Expression of the Human Aryl Hydrocarbon Receptor Complex in Yeast

ACTIVATION OF TRANSCRIPTION BY INDOLE COMPOUNDS

(Received for publication, July 16, 1997)

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The human aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator protein (ARNT) were coexpressed in the yeast Saccharomyces cerevisiae to create a system for the study of this heterodimeric transcription factor. Specific transcriptional activation mediated by AHR/ARNT heterodimer, which is a functional indicator of receptor expression, was assessed by β-galactosidase activity produced from a reporter plasmid. Yeast expressing AHR and ARNT displayed constitutive transcriptional activity that was not augmented by addition of AHR agonists in strains that required exogenous tryptophan for viability. In contrast, strains with an intact pathway for tryptophan biosynthesis responded to AHR agonists and had lower levels of background β-galactosidase activity. Hexachlorobenzene, benzo(a)pyrene, and β-naphthoflavone were effective AHR agonists in the yeast system, and had EC₅₀ values of 200, 40, and 20 nM, respectively, for β-galactosidase activity induction. Tryptophan, indole, indole acetic acid, and tryptamine activated transcription in yeast coexpressing AHR and ARNT (EC₅₀ values ~300 μM). Indole-3-carbinol was an exceptionally potent AHR agonist (EC₅₀ ~10 μM) in yeast. This yeast system is useful for the study of AHR/ARNT protein complexes, and may be generally applicable to the investigation of other multiprotein complexes.

AHR1 and its dimerization partner, ARNT, form a heterodimeric transcription factor called aryl hydrocarbon receptor complex (AHRC). AHRC enhances transcription of a number of genes in animals, including specific cytochrome P450 genes, NAD(P)H-dependent quinone reductase, specific phase II detoxifying enzymes, growth factors, and cytokines (reviewed in Refs. 1–3). AHR and ARNT are members of a subgroup of basic helix-loop-helix proteins which contain a specialized protein-protein interaction domain called the PAS motif (4, 5). AHR is present in a cytoplasmic complex that contains dimeric HSP90 prior to ligand interaction (6–8). Following ligand activation, AHR migrates into the nucleus and interacts with ARNT to form the functional AHRC. AHRC binds specific DNA response elements called DREs and mediates the regulation of gene expression (9). Many environmental pollutants, including TCDD and other polycyclic aromatic compounds, bind and activate AHR; however, an endogenous ligand of the AHR remains to be demonstrated.

Although AHR is activated by exposure to exogenous aromatic ligands, several investigations have demonstrated constitutive AHRC activation in mammalian cell lines that were not exposed to known agonists. Evidence for a possible endogenous AHR agonist comes from immunological and biochemical studies showing HeLa cells exhibit significant amounts of nuclear AHR in the absence of any treatment (10), whereas such activity is not seen in murine hepatoma cells. Similar activation of AHR resulted from changing keratinocytes from growth on a substratum to suspension culture (11). Genetic evidence suggesting endogenous AHR activation in vivo in the absence of exogenously added ligands is demonstrated by the 10-fold reduction in the AHRC-responsive hepatic CYP1A2 mRNA in mice which lack AHR (12). This result is consistent with the presence of an endogenous AHR agonist in vivo and, along with the results above, supports the hypothesis of an endogenous AHR agonist(s).

Expression of foreign proteins in yeast is a useful tool for studies of the molecular biology of transcription factors. In particular, approaches using the yeast two-hybrid system and yeast genetics have provided significant information regarding mammalian receptors and led to the discovery of new genes involved in regulation of AHR (13, 14). AHR, ARNT, and chimeric versions thereof have been studied in yeast expression systems to a limited extent. A yeast two-hybrid system was used to demonstrate interactions between chimeric AHR and ARNT proteins (15, 16) and, in one case, the interaction was ligand dependent (16). In only one study has a response of intact AHR and ARNT proteins to agonists in yeast been demonstrated (6). Dose-dependent responses to α- and β-naphthoflavones showed that intact AHR functions in yeast when coexpressed with intact ARNT protein, although the signal from the DRE-directed reporter plasmid was relatively weak. Interestingly, the behavior of chimeric AHR derivatives expressed in yeast exhibit a reduced or altered affinity for prototypic AHR agonists (7). Perhaps for reasons which may include weak transcriptional activation and the technical difficulty of expressing multiple proteins in yeast, the function of intact AHR and ARNT proteins in yeast has not been extensively examined.

