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Aryl Hydrocarbon Receptor, Cell Cycle Regulation, Toxicity, and Tumorigenesis

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Abstract Most effects of exposure to halogenated and polycyclic aromatic hydrocarbons are mediated by the aryl hydrocarbon receptor (AHR). It has long been recognized that the AHR is a ligand-activated transcription factor that plays a central role in the induction of drug-metabolizing enzymes and hence in xenobiotic detoxification. Of late, it has become evident that outside this well-characterized role, the AHR also functions as a modulator of cellular signaling pathways. In this Prospect, we discuss the involvement of the AHR in pathways critical to cell cycle regulation, mitogen-activated protein kinase cascades, immediate-early gene induction, and the functions of the RB protein. Ultimately, the toxicity of AHR xenobiotic ligands may be intrinsically connected with the perturbation of these pathways and depend on the many critical signaling pathways and effectors with which the AHR itself interacts. J. Cell. Biochem. 96: 1174–1184, 2005.

Key words: Ah receptor; xenobiotic ligands; signal transduction; retinoblastoma protein; apoptosis

Exposure to halogenated aromatic hydrocarbons (HAHs) and PAHs results in a wide range of toxic and carcinogenic responses in animals and in humans. It is widely accepted that most of these exposure effects are mediated by the aryl hydrocarbon receptor (AHR), a cytosolic ligand-activated transcription factor that upon ligand binding translocates to the nucleus, where it complexes with ARNT (a.k.a. HIF-1β). AHR/ARNT heterodimers bind to specific consensus DNA sites in the regulatory domains of genes coding for many Phase I and Phase II drug-metabolizing enzymes and activate the transcription of these genes [Hankinson, 1995]. During the last 8–10 years, it has also become evident that the AHR has a second function, involving promotion of cell cycle progression, and that this function is accomplished in the absence of an exogenous ligand. In contrast, activation of the Ah receptor by high-affinity HAH or PAH ligands such as TCDD and B[a]P has been known for many years to result in a wide range of cell cycle perturbations, including G_0/G_1 and G_2/M arrest, diminished capacity for DNA replication, and inhibition of cell proliferation [reviewed in Puga et al., 2002]. These two outcomes are diametrically opposed and raise questions for which we do not have satisfactory answers at present. For example, how does the unliganded cytosolic Ah receptor influence a nuclear function such as cell cycle progression? Does this effect involve nuclear translocation? If so, do liganded and unliganded nuclear translocation events have different molecular outcomes? What makes dioxin carcinogenic? In these and similar questions, we need to bear in mind that it is only our current

Abbreviations used: AHR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; B[a]P, benzo[a]pyrene; CDK, cyclin-dependent kinase; CKI, cyclin-dependent kinase inhibitor; DEN, diethylnitrosamine; DHFR, dihydrofolate reductase; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HAH, halogenated aromatic hydrocarbon; HIF-1β, hypoxia-inducible factor-1β; JNK, Jun N-terminal kinase; MAPK, mitogen activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; MEF, mouse embryo fibroblasts; RB, retinoblastoma protein; SRE, serum response element; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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state of ignorance that allows us to apply the term unliganded to an Ah receptor that may be liganded by an as yet uncharacterized endogenous ligand.

The fact that the AHR is involved in such a variety of outcomes indicates that it is able to modulate diverse molecular pathways in concert with the induction of Phase I and Phase II genes for the detoxification of foreign compounds. Many studies have shown that the AHR functions in the direct and indirect modulation of transcriptional programs, at least in part by associating with additional transcription factors [Ge and Elferink, 1998; Wang et al., 1999], coactivators or corepressors [Nguyen et al., 1999; Wang and Hankinson, 2002], and by altering signal transduction cascades [Tan et al., 2002, 2004]. In doing so, the unliganded receptor may modulate components critical for the regulation of cell cycle progression. In this case, the presence of a high affinity ligand might cause a toxic response because a particular regulatory function would not be carried out by virtue of the receptor's engagement with ligand. Alternatively, the unliganded AHR might be fully quiescent and its activation by ligand might simply elicit detoxification and adaptive responses. In this case, the toxicity of the ligand may be determined by the functions that the AHR would carry out by virtue of its engagement with ligand. In recent years, experimental evidence has accumulated in favor of the first alternative, whereas classical toxicological research has provided much evidence in favor of the second. In all likelihood, both alternatives are correct.

