15151.

Luster, M.I.; Hong, L.H.; Boorman, G.A.; Clark, G.; Hayes, H.T.; Greenlee, W.F.; Dold, K.; Tucker, A.N. (1985) Acute myelotoxic responses in mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 81: 156-165.

Ma, Q.; Dong, L.; Whitlock, J.P., Jr. (1995) Transcriptional activation by the mouse Ah receptor: interplay between multiple stimulatory and inhibitory functions. J. Biol. Chem. 270: 12687-12703.

Ma, Q.; Whitlock, J.M., Jr. (1996) The aromatic hydrocarbon receptor modulates the Hepa 1c1c7 cell cycle and differentiated state independently of dioxin. Mol. Cell. Biol. 16: 2144-2150.

Ma, Q.; Whitlock, J.M., Jr. (1997) A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Biol. Chem. 272: 8878-8884.

Ma, X.; Mufti, N.A.; Babish, J.G. (1992) Protein tyrosine phosphorylation as an indicator of 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure in vivo and in vitro. Biochem. Biophys. Res. Commun. 189: 59-65.

Mahon, M.J.; Gasiewicz, T.A. (1995) Ah receptor phosphorylation: localization of phosphorylation sites to the C-terminal half of the protein. Arch. Biochem. Biophys. 318: 166-174.

Maltepe, E.; Schmidt, J.V.; Baunoch, D.; Bradfield, C.A.; Simon, M.C. (1997) Abnormal angiogenesis and response to glucose and oxygen deprivation in mice lacking the protein ARNT. Nature 386: 403-407.

Manchester, D.K.; Gordon, S.K.; Golas, C.L.; Roberts, E.A.; Okey, A.B. (1987) Ah receptor in human placenta: stabilization by molybdate and characterization of binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 3-methylcholanthrene, and benzo(a)pyrene. Cancer Res. 47: 4861-4868.

Meyer, B,K.; Pray-Grant, M.G.; Vanden Heuvel, J.P.; Perdew, G.H. (1998). Hepatitis B virus X-associated protein 2 is a subunit of the unliganded aryl hydrocarbon receptor core complex and exhibits transcriptional enhancer activity. Mol. Cell. Biol. 18: 978-988.

Meyer, B.K.; Perdew, G.H. (1999) Characterization of the AhR-hsp90-XAP2 core complex and the role of the immunophilin-related protein XAP2 in AhR stabilization. Biochemistry 38: 8907-8917.

Meyer, M-E.; Gronemeyer, H.; Turcotte, B.; Bocquel, M-T.; Tasset, D.; et al. (1989) Steroid hormone receptors compete for factors that mediate their enhancer function. Cell 57: 433-442.

Michalek, JE; Tripathi, RC. (1999) Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 15 year follow-up. J. Toxicol. Environ. Health 57: 369-378.

Michalek, J.E.; Pirkle, J.L.; Caudill, S.P.; et al. (1996) Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. J. Toxicol. Environ. Health 47: 209-220.

Mimura, J.; Yamashita, K.; Nakamura, K.; Morita, M.; Takagi, T.N.; Nakao, K.; Ema, M.; Sogawa, K.; Yasuda, M.; Katsuki, M.; Fujii-Kuriyama, Y. (1997) Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. Genes Cells 2: 645-654.

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

Mocarelli, P.; Needham, L.L.; Marocchi, A.; Patterson, D.G., Jr.; Brambilla, P.; Gerthoux, P.M.; Meazza, L.; Carreri, V. (1991) Serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and test results from selected residents of Seveso, Italy. J. Toxicol. Environ. Health 33: 357-366.

Morgan, J.E.; Whitlock, J.P., Jr. (1992) Transcription-dependent and transcription-independent nucleosome disruption induced by dioxin. Proc. Natl. Acad. Sci. U.S.A. 89: 11622-11626.

Morris, D.L.; Holsapple, M.P. (1991) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral immunity. 2. B cell activation. Immunopharmacology 21: 171-181.