An anomalous result was obtained in the initial attempts to develop the yeast AHR/ARNT expression system described in this report. Coexpression of human AHR and ARNT lead to
transcription of a DRE-containing reporter plasmid in the absence of known exogenous AHR agonists. Subsequent experiments showed that constitutive activation of AHR in the yeast system was due to the combined effects of exogenous tryptophan in the culture medium and the particular genotype of the yeast strain used. Several classical AHR agonists, as well as indole compounds found in the diet or produced during normal tryptophan metabolism, activated the human AHR in the heterologous system described here. The data shown below indicate that considerable AHR activation occurs in yeast and are consistent with the hypothesis that an endogenous AHR agonist is present, even though yeast express no known homologues of AHR and ARNT.

**MATERIALS AND METHODS**

**Bacterial Culture and Plasmid Preparation**—The DH5α strain of _Escherichia coli_ was used for expansion and subcloning of plasmids. Cultures were propagated in LB medium with ampicillin (20 μg/ml) when selecting for the growth of cells containing plasmids (17). DNA mini-preps were prepared by a boiling lysis method (18). RNA was degraded by RNase A digestion (10 μg/ml) final concentration for 10 min at 37 °C and plasmid DNA was purified by phenol-chloroform-isoamyl alcohol (CSA; 25 volumes) extraction followed by precipitation in 70% ethanol and centrifugation for 10 min at 14,000 × g (17). Plasmids were dissolved in a buffer of 10 mM Tris-HCl and 1 mM Na2EDTA, pH 7.8.

**Yeast Transformation, Strains, and Culture**—Yeast were transformed with multiple plasmids using the high efficiency lithium acetate method (19). The transformed yeast were identified by the ability to form colonies on synthetic glucose medium plates that lacked supplemented uracil, tryptophan, or leucine (20). Yeast strain YPH499 (mata, ade2-101, his3-D200, leu2-3,1,2, lys2-801, trp1Δ63, ura3-52, obtained from Stratagene, La Jolla, CA) was used in all experiments shown in the figures and tables presented here. The strains A305 (mata, his3-D200, leu2-3,1,2, trp1Δ1, ura3-1, a gift from S. Lindquist, University of Chicago) were also used for the coexpression of AHR and ARNT with equivalent results to those obtained for YPH499. Yeast were transformed and maintained in a minimal synthetic medium containing 0.67% yeast nitrogen base lacking amino acids (Difco), 2% glucose, and the required amino acids and bases needed to correct for auxotrophic deficiencies (20). Dimethyl sulfoxide (Me2SO) was used as a vehicle for dissolving AHR agonists with the exception of tryptophan, which was dissolved in water. Me2SO was then dissolved in water, sterilized by filtration, heating at 85 °C, and then the dried residue was washed three times dissolved completely. The ethanol was allowed to evaporate by further heating at 37 °C for 24 h.

**Yeast Galactose Induction**—The DH5α strain of _Escherichia coli_ was used for expansion and subcloning of plasmids. Cultures were propagated in LB medium with ampicillin (20 μg/ml) when selecting for the growth of cells containing plasmids (17). DNA mini-preps were prepared by a boiling lysis method (18). RNA was degraded by RNase A digestion (10 μg/ml) final concentration for 10 min at 37 °C and plasmid DNA was purified by phenol-chloroform-isoamyl alcohol (CSA; 25 volumes) extraction followed by precipitation in 70% ethanol and centrifugation for 10 min at 14,000 × g (17). Plasmids were dissolved in a buffer of 10 mM Tris-HCl and 1 mM Na2EDTA, pH 7.8, and stored at −20 °C.