AHR LIGAND-DEPENDENT ACTIVATION OF SIGNAL TRANSDUCTION PATHWAYS

Exposure to exogenous ligands of the AHR, such as TCDD and B[a]P, causes the activation of multiple signaling pathways, although the mechanisms that connect these toxic agents to their effects on a particular signaling pathway have not been characterized. Thus, even though the available information indicates that the interaction of the ligand-activated AHR with signal transduction events enhances the toxicity of the ligand, the understanding of the connections between agent, signaling mechanism and toxic outcome remains poor.

Exposure of multiple rodent cell lines to TCDD results in an essentially immediate increase in protein kinase C levels and activity in cellular membranes [Bombick et al., 1985, 1988; Madhukar et al., 1988; Carrier et al., 1992]. TCDD specifically activates tyrosine kinases associated with the EGFR [Madhukar et al., 1984, 1988] and induces the association of adaptor proteins such as SHC, GRB2, and SOS with EGFR [Park et al., 1998]. While TCDD is not an EGFR ligand [Sewall et al., 1995], it seems that cross-talk between AHR and EGFR signaling is critical for TCDD-induced developmental toxicity [Partanen et al., 1998] and hepatocarcinogenicity [Sewall et al., 1993]. Further downstream in the MAP kinase pathway, TCDD activates the expression of the *Hras* gene coding for the small GTPase $p21^{RAS}$, probably as the result of the transmission of receptor and non-receptor phosphotyrosine kinase signals from the EGFR adaptor complex [Enan and Matsumura, 1994]. As a consequence, RAS GTP binding activity is increased in adipose tissues treated with TCDD. In a different system altogether, consisting of rat vascular smooth muscle cells in culture, *Hras* expression is induced by exposure to B[a]P, yet a different AHR ligand. [Bral and Ramos, 1997], perhaps in a tissue-specific manner [Parrish et al., 1998]. Gene expression analyses in human hepatoma cells using global microarray experiments confirm the activation of the RAS MAP kinase pathway by TCDD [Puga et al., 2000c]. Additionally, AHR-independent induction of K-RAS activity by TCDD in mouse lung tissues suggests that there are multiple mechanisms by which AHR ligands influence signal transduction pathways [Ramakrishna and Anderson, 1998].

The ERK family of proteins, in addition to the JNKs and p38, are serine/threonine kinases of the MAP kinase family. ERK activity is stimulated in human epithelial cells treated with B[a]P [Jyonouchi et al., 1999] and in endocervical cells from TCDD-exposed macaque monkeys [Enan et al., 1998b]. TCDD also induces ERK and JNK phosphorylation in cell lines lacking a functional AHR, with kinetics indicative of a so-called non-genomic effect [Tan et al., 2002]. Upstream signaling cascades of MAPKKKs and MAPKKs regulate the downstream MAP kinases [Cobb and Goldsmith, 2000]. MEK-1 and -2 are the upstream MAPKKs for ERK proteins, while the MEKs themselves are

regulated by the upstream MAPKKK RAF-1, among others. AHR agonists may stimulate ERK activity by inducing EGFR activation and the subsequent activation of RAS; however, an AHR-dependent interaction of RAS with RAF-1, which would be responsible for RAF-1 activation and progression of the signal to MEK and ERK [Barbacid, 1987], has yet to be found. Other EGFR downstream signaling molecules involved in ERK activation such as PK-C, PLC-γ, and PI-3K may also be responsive to TCDD. In fact, several studies have shown that activation of the PK-C pathway is required for AHR activation and CYP1A1 expression [Carrier et al., 1992; Chen and Tukey, 1996; Tannheimer et al., 1997; Long et al., 1998]. Exposure of cell cultures to either TCDD or B[a]P leads to increases in intracellular calcium levels as well as extracellular calcium fluxes [Puga et al., 1995, 1997; Tannheimer et al., 1997], possibly through activation of PLC-γ, a major regulator of intracellular calcium stores. In addition to the effects of AHR ligands on MAPK signaling cascades, recent studies have shown that AHR transcriptional activity is dependent upon ERK and JNK activation in a cell-lineage and gene-specific manner [Tan et al., 2004].

