Production of Novel Recombinant Cell Line Bioassay Systems for Detection of 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Like Chemicals

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INTRODUCTION

Halogenated aromatic hydrocarbons (HAHs) represent a class of widespread environmental contaminants, including: polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls. Since exposure to specific HAHs results in a wide variety of species- and tissue-specific toxic and biological effects at low concentrations (1,2), the development of techniques for the identification and quantitation of these compounds in various samples is of importance. However, the presence of HAHs in complex mixtures rather than individual congeners and the observed interactions between chemicals in the mixture that can affect the biopotency (2), has complicated their detection and has made it difficult to accurately predict the biological/toxic potency of these mixtures in animals at risk.

Many, if not all, of the toxic and biological responses of HAHs are mediated by their high affinity binding to the Ah receptor (AhR) (1-3), a ligand-dependent DNA-binding protein which alters gene expression via its interaction with a specific DNA element (the dioxin responsive enhancer (DRE)) adjacent to HAH-responsive genes (3-5). Since the action of HAHs are mediated via the AhR, calculation of the relative biological potency of complex mixtures of HAHs can be estimated by measuring the ability of the mixture to induce an AhR-dependent response. Induction of cytochrome P450IA1-dependent ethoxyresorufin-O-deethylase activity in rat hepatoma cells (H4IIe) in culture is one response has been used as a bioassay system to evaluate the biological/toxic potency of complex mixtures of HAHs (2,6).

Although the rat H4IIe assay has been used extensively to examine the biological/toxic potency of complex mixtures it may not necessarily represent an appropriate model for assessing the biopotency of HAHs in other species. Although the AhR appears to vary among species, the DNA

binding specificity of the HAH:AhR complex is highly conserved (7). Here we report construction and utilization of a recombinant vector which contains a sensitive reporter gene whose expression is inducible by AhR ligands and whose activity can be rapidly and inexpensively measured. These vectors can be utilized to produce a variety of species- and tissue-specific bioassays for detection and relative quantitation of TCDD-like chemicals.

MATERIALS AND METHODS

Expression Vectors: To construct the HAH-inducible expression vectors pMpap1.1 and pGudluc1.1, a 1810 bp HindIII fragment was isolated from the plasmid pMcat5.9 (8) and subcloned into the HindIII site of the plasmid pSV_0Apap (9) or pGL2-basic (Promega), immediately upstream of the heat-stable human placental alkaline phosphatase (PAP) gene or the Firefly luciferase (LUC) gene, respectively. Th 1810 bp fragment contains a portion of the mouse mammary tumor virus long terminal repeat, inclusive of the viral promoter, but lacking functional glucocorticoid responsive enhancers. In addition, a 480 bp fragment isolated from the 5'-flanking region of the mouse cytochrome P450IA1 gene which contains four dioxin responsive enhancers has been inserted directly upstream of the MMTV viral promoter. The resultant plasmid contains the PAP or LUC gene under control of the mouse mammary tumor virus promoter. The DREs upstream of the promoter confer TCDD responsiveness upon the promoter and reporter gene (4,5,8).

Tissue Culture - Transfections - Chemical Treatment - Reporter Assays: Mouse Hepatoma (hepa1c1c7) cell were obtained from J.P.Whitlock, Jr. and were grown as previously described (4,8). The expression plasmids were prepared using standard techniques and cotransfected into the cells with pSV2neo using polybrene (8). Stably transfected cell clones were isolated and TCDD-inducibility of confluent plates of was determined by incubation with DMSO (1µl/ml), TCDD (1nM) in DMSO, or the indicated chemicals for 24h, followed by measurement of PAP/LUC activity in cell sonicates (9,10).

RESULTS

Preliminary experiments (data not shown) demonstrated that TCDD would induce PAP/LUC expression from cells transiently transfected with the reporter constructs, but not those transfected with pMpap1.0/pGudluc1.0 (the respective constrcuts which lack DREs). This indicated the DRE-dependent nature of the induction response. In addition, TCDD failed to induce PAP/LUC activity in variant mouse hepatoma cells (data not shown) which have a defective receptor that fails to bind to DNA and thus fail to induce (14). These latter results demonstrate that TCDD induction of the reporter gene requires functional AhRs for its action.