**β-Galactosidase Assays**—Three independently derived transformants containing a particular group of plasmids were selected and grown overnight at 30 °C in a shaking incubator in synthetic glucose medium. The next day 50 μl from each of the cultures was added to 2 ml of synthetic galactose medium. Agonists were added to achieve the final concentrations indicated. Approximately 18 h later the cell densities were determined by reading the absorbance at 600 nm (1 absorbance unit is ~15 million cells/ml). One ml of exponentially growing cells (~5–10 million cells/ml) was added to a microcentrifuge tube and sedimented at 14,000 × g for 1 min. All but the last 0.5 ml of the supernatant were aspirated away, and 700 μl of Z-buffer were added. Z-buffer is composed of 60 mM NaHPO4, 40 mM NaH2PO4, 1 mM MgCl2, 10 mM KCl, 50 mM 2-mercaptoethanol, and is adjusted to a pH of 7 (23). Twenty μl of a sodium dodecyl sulfate solution (0.1% stock solution) and 20 μl of chloroform were added to lyse the cells. The reaction starting time was noted, and 0.2 ml of o-nitrophenol-β-D-galactopyranoside (ONPG, 4 mg/ml) stock solution was added. The mixture was mixed thoroughly, placed in a 37 °C water bath, and incubated for 5 min at 3 h as needed to generate a moderate yellow color. Four hundred μl of 1 M sodium carbonate solution were added to stop the reaction, the stopping time was noted, and the samples were clarified by centrifugation for 2 min at 14,000 × g. The absorbances of the supernatants were read in a spectrophotometer at 420 nm. The activity of β-galactosidase (reported as Miller units) was calculated by the following formula: A

**Results**

The diagrams shown in Fig. 1 and the plasmids listed in Table I describe the essential features of the AHRC expression system described here. Coexpression of AHR and ARNT from a single plasmid was accomplished by using the bidirectional GAL1,10 promoter (Fig. 1A). The DRE2,3 sequence from the regulatory region of the marine CYP1A1 gene places the β-ga-
**AHRC Function in Yeast**

**TABLE I**

| Plasmid Name | Features |
|--------------|----------|
| 1. YEplac181 | High copy number plasmid with LEU2 selection gene |
| 2. YEplac112 | High copy number plasmid with TRP1 selection gene |
| 3. YEp181GAL1,10 | YEp181 containing the divergent promoter from the region between GAL1 and GAL10 genes and a LEU2 selection gene |
| 4. pGARN | Derived from #3, expresses human ARNT from the GAL1,10 promoter |
| 5. pGAHR | Derived from #3, expresses human AHR from the GAL1,10 promoter |
| 6. pGAHR/ARNT | Derived from #3, expresses human ARNT and AHR from GAL1,10 promoters |
| 7. pBEVY-GT | Expression plasmid derived from #2 with GAL1,10 promoter and a TRP1 selection gene |
| 8. pBEVY-GT-AHR/ARNT | Expression plasmid derived from #7, expressing human ARNT and AHR from GAL1,10 promoters |
| 9. p2uGZ | Parent lacZ reporter plasmid with URA3 selection gene |
| 10. pDRE23Z | Reporter plasmid derived from #9, contains DREs 2 and 3 from the murine CYP1A1 gene positioned 5’ of the lacZ gene (6) |

Lactosidase gene (lacZ gene) of *E. coli* under control of the AHRC (Fig. 1B). Expression of either AHR or ARNT in yeast revealed very low levels (~0.1 unit or less) of β-galactosidase expression in the presence or absence of AHR ligands (Fig. 2). This result was expected because expression from the lacZ reporter plasmid requires the interaction of the heterodimeric AHR-ARNT complex (AHRC) with 5’-DREs to stimulate transcription. When AHR and ARNT were co-expressed in yeast, there was significantly greater (~20-fold) β-galactosidase activity relative to the cells expressing either AHR or ARNT alone (Fig. 2). The β-galactosidase activity induced by AHRC was observed when cells were grown in medium containing galactose, as expected for gene expression under control of the bidirectional GAL1,10 promoter. However, there was not a significant response upon addition of exogenous AHR ligands, β-naphthoflavone (NF) or TCDD (Fig. 2). In these experiments the pGAHR, pGARN, and pGAHR/ARNT LEU2 expression plasmids were used, and the reporter plasmid, pDRE23Z, contained the URA3 gene (Fig. 2, Table I).

A number of factors which could be responsible for the constitutively active AHRC were examined. The possibilities of agonistic aromatic hydrocarbons leaching from the plastic culture tubes, the generation of agonistic pyrolysis products during the autoclaving of medium components, and strain differences among yeast were tested and eliminated as contributors to the high level of constitutive activity of AHRC in this system. Genetic experiments to identify possible proteins that might alleviate the high constitutive AHRC activation were conducted. During these experiments it was accidentally discovered that lower background AHRC activity and agonist responsiveness occurred in tryptophan prototropic strains, which did not require addition of exogenous tryptophan. A review of the literature in which others have shown the function of ligand-dependent AHRC or AHR chimeric proteins in yeast revealed that in all cases the cells are prototrophic for tryptophan synthesis (6, 7, 13–16). In the initial experiments shown in Fig. 2, a trp1 strain of yeast requiring supplementation with exogenous tryptophan was used.