Still other signaling pathways are affected following exposure to AHR ligands. The tyrosine kinase activity of c-SRC is triggered in multiple in vitro and in vivo systems in response to AHR activation. SRC may be activated through several alternative pathways, including by signals initiated from cell surface receptors such as EGFR, or from G protein-coupled receptors and intracellular receptors [Cao et al., 2000; Watters et al., 2000], as well as via ligand-induced disruption of the AHR–HSP90 complex, to which SRC may be functionally associated [Enan and Matsumura, 1995; Enan et al., 1998a; Dunlap et al., 2002]. TCDD fails to suppress the differentiation of c-SRC-deficient MEF cells, but not of wild type cells [Vogel and Matsumura, 2003; Vogel et al., 2003]. Furthermore, the overall toxicity of TCDD is dependent in part on the activation of c-SRC, as SRC-deficient, TCDD-treated mice exhibit reduced toxicity [Dunlap et al., 2002]. Lastly, a signal transduction pathway that involves the c-SRC protein tyrosine kinase has been shown to be responsible for the activation of the AHR by pharmacologic agents in a ligand-independent manner by mechanisms unlike those exerted by high-affinity ligands [Backlund and Ingelman-Sundberg, 2005].

AHR AGONISTS ACTIVATE IMMEDIATE-EARLY RESPONSE GENES

As outlined above, many AHR ligands activate signaling cascades initiated and propagated by trans-membrane and intracellular ion fluxes, and by protein kinase and phosphatase activation. Transduction of such signals to the nucleus of quiescent cells induces the expression of multiple immediate-early response genes, including *MYC*, *MYB*, and members of the FOS and JUN families, which coordinate the expression of additional genes required for subsequent cell cycle progression [Kohn, 1999]. The untimely expression of such genes and the successive cycling of normally quiescent cells may in part explain the ability of TCDD and other ligands of the AHR to act as powerful tumor promoters and carcinogens [Schwarz et al., 2000].

AHR ligand-dependent activation of the *c-MYC* gene in human breast cancer cells results from the binding of an AHR–RelA protein complex to an NF-κB DNA binding element in the c-MYC promoter [Kim et al., 2000]. The AHR may therefore contribute indirectly in this context to entry of these cells into the cell cycle. NF-κB controls many physiological functions adversely affected by PAHs, and the formation of AHR–RelA complexes may also help to explain some of the adverse toxicological outcomes of AHR ligands such as immune suppression, thymic involution, hyperkeratosis, and carcinogenesis. In addition to the effect of AHR activation on NF-κB-mediated transcriptional activity, the formation of AHR–RelA complexes also results in the functional repression of AHR/ARNT activities [Tian et al., 1999; Ke et al., 2001]. Increased p50 homodimer binding to NF-κB sites may also be explained by sequestration of RelA by the AHR [Puga et al., 2000a].