Morris, D.L.; Jordan, S.D.; Holsapple, M.P. (1991) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral immunity. 1. Similarities to Staphylococcus aureus cowan Strain I (SAC) in the in vitro T-dependent antibody response. Immunopharmacology 21: 159-169.

Morris, D.L.; Snyder, N.K.; Gokani, R.E.; Blair, R.E.; Holsapple, M.P. (1992) Enhanced suppression of humoral immunity in DBA/2 mice following subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 112: 128-132.

Murre, C.; Bain, G.; van Dijk, M.A.; Engel, I.; Furnari, B.A.; Massari, M.E.; Matthews, J.R.; Quong, M.W.; Rivera, R.R.; Stuiver, M.H. (1994) Structure and function of helix-loop-helix proteins. Biochim. Biophys. Acta 1218: 129-135.

Nebert, D.W.; Peterson, D.D.; Puga, A. (1991) Human Ah locus polymorphism and cancer: inducibility of CYPIA1 and other genes by combustion products and dioxin. Pharmacogenetics 1: 68-78.

Nebert, D.W.; Roe, A.L.; Dieter, M.Z.; Solis, W.A.; Yang, Y.; Dalton, T.P. (2000) Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. Biochem. Pharmacol. 59: 65-85.

Neuhold, L.A.; Gonzales, F.J.; Jaiswal, A.K.; Nebert, D.W. (1986) Dioxin-inducible enhancer region upstream from the mouse P1-450 gene and interaction with a heterologous SV40 promoter. DNA 5: 403-411.

Neuhold, L.A.; Shirayoshi, Y.; Ozato, K.; Jones, J.E.; Nebert, D.W. (1989) Regulation of mouse Cyp1A1 gene expression by dioxin: requirement of two cis-acting elements during induction. Mol. Cell. Biol. 9: 2378-2386.

Neumann, C.M.; Steppan, L.B.; Kerkvliet, N.I. (1992) Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in splenic tissue of C57BL/6J mice. Drug Metab. Disp. 20: 467-469.

Nguyen, T.A.; Hoivik, D.; Lee, J-E.; Safe, S. (1999) Interactions of nuclear receptor coactivator/corepressor proteins with the aryl hydrocarbon receptor complex. Arch. Biochem. Biophys. 367: 250-257.

Okino, S.T.; Pendurthi, U.R.; Tukey, R.H. (1992) Phorbol esters inhibit the dioxin receptor-mediated transcriptional activation of the mouse Cyp1a1 and Cyp1a2 genes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J. Biol. Chem. 267: 6991-6998.

Okino, S.T.; Whitlock, J.P., Jr. (1995) Dioxin induces localized, graded changes in chromatin structure: implications for CYP1A1 gene transcription. Mol. Cell. Biol. 15: 3714-3721.

Park, S-K.; Henry, E.C.; 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.

Pavylak, A.L.; Wielgosz, S.M.; Huttner, E. (1989) Proliferation of splenic lymphocyte is inhibited more strongly by 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/10 (Ah+Ah+) mice than in DBA/2 (Ah-Ah-) mice. Exp. Biol. 107: 365-372.

Perdew, G.H. (1988) Association of the Ah receptor with the 90-kDa heat shock protein. J. Biol. Chem. 263: 13802-13805.

Perdew, G.H.; Hollenback, C.E. (1995) Evidence for two functionally distinct forms of the human Ah receptor. J. Biochem. Toxicol. 10: 95-102.

Peters, J.M.; Narotsky, M.G.; Elizondo, G.; Fernandez-Salguero, P.M.; Gonzalez, F.J.; Abbott, B.D. (1999) Amelioration of TCDD-induced teratogenesis in aryl hydrocarbon receptor (AhR)-null mice. Toxicol. Sci. 47: 86-92.

Petrulis, J.R.; Hord, N.G.; Perdew, G.H. (2000) Subcellular localization of the aryl hydrocarbon receptor is modulated by the immunophilin homolog hepatitis B virus X-associated protein 2. J. Biol. Chem. 275: 37448-37453.