The conditions that determine the levels of AHRC activity in yeast are shown in Fig. 3. Cells that were supplemented with exogenous tryptophan and contained both the pGAHR/ARNT expression plasmid and the pDRE23Z reporter plasmid did not respond significantly to the exogenous agonist NF (Fig. 3, condition 1). Thus, in the absence of intentional ligand addition AHRC was activated. Addition of a TRP1 plasmid, YEplac112, to cells containing pGAHR/ARNT and pDRE23Z, eliminated the need for exogenous tryptophan in the culture medium and resulted in lower basal AHRC activity in untreated cells (Fig. 3, condition 2), and AHRC activity was induced 8-fold when the agonist NF was added. A similar (~4X) ligand-dependent induction of transcription was observed when a TRP1 plasmid expressing AHR and ARNT (pBEVY-GT-AHR/ARNT) was used in conjunction with the pDRE23Z reporter plasmid (Fig. 3, condition 5). Elimination of either the DREs from the reporter plasmid or AHRC expression (conditions 3 and 4) produced ~11- and 25-fold decreases, respectively, in background β-galactosidase activity relative to the untreated control in which all elements required for the signaling pathway were present (condition 5). The untreated controls of conditions 2 and 5 showed a considerable level of β-galactosidase induction in the tryptophan prototrophic yeast, whereas addition of exogenous NF enhanced the activity of the AHRC signaling pathway. Data from experimental conditions 3 and 4 demonstrate a requirement for AHR, ARNT, and DREs in the signal transduction pathway leading to lacZ gene expression.

If this heterologous system is to be useful for the study of...
AHRC, then known AHR agonists should activate transcription and produce β-galactosidase at concentrations similar to those reported for mammalian assay systems. Hexachlorobenzene (HCB), benzo(a)pyrene (BP), and NF represent three structurally distinct AHR agonists. Analysis of yeast β-galactosidase responses showed NF and BP had EC50 values of 20 and 40 nM, respectively, whereas HCB had an EC50 of ~200 nM in the yeast system (Fig. 4). This agonist hierarchy is consistent with the abilities of these aromatic agonists to activate AHR, with HCB being reported as a weak agonist in other systems (25). Additionally, preliminary experiments show that TCDD is a potent activator of AHR in this system. No inhibition of cell growth (e.g. toxicity) was observed for any of these aromatic agonists at the concentrations shown. Collectively, these results indicate that the expression of human AHR and ARNT in yeast recapitulates the AHR agonist responsiveness observed in mammalian systems, thus validating this system as a model for the study of AHRC.

The experiments shown in Figs. 2 and 3 suggest that tryptophan, a tryptophan metabolite, a contaminant in tryptophan, or some indirect cellular or metabolic effect resulting from tryptophan addition was responsible for the AHR agonist activity in yeast. Recrystallization of tryptophan was performed to minimize the possibility of an agonistic contaminant because oxidized derivatives of tryptophan are activators of AHR (26, 27). Dose-response experiments comparing a stock solution of tryptophan to a solution made from freshly recrystallized tryptophan were performed to test for the presence of a possible contaminant ligand. There was a dose-response relationship between tryptophan concentration and the reporter gene response mediated by AHRC at concentrations that ranged from 100 to 1000 μM (EC50 = 300 μM, Fig. 5). Furthermore, the dose-response relationships produced by the two tryptophan treatments were very similar, suggesting that there was not a contaminant agonist in the tryptophan, or that the contaminant agonist copurified with the recrystallized tryptophan.

Is the response of AHR expressed in yeast specific for tryptophan or does it extend to other indole compounds? Yeast containing the pBEVY-GT-AHR/ARNT reporter plasmid and the pDRE23Z reporter plasmid were used to determine whether other indole derivatives besides tryptophan could stimulate AHRC-mediated transcription (Fig. 6). The compounds indole (IND), indole-3-carbinol (I3C), indole acetic acid (IAA), tryptamine (TA), serotonin, and melatonin were tested for AHR agonist activity in the yeast system. Melatonin and serotonin did not produce a detectable activation of AHRC-mediated reporter expression, perhaps because the modification at position 5 on the indole ring is incompatible with AHR interaction. Treatment of yeast with IND, IAA, and TA induced reporter gene expression at concentrations of 100 μM or more and were similar to tryptophan in potency (EC50 = 200–300 μM). The ability of the indole derivatives to stimulate reporter expression in this system required the presence of DREs on the reporter plasmid, thus demonstrating specificity of the signaling mechanism. Interestingly, IND and IAA increased the doubling time of the yeast, but the growth inhibition was not correlated with the activation of AHR. IAA strongly inhibited cell growth at concentrations of 500 μM or more, and it was not possible to test for further AHR activation beyond this point.

