

plasmid expressing a chimeric Ah receptor and a plasmid expressing a reporter gene driven by a suitable operator.

[0012] The genetically engineered cells of this invention can be used in an assay to detect agonists to the Ah receptor. The assay can be used to detect agonists in environmental samples such as air, water and soil. Such an assay can be conducted on agar plates or in a liquid media. Such an assay would involve preparing a culture of the genetically engineered viable cells, incorporating the sample to be tested into the culture, growing the culture for several hours, determining Ah receptor activation by detecting reporter gene expression and monitoring agonists based on Ah receptor activation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 shows detection of the Ah receptor in Wild-Type and Mutant Hepa-1c1c7 cells.

[0014] FIG. 2 shows a restriction map and location of cDNA clones.

[0015] FIG. 3 shows Northern blot analysis of wild type and class I mutant Hepa 1c1c7 cells.

[0016] FIG. 4 shows peptide mapping and amino acid sequencing of internal fragments generated by CNBr.

[0017] FIG. 5 shows the alignment of the basic helix-loop-helix domains of Ah-receptor (AHR), Sim, and ARNT.

[0018] FIG. 6A shows a partial restriction map and location of human Ah-receptor cDNA clones.

[0019] FIG. 6B shows the amino acid sequence of the human Ah-receptor (Hu) and comparison with the murine Ah-receptor.

[0020] FIG. 7 shows the ligand binding of the murine and Ah receptors.

[0021] FIGS. 8A, 8B, and 8C show gel shift assays demonstrating the binding of Ah receptor (AhR)-ARNT heterodimers to DRE3.

[0022] FIG. 9 shows deletion analysis of the human and murine Ah-receptors.

[0023] FIG. 10 shows an example of a mammalian expression vector for human AhR.

[0024] FIG. 11 shows an example of a receptor expression plasmid and a reporter plasmid.

[0025] FIG. 12 shows a plasmid map of pSV.Sport1.

[0026] FIG. 13 shows a plasmid map of pSport M'AhR.

[0027] FIGS. 14A, 14B, and 14C show the pharmacology of the Ah Receptor expressed in yeast. FIG. 14A shows the structure of ligands used in the dose-response assay and the key to symbols. The square refers to β NF, the triangle, α NF, and the diamond, dexamethasone. FIG. 14B shows the dose-response curves for AHR/ARNT/DRE-2 system. Cultures containing strain A303 transformed with plasmids pCWhuAHR, pY2ARNT, and pDRE23-Z were exposed to agonist for 16-18 hours and β -galactosidase assays performed to measure reporter activity; β -Galactosidase units were converted to percent of the maximal activity of β NF and plotted against concentration. FIG. 14C shows the dose-response curve for the chimeric AHR-LexA signaling

system. Strain GRS4 transformed with plasmids pEGAYRNA166 and pSH18-34 were grown in 2% galactose selection media containing agonists for 16-18 hours. β -Galactosidase activity was measured to determine reporter gene expression. β -Galactosidase units for all ligands were compared to the maximum response of β NF and plotted against agonist concentrations.

[0028] FIG. 15 shows a representative CAT assay of extracts from cells transfected with selected Gal4-fusion chimeras. The (-) means without β NF, the (+) means with β NF. Due to the high level of activity, extracts from the following plasmids were diluted 10-fold: pGAHRN Δ 409, pGAHR Δ 418/VP, pGAHRN Δ 520. Extracts from plasmids pGAHRN Δ 409/C Δ 165 and pGARNTN Δ 581 were diluted 20-fold.

[0029] FIG. 16 shows a schematic diagram of amino- and carboxyl-terminal deletion GAL4-AHR fusion constructs and the average of their CAT assay results. The values reported are the average of two to four independent experiments with standard error never greater than 25%. The box marked GAL4 represents yeast GAL4 (1-147 amino acids) vertical bars represent the basic helix loop helix (bHLH) the striped box represents the PAS domain with the "A" and "B" repeats indicated therein with black boxes (2); box with left-to-right diagonal lines represents the glutamine-rich region (Q) and the gray shaded box corresponds to TAD of the herpes simplex virus VP16 protein (VP16). The positions of the PAS, ligand binding domain, and TAD are indicated with horizontal bars. Fold induction, reported in the bar graph on the right, is relative to the control pS6424. Bars with a gray diagonal lines represent experiments without β NF, and black bars are those with β NF. Ligand-dependent induction is indicated to the right of bars when relevant.

DETAILED DESCRIPTION OF THE INVENTION

[0030] For purposes of clarity of disclosure, and not by way of limitation, the detailed disclosure is divided into the following subsections:

[0031] (i) Cloning the Murine Ah-receptor;

[0032] (a) Cloning the Murine Ah-Receptor

[0033] (b) Cloning the Human Ah-Receptor

[0034] (ii) The Genes and Proteins of this invention;

[0035] (iii) Expression of the Ah receptor;

[0036] (iv) The Utility of the invention; and

[0037] (v) Genetically Engineered Cell Systems and Assays for Detecting Agonists to the Ah receptor.

[0038] (i) Cloning the Ah-Receptor

[0039] (a) Cloning the Murine Ah-Receptor

[0040] The Ah receptor gene is defined herein as the nucleic acid sequences encoding the Ah receptor proteins and may be identified according to the invention by cloning cDNA transcripts of Ah protein and identifying clones containing full length Ah receptor protein-encoding sequences or using oligonucleotide probes designated as Sequence ID. NOS. 5, 6, & 7.