Phelan, D.M.; Brackney, W.R.; Denison, M.S. (1998) The Ah receptor can bind ligand in the absence of receptor-associated heat-shock protein 90. Arch. Biochem. Biophys. 353: 47-54.

Picard, D.; Khursheed, B.; Garabedian, M.J.; Fortin, M.G.; Lindquist, S.; Yamamoto, K.R. (1990) Reduced levels of hsp90 compromise steroid receptor action in vivo. Nature 348: 166-168.

Piechocki, M.P.; Hines, R.N. (1998) Functional characterization of the human CYP1A1 negative regulatory element: modulation of Ah receptor mediated transcriptional activity. Carcinogenesis 19: 771-780.

Pimental, R.A.; Liang, B.; Yee, G.K.; Wilhelmsson, A.; Poellinger, L.; Paulson, K.E. (1993) Dioxin receptor and C/EBP regulate the function of the glutathione S-transferase Ya gene xenobiotic response element. Mol. Cell Biol. 13: 4365-4373.

Pirkle, J.L.; Wolfe, W.M.; Patterson, D.G., Jr.; Needham, L.L.; Michalek, J.E.; Miner, J.C.; Peterson, M.R.; Phillips, D.L. (1989) Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam veterans of Operation Ranch Hand. J. Toxicol. Environ. Health 27: 165-171.

Poellinger, L.; Göttlicher, M.; Gustafsson, J.-A. (1992) The dioxin and peroxisome proliferator-activated receptors: nuclear receptors in search of endogenous ligands. Trends Pharmacol. Sci. 13: 241-245.

Poland, AD. (1996) Meeting report. Receptor-acting xenobitics and their risk assessment. Drug Metab. Disp. 24: 1385-1388.

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

Poland, A.; Palen, D.; Glover, E. (1982) Tumor promotion by TCDD in skin of HRS/J hairless mice. Nature 300: 271-273.

Poland, A.; Teitelbaum, P.; Glover, E. (1989) [125I]2-Iodo-3,7,8-trichlorodibenzo-p-dioxin-binding species in mouse liver induced by agonists for the Ah receptor: characterization and identification. Mol. Pharmacol. 36: 113-120.

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.

Pollenz, R.S.; Davirinos, N.A.; Shearer, T.P. (1999) Analysis of aryl hydrocarbon receptor-mediated signaling during physiological hypoxia reveals a lack of competition for the aryl hydrocarbon nuclear translocator transcription factor. Mol. Pharmacol. 56: 1127-1137.

Pollenz, R.S.; Sattler, C.A.; 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.

Pollenz, R.S.; Sullivan, H.R.; Holmes, J., Necela, B.; Peterson, R.E. (1996) Isolation and expression of cDNAs from rainbow trout (Oncorhynchus mykiss) that encode two novel basis helix-loop-helix/PER-AENT-SIM (bHLH/PAS) proteins with distinct functions in the presence of the aryl hydrocarbon receptor. J. Biol. Chem. 271: 30886-30896.

Pongratz, I.; Mason, G.G.F.; Poellinger, L. (1992) Dual roles of the 90 kDa heat shock protein hsp90 in modulating functional activities of the dioxin receptor. J. Biol. Chem. 267: 13728-13734.

Pongratz, I.; Stromstedt, P.-E.; Mason, G.G.F.; Poellinger, L. (1991) Inhibition of the specific DNA binding activity of the dioxin receptor by phosphatase treatment. J. Biol. Chem. 266: 16813-16817.

Probst, M.R.; Fan, C.M.; Tessier-Lavigne, M.; Hankinson, O. (1997) Two murine homologs of the Drosophila single-minded protein that interact with the mouse aryl hydrocarbon receptor nuclear translocator protein. J. Biol. Chem. 272: 4451-4457.

Puga, A.; Nebert, D.; Carrier, F. (1992) Dioxin induces expression of c-fos and c-jun proto-oncogenes and a large increase in transcription factor AP-1. DNA Cell. Biol. 11: 269-281.