I3C, a weak AHR agonist, was exceptionally effective in inducing β-galactosidase activity in comparison to the other indole compounds described above. The relationship between I3C concentration, AHRC-dependent lacZ gene expression, and

**Fig. 3.** Yeast genotype and exogenous tryptophan affect AHRC activity in yeast. Yeast transformed with plasmids were exposed to NF and assayed for β-galactosidase activity as described in Fig. 2. **Shaded bars** indicate responses of cultures that were treated with MeSO alone and **solid black bars** indicate responses of cultures treated with 1 μM NF. The legend below summarizes the experimental conditions resulting from the various plasmid combinations. Yeast contain pBEVY-GT in condition 1; pGAHR/ARNT, YEplac112, and pDRE23Z in condition 2; pBEVY-GT-AHR/ARNT and pβaGZ in condition 3; pBEVY-GT and pDRE23Z in condition 4; and pBEVY-GT-AHR/ARNT and pDRE23Z in condition 5.

**Fig. 4.** Activation of AHRC-mediated reporter gene expression by aromatic hydrocarbons. β-Galactosidase assays were conducted as described in Fig. 2, and established AHR agonists were used to stimulate yeast expressing the minimal components of the AHRC signaling pathway. Yeast contain the pBEVY-GT-AHR/ARNT expression plasmid and the pDRE23Z reporter plasmid in this experiment. **Square symbols** indicate the responses of cells exposed to NF, **triangles** indicate BP, and **circles** indicate HCB responses to exposures, respectively. The background activity of untreated cells was 2.1 ± 0.2 Miller units.

**Fig. 5.** Tryptophan exposure activates AHRC-mediated signaling in yeast. A stock solution of tryptophan was compared with a solution of recrystallized tryptophan for the ability to activate AHRC-mediated lacZ transcription. Tryptophan was diluted into cultures of yeast which contained the plasmids pGAHR/ARNT, pDRE23Z, and YEplac112. β-Galactosidase assays were conducted as described in Fig. 2. **Square symbols** indicate responses from cultures treated with the tryptophan stock solution and **circle symbols** indicate responses from treatments with the purified tryptophan solution.
inhibition of yeast growth is shown in Fig. 7. I3C (EC50 ~ 10 μM) was approximately 30 times more effective in inducing reporter expression relative to tryptophan. The greatest concentration of I3C shown (250 μM) increased the doubling time of the yeast from approximately 4 h/generation to 5 h/generation in synthetic galactose medium. Concentrations of I3C >250 μM produced marked inhibition of cell growth and were not tested for β-galactosidase activity. Since maximal activation of lacZ gene expression occurs at lower concentrations of I3C than those required to slow cell growth, the effect of I3C on cell proliferation is independent of AHRC activation. Additionally, the presence of AHRC was not required to observe growth inhibition due to indoles.

**DISCUSSION**

A yeast system containing the minimal components of the human pathway used in the response to aromatic hydrocarbon agonists has been constructed. The work presented here is important in defining the optimal parameters for AHR and ARNT expression in yeast. The observation that the human AHR and ARNT genes function in yeast is remarkable when one considers the many complex processes that must be conserved for human AHRC to function as a transcription factor when expressed in yeast. For example, yeast HSP90 homologues and probably other chaperones must correctly interact with the human AHR to create a receptor which is competent in signal transduction (6, 7, 13, 14). The ligand-activated AHR must translocate to the yeast nucleus for association with ARNT (1–3), DRE binding (9), and interaction with the transcriptional apparatus (28) to occur. Both AHR and ARNT are thought to require post-translational phosphorylation to function correctly (29), therefore yeast probably carry out these reactions. It is possible that specific phosphorylation events for AHR and ARNT are not of consequence in the yeast system; however, the demonstration that dephosphorylation of AHR and ARNT disrupts binding to DREs in vitro favors the possibility that AHR and ARNT are correctly phosphorylated by the yeast (29). It is important to note that the transcription factor Sp1, which is a frequent component of the basal promoters of DRE-containing genes and interacts with AHR and ARNT (30), apparently does not function as a transcriptional activator when expressed in yeast (31). It may therefore be fortuitous that the human AHRC does indeed function in yeast.