The expression of additional immediate-early genes, specifically members of the FOS and JUN families of protooncogenes, has been shown to be induced by AHR agonists, with a resulting increase in AP-1 DNA-binding activity. This effect has been observed in multiple liver cell types [Puga et al., 1992; Enan et al., 1998b; Ashida et al., 2000], but not in all [Gohl et al., 1996]. In other cells, such as

LPS-activated B cells, TCDD downregulates AP-1 expression [Suh et al., 2002], suggesting a cell type-dependent effect of AHR ligands on immediate-early protooncogene induction. The induction of c-Jun and Jun-D expression by the AHR appears to result from AHR-complex binding sites in the promoters of these genes. In contrast, c-Fos induction by TCDD is dependent on a SRE motif in its promoter, and is not dependent on the presence of the AHR [Hoffer et al., 1996]. Activation of the ERK MAP kinases leads to ELK-1 phosphorylation and to binding of the ternary ELK-1/TCF complex to the SRE motif [Gille et al., 1996], potentially connecting ERK activation by TCDD to AHR-independent downstream effects on immediate-early gene expression [Tan et al., 2004]; however, neither ELK-1 phosphorylation nor formation of the ternary complex have been observed after AHR activation.

LIGAND-INDEPENDENT CELL CYCLE CONTROL THROUGH THE AHR

Cell cycle progression, through the controlled process of DNA replication and cell division, is initiated in quiescent cells by mitogen stimulation. Typically, eukaryotic cells progress through cell cycle stages by the activities of cyclins, CDKs, and CKIs, which are responsible for the ordered transition from one phase of the cycle to the next. Their expression and activities are in turn controlled and modulated by members of the RB and E2F families of proteins [Coqueret, 2002; Trimarchi and Lees, 2002; Murray, 2004].

It has long been recognized that the AHR plays a role in cell cycle regulation. Ah receptor-null mice exhibit epidermal hyperplasia and hyperproliferation of hair follicles, hyperproliferation of liver blood vessels, and an age-dependent incidence of adenocarcinomas of liver and lung; paradoxically, these mice also show accelerated rates of apoptosis in the liver [Gonzalez and Fernandez-Salguero, 1998]. Fetal fibroblasts from AHR-null mice show slower proliferation rates and increased apoptosis, concomitant with the accumulation of cells in G_2/M, possibly due to altered expression of the G_2/M kinases CDC2 and PLK. The increase in apoptosis of AHR-null cells was attributed to increased levels of TGF-β, an inhibitor of cell proliferation [Elizondo et al., 2000]. Retinoic acid levels are increased in the livers of AHR-null mice, possibly due to the absence of some AHR-regulated P450 enzyme, and this elevation in retinoic acid content is thought to cause the higher levels of TGF-β [Gonzalez and Fernandez-Salguero, 1998]. However, other studies in AHR-null embryo fibroblasts have shown that p300-dependent stimulation of DNA synthesis by the adenovirus E1A protein does not take place in the absence of AHR, suggesting the possibility that the AHR exerts its influence on cell cycle regulation by other mechanisms [Tohkin et al., 2000].

Evidence that the absence of the AHR results in prolongation of the cell cycle has grown in recent years. Both AHR-negative mouse hepatoma Hepa1c1c7 cell variants [Ma and Whitlock, 1996], and human HepG2 hepatoma cells transfected with AHR siRNA [Abdelrahim et al., 2003] show a slower progression through the cell cycle, attributed to a delay in the transition from G_1 to S. These results suggest that the AHR plays an endogenous role in the promotion of cell cycle progression and that this role is independent of activation by exogenous ligands. This conclusion is significantly strengthened by the findings that, in the absence of ligand, expression of a constitutively active AHR variant in transgenic mice causes pro-proliferative effects, such as induction of stomach tumors [Andersson et al., 2002], and promotion of hepatocarcinogenesis [Moennikes et al., 2004]. Paradoxically, expression of the same variant AHR in human Jurkat cells causes growth inhibition and apoptosis [Ito et al., 2004].