Puga, A.; Barnes, S.J.; Dalton, T.P.; Chang, C.; Knudsen, E.S.; Maier, M.A. (2000a) Aromatic hydrocarbon receptor interaction with the retinoblastoma protein potentiates repression of E2F-dependent transcription and cell cycle arrest. J. Biol. Chem. 275: 2943-2950.

Puga, A.; Maier, A.; Medvedovic, M. (2000b) The transcriptional signature of dioxin in human hepatoma HepG2 cells. Biochem. Pharmacol. 60: 1129-1142.

Quattrochi, L.C.; Tukey, R.H. (1989) The human cytochrome Cyp1A2 gene contains regulatory elements responsive to 3-methylcholanthrene. Mol. Pharmacol. 36: 66-71.

Rannung, A.; Rannung, U.; Rosenkratz, H.S.; Winqvist, L.; Westerholm, R.; Agurell, E.; Grafstrom, A.K. (1987) Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. J. Biol. Chem. 262: 15422-15427.

Reisz-Porszasz, S.; Probst, M.R.; Fukunaga, B.N.; Hankinson, O. (1994) Identification of functional domains of the aryl hydrocarbon nuclear translocator protein (ARNT). Mol. Cell. Biol. 14: 6075-6086.

Richardson, V.M.; Santostefano, M.J.; Birnbaum, L.S. (1998) Daily cycle of bHLH-PAS proteins, Ah receptor and Arnt, in multiple tissues of female Sprague-Dawley rats. Biochem. Biophys. Res. Commun. 252: 225-231.

Roberts, EA.; Golas, CL.: Okey, AB. (1986) Ah receptor mediating induction of aryl hydrocarbon hydroxylase: detection in human lung by binding of 2,3,7,8-[^{3}H]tetrachlorodibenzo-p-dioxin. Cancer Res. 46: 3739-3743.

Roberts, EA.; Johnson, KC.; Dippold, WG. (1991) Ah receptor mediating induction of cytochrome P450IA1 in a novel continuous human liver cell line (Mz-Hep-1). Detection by binding with [^{3}H]2,3,7,8-tetrachlorodibenzo-p-dioxin and relationship to the activity of aryl hydrocarbon hydroxylase. Biochem. Pharmacol. 42: 521-528.

Roberts, E.A.; Johnson, K.C.; Harper, P.A.; Okey, A.B. (1990) Characterization of the Ah receptor mediating aryl hydrocarbon hydroxylase induction in the human liver cell line HepG2. Arch. Biochem. Biophys. 276: 442-450.

Robles, R.; Morita, Y.; Mann, K.K.; Perez, G.I.; Yang, S.; Matikainen, T.; Sherr, D.H.; Tilly, J.L. (2000) The aryl hydrocarbon receptor, a basic helix-loop-helix transcripton factor of the PAS gene family, is required for normal ovarian germ cell dynamics in the mouse. Endocrinol. 141: 450-453.

Roman, B.L.; Sommer, R.J.; Shinomiya, K.; Peterson, R.E. (1995) In utero and lactational exposure of the male rat to 2,3,7,8-tetrachlorodibenzo-p-dioxin: impaired prostate growth and development without inhibited androgen production. Toxicol. Appl. Pharmacol. 134: 241-250.

Rowlands, J.C.; Gustafsson, J-A. (1997) Aryl hydrocarbon receptor-mediated signal transduction. Crit. Rev. Toxicol. 27: 109-134.

Rowlands, J.C.; McEwan, I.J.; Gustafsson, J-A. (1996) Trans-activation by the human aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator proteins: direct interactions with basal transcription factors. Mol. Pharmacol. 50: 538-548.

Rushmore, T.H.; Pickett, C.B. (1993) Glutathione S-transferases, structure, regulation, and therapeutic implications. J. Biol. Chem. 268: 11475-11478.

Saatcioglu, F.; Perry, D.J.; Pasco, D.S.; Fagan, J.B. (1990a) Aryl hydrocarbon (Ah) receptor DNA-binding activity: sequence specificity and Zn^{2+} requirement. J. Biol. Chem. 265: 9251-9258.