The yeast system described here for the coexpression of AHR and ARNT differs from those described by others. In several investigations AHR was expressed in yeast as a chimeric lexA or glucocorticoid receptor fusion protein, thus eliminating the need for the coexpression of ARNT or for recognition of DREs (6, 7, 15, 16). In this report the full-length AHR and ARNT are expressed from a single plasmid containing the strongly inducible GAL1,10 promoter rather than constitutively active promoters. Chimeric constructions which remove the basic helix-loop-helix domain of AHR and replace it with a foreign sequence result in a receptor protein with altered HSP90, ARNT, and DRE interactions (6, 7). The dose-response relationships of β-galactosidase induction by well characterized aromatic AHR agonists (Fig. 4) show that coexpression of intact AHR and ARNT in yeast leads to induction of signal transduction that is similar to that observed in human cells. Thus, expression of full-length proteins rather than chimeric protein constructions in yeast provides a system which better reflects the actual biology of AHRC.

During the initial characterization of AHRC activity, results were obtained that differed from those reported by other investigators. Under specific conditions, a constitutive activation of the AHRC was observed (Figs. 2 and 3). Other investigators have reported agonist-dependent AHR activation in yeast. However, in those studies tryptophan prototrophic strains were used (6, 7, 13–16). This is likely to account for the observation that strains which respond to exogenous agonists do not require supplementation with tryptophan. The experimental results shown in Figs. 3 and 5–7 provide an explanation for the constitutive AHRC activity and point to the possibility of tryptophan derivatives as AHR agonists. The initial manner in which AHRC was expressed (Figs. 2 and 3) was fortuitous in that it lead to the observation that genetic background and the presence of exogenous tryptophan were related to the activated state of human AHR. In retrospect this observation is not surprising because there is considerable evidence for tryptophan-related compounds functioning as AHR agonists. The carcinogenic aromatic compounds that arise during the cooking of meat and other tryptophan-rich foods are known agonists of AHR (32). Vegetables such as broccoli are also important dietary sources of indole compounds such as I3C, which is a weak AHR agonist that may self-react to form agonists of greater potency (33). Tryptophan-derived agonists generated by the photooxidation of tryptophan are potent ligands for AHR, and
are capable of interacting with AHR at concentrations comparable to effective concentrations of TCDD (26, 27). Thus, there are numerous derivatives of tryptophan that display variable potencies in AHR binding and activation. The experiments shown in Figs. 5–7 were performed to address the possibility of oxidized tryptophan derivatives or other contaminants being a source of agonist activity. The fact that purified tryptophan and other specific indoles (TA, IAA, I3C, and IND) induced β-galactosidase activity argues against, but does not rule out, the possibility of contaminant agonists.

It is important to note that the molecule(s) which actually activated the AHR in the experiments presented here is not known. Metabolism of compounds by yeast might either activate or eliminate AHR agonist activity. Determination of specific AHR agonists in the yeast system is further complicated by acidic culture medium. The low pH of the yeast medium could cause I3C to undergo acidic condensation reactions, generating more potent forms of AHR agonists such as 3,3′-diantidomethane and indolo[3,2-b]carbazole (33). These condensation products could account for the relative potency of I3C as an AHR agonist in the yeast system. I3C is reported to be a weak AHR agonist in mammalian cells. For example, no significant induction of P450 genes occurs in rat hepatocytes treated with indole compounds (36), but the molecular basis for the induction was not determined conditions, this too may reflect some biological function of an endogenous ligand that is not recognized in the mammalian system. I3C is reported to be a weak AHR agonist in the yeast system. I3C is known to block the cell cycle in yeast, and strains having mutations in the DRE-regulated genes through a pathway which requires DREs and AHRC are both present in cells. This data shown here indicate that indoles or indole metabolites may act as AHR agonists in yeast and that this AHR agonist activity is not due to metabolic activation of compounds to more effective intermediates. It is important to note that the molecule(s) which actually activated the AHR in the experiments presented here is not known.

ACKNOWLEDGMENTS—I thank William Toseano, Stacey Ricci, Kevin Lorick, Carolyn Mattingly, Steve Arnold, and Naydu Arana for helpful suggestions regarding this work. I thank Michelle Martin for technical assistance with plasmid construction and those named in the methods section for supplying reagents.

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