CELL CYCLE ARREST INDUCED BY AHR LIGANDS

There is a large body of evidence showing that exogenous AHR ligands, especially TCDD, actually inhibit cell proliferation and induce cell cycle arrest in normally cycling cell populations [reviewed in Puga et al., 2002]; however, the mechanisms controlling this effect remain indistinct and ill-defined. TCDD was shown to inhibit DNA synthesis in confluent mouse epithelial cells [Gierthy and Crane, 1984], in partially hepatectomized rat liver [Bauman et al., 1995], and in rat primary hepatocytes [Hushka and Greenlee, 1995] by mechanisms that were independent of TGF-β or the mitogenic activity of EGF. TCDD also inhibited 17β-estradiol-induced growth of MCF-7 human

breast cancer cells, concomitantly with decreases in RB phosphorylation, cyclin D1 protein levels, and CDK-dependent kinase activities [Wang et al., 1998]. In mouse intrathymic progenitor cells, TCDD blocked S-phase progression and caused persistent thymic atrophy [Laiosa et al., 2003]. TCDD also inhibited proliferation of the fish hepatocellular carcinoma PLHC-1 cell line [Hestermann et al., 2002] and the androgen-induced proliferation of G_0/G_1-synchronized human prostate cancer LNCaP cells [Barnes-Ellerbe et al., 2004]. Treatment with TCDD also induced the AHR-dependent G_1 arrest of SK-N-SH human neuronal cells concomitant with the increased expression of p27 and the hypophosphorylation of RB [Jin et al., 2004].

Similar effects on the cell cycle and cell proliferation have been observed with AHR ligands other than TCDD. B[a]P suppressed cell proliferation in Swiss mouse 3T3 cells [Vaziri and Faller, 1997]. Treatment of rat 5L hepatoma cells with several different flavonoids known to be AHR ligands resulted in G_1 arrest [Reiners et al., 1999]. The novel AHR agonist 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole also induced an AHR-dependent cell cycle arrest in MCF-7 cells [Trapani et al., 2003]. These results are consistent with findings in mouse skin carcinogenesis assays that showed that TCDD pretreatment is anticarcinogenic under conditions of low doses of the tumor inducer DMBA [Lesca et al., 1994]. AHR is also expressed at high levels in pancreatic cancer tissues, and TCDD and other AHR agonists inhibit pancreatic cancer cell growth [Koliopanos et al., 2002].

Like treatment with flavonoids, TCDD treatment of asynchronously growing 5L hepatoma cell cultures leads to a delay in G_1- to S-phase progression [Wiebel et al., 1991]. This effect depends on the presence of the Ah receptor, since variant 5L clones lacking AHR expression do not show delayed G_1 to S progression [Gottlicher et al., 1990; Göttlicher and Wiebel, 1991; Wiebel et al., 1991], and expression of ectopic AHR in these variant cell lines reconstitutes the ability of TCDD to delay cell cycle progression [Weiss et al., 1996]. Several observations may explain these findings. TCDD was found to induce expression of the $p27^{Kip1}$ CDK inhibitor in an AHR-dependent manner, as the effect was lost in cells lacking AHR expression [Kolluri et al., 1999]. The observed induction of $p27^{Kip1}$ occurred concurrently with reduced cell proliferation, which was reversed by transient expression of a *Kip1* antisense RNA. Independent studies have shown that genes of the Ah battery are regulated in a cell cycle-dependent manner, and that the greatest induction of CYP1A1 by TCDD occurs during late G_1 to early S-phases [Santini et al., 2001]. Serum-mediated release of G_0/G_1-synchronized 5L cells into the cell cycle results in transient activation of the AHR and subsequent CYP1A1 expression, followed by progression of the cells into S-phase [Levine-Fridman et al., 2004]. This is in contrast to treatment of the same cells with TCDD, which results in sustained AHR activation, increased $p27^{Kip1}$ expression, and G_1 arrest. Simultaneous treatment of G_0/G_1-synchronized 5L cells with serum and the CYP1A1 suicide substrate 1-PP triggers sustained AHR activation and $p27^{Kip1}$ induction, similar to the action of TCDD alone. Thus, CYP1A1 activity appears to negatively regulate the length of AHR activation through the metabolism of an as yet unknown AHR agonist and CYP1A1 substrate, allowing cells to progress through the cell cycle in response to serum stimulation. It is possible that the lack of metabolism of TCDD and other persistent AHR agonists is responsible not only for the sustained induction of AHR activation but also for the induction of cell cycle arrest.