Saatcioglu, F.; Perry, D.J.; Pasco, D.S.; Fagan, J.B. (1990b) Multiple DNA-binding factors interact with overlapping specificities at the aryl hydrocarbon response elements of the cytochrome P4501A1 gene. Mol. Cell. Biol. 10: 6408-6416.

Sadek, C.M.; Allen-Hoffman, B.L. (1994) Suspension-mediated induction of hepa1c1c7 CYP1a1 expression is dependent on the Ah receptor signal transduction pathway. J. Biol. Chem. 269: 31505-31509.

Safe, S.H. (1986) Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Annu. Rev. Pharmacol. Toxicol. 26: 371-398.

Safe, S.H. (1990) Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. 21:51-88.

Schaldach, C.M.; Riby, J.; Bjeldanes, L.F. (1999) Lipoxin A4: a new class of ligand for the Ah receptor. Biochemistry 38: 7594-7600.

Schecter, A. (ed). (1994) Dioxins and health. New York: Plenum Press.

Schlezinger, J.J.; Blickarz, C.E.; Mann, K.K.; Doerre, S,; Stegeman, J.J. (2000) Identification of NF-kB in the marine fish Stenotomus chrysops and examination of its activation by aryl hydrocarbon receptor agonists. Chem.-Biol. Interact. 126: 137-157.

Schmidt, J.V.; Bradfield, C.A. (1996) AH receptor signaling pathways. Annu. Rev. Cell. Dev. Biol. 12: 55-89.

Schmidt, J.V.; Su, G. H.-T.; Reddy, J.K.; Simon, M.C.; Bradfield, C.A. (1996) Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. Proc. Natl. Acad. Sci. USA 93: 6731-6736.

Shen, E.S.; Whitlock, J.P., Jr. (1989) The potential role of DNA methylation in the response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J. Biol. Chem. 264: 17754-17758.

Shen, E.S.; Whitlock, J.P., Jr. (1992) Protein-DNA interactions at a dioxin-responsive enhancer: mutational analysis of the DNA-binding site for the liganded Ah receptor. J. Biol. Chem. 267: 6815-6819.

Shimba, S.; Todoroki, K.; Aoyagei, T.; Tezuka, M. (1998) Depletion of arylhydrocarbon receptor during adipose differentiation in 3T3-L1 cells. Biochem. Biophys. Res. Commun. 249: 131-137.

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

Silkworth, J.B.; Antrim, L. (1985) Relationship between Ah receptor-mediated polychlorinated biphenyl (PCB)-induced humoral immunosuppression and thymic atrophy. J. Pharmacol. Exp. Ther. 235: 606-611.

Silkworth, J.B.; Cutler, D.S.; O'Keefe, P.W.; Lipinskas, T. (1993) Potentiation and resolution of 2,3,7,8-tetrachlorodibenzo-p-dioxin effects in a complex environmental mixture. Toxicol. Appl. Pharmacol. 119: 236-247.

Sinal, C.J.; Bend, J.R. (1997) Aryl hydrocarbon receptor-dependent induction of Cyp1a1 by bilirubin in mouse hepatoma Hepa 1c1c7 cells. Mol. Pharmacol. 52: 590-599.

Spink, D.C.; Lincoln, D.W., II; Dickerman, H.W.; Gierthy, J.F. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an extensive alteration of 17β-estradiol metabolism in MCF-7 breast tumor cells. Proc. Natl. Acad. Sci. U.S.A. 87: 6917-6921.

Stephenson, R.P. (1956) A modification of receptor theory. Br. J. Pharmacol. 11: 379.

Sutter, T.R.; Tang, Y.M.; Hayes, C.L.; Wo, Y-YP.; Jabs, E.W.; Li, X.; Yin, H.; Cody, C.W.; Greenlee, W.F. (1994) Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. J. Biol. Chem. 269: 13092-13099.

Swanson, H.I.; Bradfield, C.A. (1993) The AH receptor: genetics, structure and function. Pharmacogenetics 3: 213-230.