TCDD treatment of stably-transfected PAP/LUC cell clones resulted in a dose-dependent increase in reporter enzyme activity (Fig. 1) with an apparent minimal detection limit of at least 1-10 pM TCDD. Limited structure activity studies (data not shown) demonstrate the AhR ligand-dependent nature of this response and indicate its utility as a bioassay.

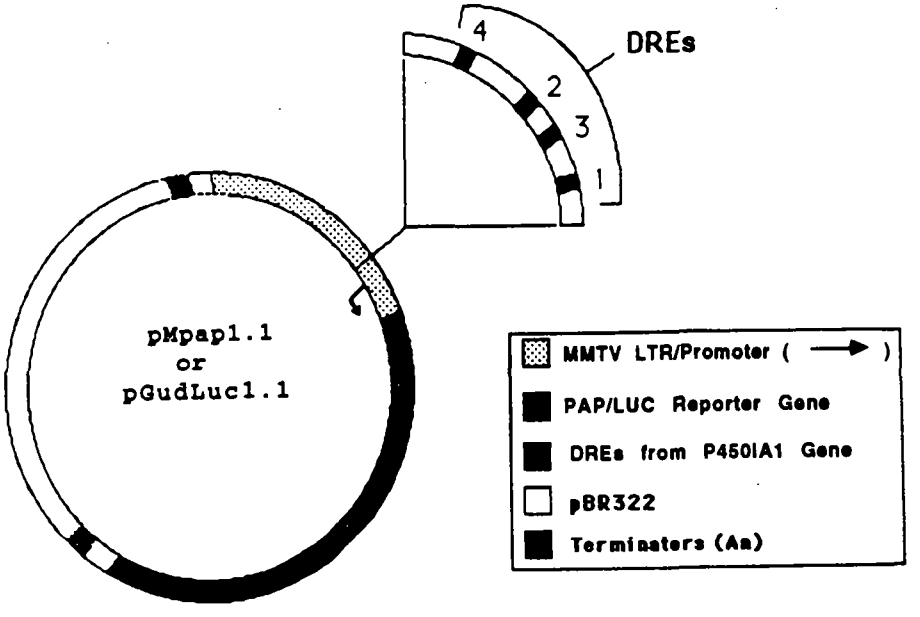

Figure 1. Structure of the HAH-inducible expresssion vectors (see text).

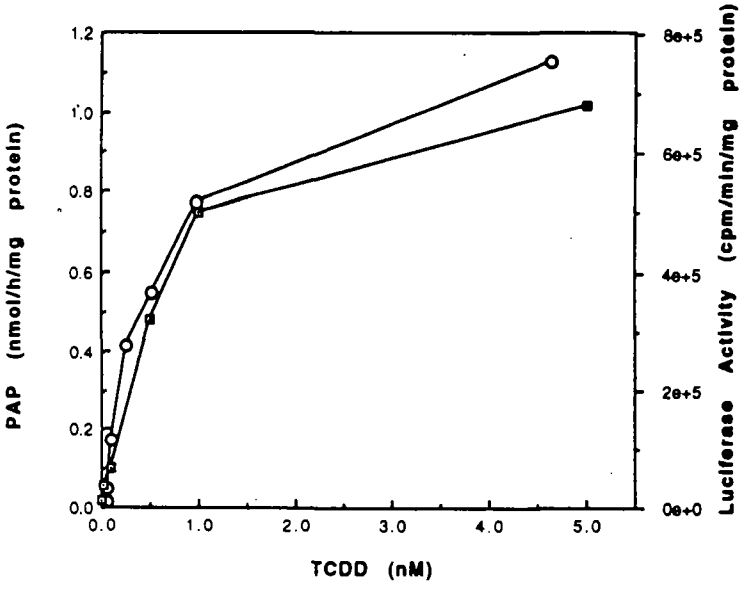

Figure 2. TCDD-dose dependent increase in expression of recombinant PAP (squares) or LUC (circles) from mouse hepatoma cells stably transfected with the expression vectors pMpap1.1 or pGudluc1.1, respectively.

CONCLUSIONS

We have prepared novel cell lines which have been stably transfected with a recombinant expression vector which responds to various aromatic hydrocarbons with the induction of a sensitive reporter enzyme (PAP or LUC). These expression vectors and cell lines can be readily be utilized for a variety of screening and monitoring purposes. These reporter vectors have been used to detect novel TCDD-like chemicals produced by UV-irradiation of tryptophan (12) as well as to identify new AhR antagonists.

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