While these observations provide a plausible mechanistic rationale for the role of AHR in cell cycle regulation, additional data suggest that other mechanisms are equally important. Several reports have shown that the AHR forms complexes with the RB protein, detected by yeast two-hybrid assays as well as by co-immunoprecipitation [Ge and Elferink, 1998; Puga et al., 2000b]. RB acts as a negative regulator of cell cycle progression by preventing the expression of genes required for cell cycle entry through the inhibition of E2F-dependent transcriptional activity. At least two Ah receptor domains interact with RB, including an L*X*C*X*E motif common to many RB-interacting proteins [Chan et al., 2001], and a glutamine-rich region within the transactivation domain of the receptor. Further analysis of the biological consequences of this interaction revealed that the AHR acts in synergy with RB to repress E2F-dependent gene expression and to slow down cell cycle progression, particularly in the G_1- to S-phase transition [Puga et al., 2000b].

In addition, work in human C33A cells, which are insensitive to RB-mediated active gene repression, has shown that the combination of RB with AHR or BRG-1 restores repression of CDK2 and cyclin A and causes cell cycle arrest [Strobeck et al., 2000]. These results suggest that AHR activation may inhibit cell cycle progression not only by inducing $p27^{Kip1}$ expression to directly inhibit CDK2 activity and therefore RB inactivation, but also by directly interacting with RB to repress expression of genes required for entry into S-phase and cell cycle progression. Recent data from our laboratory [Marlowe et al., 2004] and from Elferink and colleagues [Huang and Elferink, 2005] have confirmed this conclusion. AHR activation in cycling Hepa-1c1c7 cells results in the accumulation of cells in G_1 [Marlowe et al., 2004]. Preceding this effect there is a significant increase in the expression of $p27^{Kip1}$ and reduction in the expression of specific E2F-regulated genes, including Cyclin E, CDK2, DNA polymerase α, and DHFR. Chromatin immunoprecipitation assays showed that activation of the AHR by ligand causes it to be recruited to the promoters of these genes with the exclusion of p300 from the same promoters. These data suggest a novel mechanism by which the AHR, a potent transcriptional activator, may act as a repressor of transcription through the formation of specific protein–protein interactions, and in doing so, induce G_1 arrest in normally cycling cells by preventing the expression of genes required for S-phase progression.

AHR-MEDIATED INHIBITION OF APOPTOSIS

Paradoxically, TCDD is one of the most potent tumor promoters known in animal model systems, including the liver and the skin in two-stage carcinogenesis assays [Pitot et al., 1980; Dragan and Schrenk, 2000]. Tumor promoters are believed to act by affecting the rate of proliferation, terminal differentiation, or death of tumor precursor cells. One widely accepted mechanism of tumor promotion/progression is the inhibition of apoptosis [Roberts et al., 1997]. The capacity of AHR ligands such as TCDD to act as tumor promoters, particularly in rodent liver, has been attributed to their ability to inhibit the apoptotic elimination of initiated cells bearing genotoxic lesions [Schwarz et al., 2000]. However, the precise mechanisms responsible for this effect remain elusive, and likely differ with the organism, tissue, or cell type examined. In DEN-initiated rats, both acute and chronic TCDD treatment results in an approximate 10-fold decrease in the rate of apoptosis in preneoplastically transformed liver foci, with no effect on the background rate of apoptosis in normal hepatocytes [Stinchcombe et al., 1995]. The overall effect of TCDD in this system is thus to accelerate the rate at which DNA-damaged cells convert to a neoplastic phenotype [Luebeck et al., 2000]. Stimulation of cell division in these assays is negligible [Buchmann et al., 1994], hence, the primary effect of TCDD is the inhibition of apoptosis, which has also been shown to occur in *Myc* transgenic mice [Buchmann et al., 1994; Schwarz et al., 2000] and in the promotion of ovarian tumors in rats [Davis et al., 2000a]. Absence of tumor promotion by TCDD treatment in rat strains lacking a functional AHR suggests that the Ah receptor is required for this effect, which has been shown to include activation of MDM2 and attenuation of p53 probably by increased ubiquitination [Viluksela et al., 2000; Paajarvi et al., 2005].