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

Swanson, H.I.; Yang, J.H. (1998) The aryl hydrocarbon receptor interacts with transcription factor IIB. Mol. Pharmacol. 54: 671-677.

Takimoto, K.; Lindahl, R.; Pitot, H.C. (1992) Regulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inducible expression of aldehyde dehydrogenase in hepatoma cells. Arch. Biochem. Biophys. 298: 492-497.

Theobald, H.M.; Peterson, R.E. (1994) Developmental and reproductive toxicity of dioxins and Ah receptor agonists. In: Schecter, A. (ed). Dioxins and human health. New York: Plenum Press, pp. 199-225.

Thurmond, T.S.; Silverstone, A.E.; Baggs, R.B.; Quimby, F.W.; Staples, J.E.; Gasiewicz, T.A. (1999) A chimeric aryl hydrocarbon receptor knockout mouse model indicates that aryl hydrocarbon receptor activation in hematopoietic cells contributes to the hepatic lesions induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 158: 33-40.

Thurmond, T.S.; Staples, J.E.; Silverstone, A.E.; Gasiewicz, T.A. (2000) The aryl hydrocarbon receptor has a role in the in vivo maturation of murine bone marrow B lymphocytes and their responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 165: 227-236.

Tian, Y.; Ke, S.; Denison, M.S.; Rabson, A.B.; Gallo, M.A. (1999) Ah receptor and NF-kB interactions, a potential mechanism for dioxin toxicity. J. Biol. Chem. 274: 510-515.

Tomita, S.; Sinai, C.J.; Yim, S.H.; Gonzalez, F.J. (2000) Conditional disruption of the aryl hydrocarbon receptor nuclear translocator (Arnt) gene leads to loss of target gene induction by the aryl hydrocarbon receptor and hypoxia-inducible factor 1 alpha. Mol. Endocrinol. 14: 1674-1681.

Tucker, A.N.; Vore, S.J.; Luster, M.I. (1986) Suppression of B cell differentiation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol. Pharmacol. 29: 372-377.

Van Antwerp, D.J.; Martin, S.J.; Kafri, T.; Green, D.R., Verma, I.M. (1996) Suppression of TNF-α-induced apoptosis by NF-κB. Science 274: 787-789.

Vanden Heuvel, J.P.; Clark, G.C.; Kohn, M.C.; Tritscher, A.M.; Greenlee, W.F.; Lucier, G.W.; Bell, D.A. (1994) Examination of dose-response relationships using quantitative reverse transcriptase-polymerase chain reaction. Cancer Res. 54: 62-68.

Vaziri, C.; Schneider, A.; Sherr, D.H.; Faller, D.V. (1996) Expression of the aryl hydrocarbon receptor is regulated by serum and mitogenic growth factors in murine 3T3 fibroblasts. J. Biol. Chem. 271: 25921-25927.

Voegel, J.J., Heine, M.J.S.; Zechel, C.; Chambon, P., Gronemeyer, H. (1996) TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J. 15: 3667-3675.

Waller, C.L.; McKinney, J.D. (1995) Three-dimensional quantitative structure-activity relationships of dioxins and dioxin-like compounds: model validation and Ah receptor characteristics. Chem. Res. Toxicol. 8: 847-858.

Walsh, A.A.; Tullis, K.; Rice, R.H.; Denison, M.S. (1996) Identification of a novel cis-acting negative regulatory element affecting expression of the CYP1A1 gene in rat epidermal cells. J. Biol. Chem. 271: 22746-22753.

Wanner, R.; Brommer, S.; Czarnetzki, B.; Rosenbach, T. (1995) The differentiation-related upregulation of aryl hydrocarbon receptor transcript is suppressed by retinoic acid. Biochem. Biophys. Res. Commun. 209: 706-711.

Wanner, R.; Zober, A.; Abraham, K.; Kleffe, J.; Hanz, B.M.; Wittig, B. (1999). Polymorphism at codon 554 of the human Ah receptor: different allelic frequencies in Caucasians and Japanese and no correlation with severity of TCDD induced chloracne in chemical workers. Pharmacogenet. 9: 777-780.

Watson, A.J.; Hankinson, O. (1988) DNA transfection of a gene repressing aryl hydrocarbon hydroxylase induction. Carcinogenesis 9: 1581-1586.

Watson, A.J.; Weir-Brown, K.I.; Bannister, R.M.; Chu, F.-F.; Reinz-Porszasz, S.; Fujii-Kuriyama, Y.; Sogawa, K.; Hankinson, O. (1992) Mechanism of action of a repressor of dioxin-dependent induction of Cyp1a1 gene transcription. Mol. Cell. Biol. 12: 2115-2123.

Weinberg, R.A. (1995) The retinoblastoma protein and cell cycle control. Cell 81: 323-330.

Weiss, C.; Kolluri, S.K.; Kiefer, F.; Gottlicher, M. (1996) Complementation of Ah receptor deficiency in hepatoma cells: negative feedback regulation and cell cycle control by the Ah receptor. Exp. Cell Res. 226: 154-163.

Whitelaw, M.; Pongratz, I.; Wilhelmsson, A.; Gustafsson, J.-A.; Poellinger, L. (1993) Ligand-dependent recruitment of the Arnt coregulator determines DNA recognition by the dioxin receptor. Mol. Cell. Biol. 13: 2504-2514.

Whitelaw, M.L.; Gottlicher, M.; Gustafsson, J-A.; Poellinger, L. (1993a) Definition of a novel ligand binding domain of a nuclear bHLH receptor: co-localization of ligand and hsp90 binding activities within the regulable inactivation domain of the dioxin receptor. EMBO J. 12: 4169-4179.

Whitelaw, M.L.; McGuire, J.; Picard, D.; Gustafsson, J-A.; Poellinger, L. (1995). Heat shock protein hsp90 regulates dioxin receptor function in vivo. Proc. Natl. Acad. Sci. U.S.A. 92: 4437-4441.

Whitlock, J.P., Jr. (1999) Induction of cytochrome P4501A1. Annu. Rev. Pharmacol. Toxicol. 39: 103-125.

Wilson, C.L.; Safe, S. (1998) Mechanisms of ligand-induced aryl hydrocarbon receptor-mediated biochemical and toxic responses. Toxicol. Pathol. 26: 657-671.

Wu, L.; Whitlock, J.P., Jr. (1992) Mechanism of dioxin action: Ah receptor-mediated increase in promoter accessibility in vivo. Proc. Natl. Acad. Sci. U.S.A. 89: 4811-4815.

Wu, L.; Whitlock, J.P., Jr. (1993) Mechanism of dioxin action: receptor-enhancer interactions in intact cells. Nucleic Acids Res. 21: 119-125.

Xu, L.; Ruh, T.S.; Ruh, M.F. (1997) Effect of the histone deacetylase inhibitor trichostatin A on the responsiveness of rat hepatocytes to dioxin. Biochem. Pharmacol. 53: 951-957.

Yang, J-H.; Vogel, C.; Abel, J. (1999) A malignant transformation of human cells by 2,3,7,8-tetrachlorodibenzo-p-dioxin exhibits altered expressions of growth regulatory factors. Carcinogenesis 20: 13-18.

Yao, E.F.; Denison, M.S. (1992) Sequence determinants for binding of transformed Ah receptor to a dioxin-responsive enhancer. Biochemistry 31: 5060-5067.

Zacksenhaus, E.; Jiang, Z.; Chung, D.; Marth, J.D.; Phillips, R.A.; Gallie, B.L. (1996) pRb controls proliferation, differentiation, and death of skeletal muscle cells and other lineages during embryogenesis. Genes Dev. 10: 3051-3064.

Zaher, H.; Fernandez-Salguero, M.; Letterio, J.; Sheikh, M.S.; Fornace, A.J., Jr.; Roberts, A.B.; Gonzalez, F.J. (1998) The involvement of aryl hydrocarbon receptor in the activation of transforming growth factor-β and apoptosis. Mol. Pharmacol. 54: 313-321.