Effects of TCDD on apoptosis have also been documented in cultured cells. TCDD inhibits apoptosis in hepatocytes treated with UV light or 2-acetylaminofluorene, an effect that was also attributed in part to attenuation of p53 activity [Worner and Schrenk, 1996; Schrenk et al., 2004]. Apoptosis induced by growth factor withdrawal in human epithelial cells is inhibited by TCDD treatment, in correlation with activation of the EGF signaling pathway [Davis et al., 2000b]. Studies with AHR-null mice confirm the importance of the AHR in tissue homeostasis, as hepatocytes from these mice exhibit accelerated rates of apoptosis associated with increased production of TGF-β [Gonzalez and Fernandez-Salguero, 1998]. In vitro cell populations lacking the Ah receptor also have higher rates of apoptotic death [Elizondo et al., 2000].

CONCLUSIONS

As we have briefly described above, the Ah receptor pathway cross-talks with many cellular signal transduction cascades and ultimately leads cells in alternative directions of proliferation, cell cycle arrest, or apoptosis. It appears that whether one or the other outcome is reached might depend on the presence or absence of ligand, but the specific mechanisms

by which ligands inhibit apoptosis or promote proliferation in preneoplastic liver cells, or in any system for that matter, are unknown. We believe that interactions of the AHR with the RB/E2F axis are a critical element of these mechanisms. RB suppresses apoptosis as a result of the repression of a distinct set of proapoptotic E2F target genes, which includes Apaf-1 and several caspases [Nahle et al., 2002]. Excess E2F-1 expression unchecked by RB forces the cells to enter S-phase and promotes p53-dependent and independent apoptosis [Nahle et al., 2002]. This is a specific function of E2F-1 that depends on its binding to RB at a second binding site located entirely in the C-terminal domain of RB. E2F-1/RB complexes formed through this site have low affinity for DNA, but their interaction is sufficient for RB to repress E2F1-induced apoptosis. It follows that in cells lacking RB, E2F-1 proapoptotic activity is unchecked [Hallstrom and Nevins, 2003; Stevens and La Thangue, 2004]. In addition, E2F-1 becomes proapoptotic in response to DNA damage as a result of phosphorylation by ATM/chk2, thereby blocking its interaction with the RB C-terminal site and causing the stabilization of E2F-1 [Dick and Dyson, 2003].

Interaction of AHR with RB mediates active repression of E2F-responsive genes, thereby cooperating in the inhibition of cell cycle progression [Marlowe et al., 2004]. In addition, the activated AHR affects E2F transcriptional activity in the absence of RB binding [Marlowe et al., 2004]. The proapoptotic activity of E2F-1 suggests that its deregulation may constitute an oncogenic stress that targets pre-malignant

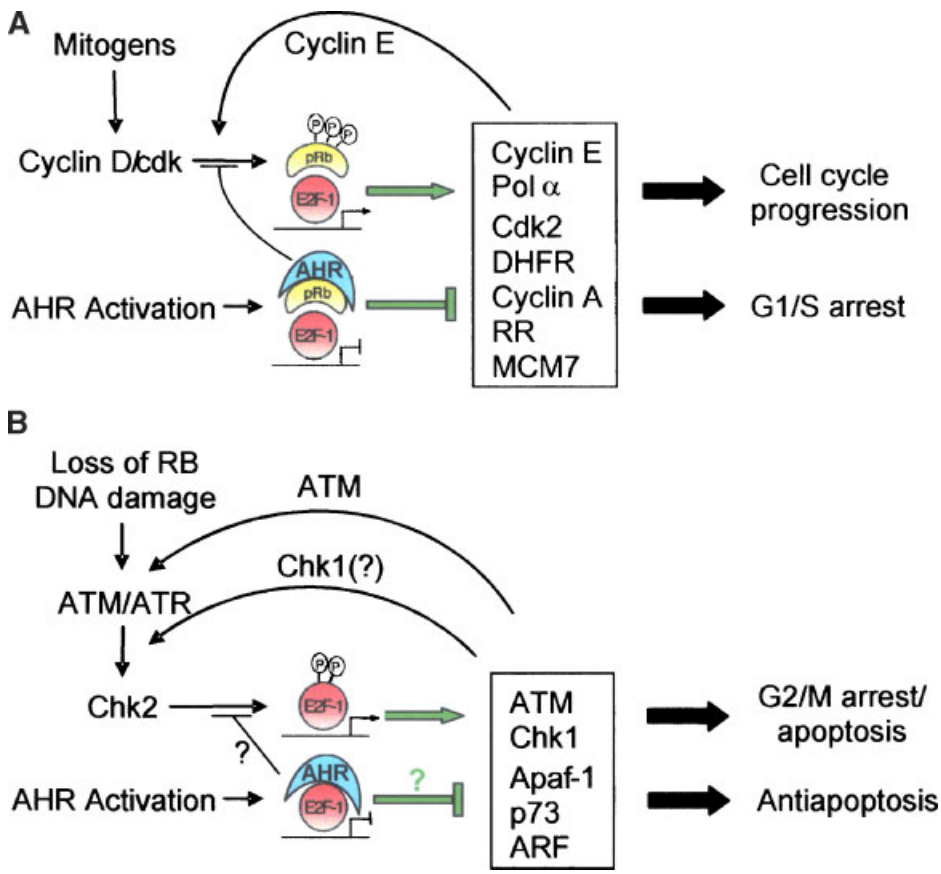

Fig. 1. Postulated yin-yang role of the Ah receptor a in cell cycle regulation. **A**: Under normal conditions, that is, in quiescent or in normal cycling cells, RB/E2F-1 interactions downregulate S-phase genes and mitogens activate cell cycle progression; under these conditions, activation of aryl hydrocarbon receptor (AHR) causes its translocation to the nucleus where it functions as an environmental checkpoint in cooperation with RB/E2F, inhibiting cyclin D, E/cdk-dependent RB phosphorylation, promoting repression of S-phase specific genes and causing cell cycle arrest. **B**: Under abnormal conditions of loss of RB or DNA damage, E2F-1 is stabilized by ATM/ATR- and chk2-dependent phosphorylation and upregulates expression of pro-apoptotic genes, promoting apoptosis; under these conditions, activation of AHR causes it to interact with E2F-1 and block its apoptotic properties, causing an antiapoptotic, pro-proliferative response. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cells to undergo apoptosis, thus preventing tumor development [Ginsberg, 2002; Stevens and La Thangue, 2003; Bell and Ryan, 2004]. We propose that, by signaling through the RB/E2F-1 axis, which can induce cell cycle progression and proliferation, cell cycle arrest, apoptosis, and DNA damage repair, the activated Ah receptor plays a critical role in all of these processes. Our working hypothesis, summarized in Figure 1, proposes that the Ah receptor has a yin-yang role in cell cycle regulation, whereby under some circumstances its activation is pro-proliferative and under others, anti-proliferative. For example, if environmental mitogens induce unscheduled cell cycle progression, activation of the AHR would cause its translocation to the nucleus, where it would function as an environmental checkpoint in cooperation with RB, inhibit S-phase gene expression by interacting with RB/E2F-1/DP1 complexes, and promote cell cycle arrest. On the other hand, under abnormal conditions of loss of RB or of DNA damage, stabilization of E2F-1 would upregulate its pro-apoptotic functions, but activation of AHR under these conditions would cause it to interact with E2F-1 and block its apoptotic properties, causing a pro-proliferative, anti-apoptotic response. This yin-yang activity of the AHR and its ligand could be at the heart of the abnormal proliferative and apoptotic responses that characterize the carcinogenicity of TCDD and other Ah receptor ligands.

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