

AH RECEPTOR CDNA AND GENETICALLY ENGINEERED CELLS FOR DETECTING AGONISTS TO THE AH RECEPTOR

[0001] This application is a continuation-in-part application of U.S. Ser. No. 08/045,806, filed Apr. 8, 1993, now U.S. Pat. No. 5,378,822.

[0002] This invention was made with Government support under Grant Number: ES-05703 awarded by the National Institute of Environmental Health Sciences, and NIH grant E505703. The Government has certain rights in the invention.

FIELD OF INVENTION

[0003] This invention relates to cDNA molecules encoding the murine and human Ah-receptors (Ah^{b-1} allele) that have been isolated and characterized. More specifically, the cDNAs of this invention can be used to make Ah-receptors which can be used inserted into cells for use in bioassays to detect environmental pollutants. Additionally, these cDNAs can be used in the generation of recombinant organisms that serve as biomonitors for environmental pollutants and as probes for detecting human and wildlife populations that have high susceptibility to environmental pollutants and polycyclic aromatic hydrocarbons.

BACKGROUND OF THE INVENTION

[0004] The Ah-receptor is a soluble protein which mediates an individual's response to a variety of drugs, carcinogens and toxic agents. Chemicals which interact with the Ah-receptor, include a variety of environmental contaminants (dioxins, PCBs, PBBs, benzo(a)pyrene and a variety of natural products (flavones, carbazoles etc). One of the most potent agonists of the Ah-receptor is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or "dioxin"). TCDD is the prototype for a large family of highly toxic carcinogenic and teratogenic environmental contaminants. Poland A., Knutson, J. C., *Ann. Rev. Pharmacol. Toxicol.* 22:517-554 (1982). Members of this family include a number of halogenated dibenzo-p-dioxin, dibenzofuran, and biphenyl isomers which induce a variety of receptor-mediated toxic responses, including a severe wasting syndrome, epidermal hyperplasia and metaplasia, tumor promotion and thymic involution.

[0005] The Ah receptor is believed to reside primarily in the cytosol. While in the cytosol, the Ah receptor is associated with a dimer of the 90 kDa heat shock protein (hsp 90). It is believed that hsp 90 holds the Ah receptor in a conformation capable of binding ligand, but unable to bind to DNA. Upon binding of a ligand, the Ah receptor undergoes a temperature dependent activation, dissociates from the hsp 90, translocates from the cytosol to the nucleus, displays an increased affinity for specific DNA enhancer elements, known as the dioxin responsive elements (DRE) found in the nucleus. Enhancer elements increase transcriptional efficiency, often independent of their orientation and distance with respect to the promoter.

[0006] Once translocated to the nucleus, the Ah receptor dimerizes with the Ah receptor nuclear translocator (ARNT) protein. The Ah receptor-ARNT complex exhibits enhanced affinity for the DREs. The binding of the Ah receptor-ARNT complex to the DRE initiates transcription of the mRNA for the CYP1A1 gene. See Durrin, L. K., Jones, P., B. C., Fisher,

J. M. Galeazzi, D. R., and Whitlock, J. P., Jr., *J. of Cell. Biochem.* 35:153-160 (1987); citing Adesnick, M., Atchison, M., *Crit. Rev. Biochem.* 19:247-305 (1985) and Lu Ayh, Wet S. B., *Pharmacol. Rev.* 31:277-295 (1979). The CYP1A1 gene encodes an isozyme of the cytochrome P450 enzyme family. Cytochrome P450 enzymes catalyze the oxygenation of many endogenous and exogenous lipophilic substrates and are involved in a variety of metabolic activities.

[0007] The photoaffinity ligand, [¹²⁵I]-2-azido-3-iodo-7,8-dibromodibenzo-p-dioxin, covalently labels the Ah-receptor from a number of species, tissues and cell types. Poland, A., Glover, E., Ebetino, F. H. & Kende, A. S., *J. Biol. Chem.* 261:6352-6365 (1986). These photoaffinity labeling studies demonstrated that the Ah-receptor exhibits significant polymorphism, both between species and within different strains of the same species. For example, four different allelic forms of the Ah-receptor have been identified in inbred strains of mice: Ah^{b-1} allele (C57 strains)=95 kD, Ah^{b-2} allele (e.g., C3H strain)=104 kD, Ah^{b-3} allele (*Mus spretus*)=105 kD, and Ah^d allele (e.g., DBA strain)=104 kD. The Ah^d allele encodes a receptor with a 10-100-fold lower affinity for agonist than the Ah^{b-1} or Ah^{b-2} alleles. Poland, A. & Glover, E., *Mol. Pharm.* 11:389-398 (1975); Okey, A. B., Vella, L. M. & Harper, P. A., *Mol. Pharm.* 35:823-830 (1989); Poland, A., Palen, D., Glover, E., *Mol. Pharm.* 46:915-921 (1994).

[0008] The purification of the Ah-receptor from C57BL/6J mouse liver has been described. Bradfield, C. A., Glover, E. & Poland, A., *Mol. Pharm.* 39:13-9 (1991). To confirm the identity of this purified protein, its N-terminal amino acids has been sequenced and the corresponding peptide synthesized. Poland, A., Glover, E. & Bradfield, C. A., *Mol. Pharmacol.* 39:20-6 (1991).

SUMMARY OF THE INVENTION

[0009] The present invention involves the isolation and characterization of cDNA sequences which encode the murine rat and human Ah receptors. These Ah receptor cDNAs have the sequences set out in Sequence ID. Nos. 1 and 3 and can be used to generate large quantities of the Ah receptor for use in assays and for insertion in yeast and animal cell systems.

[0010] The present invention also involves genetically engineered viable cells. According to this invention, two types of genetically engineered cells can be formulated. The first type of cells that can be transformed are yeast cells, such as *Saccharomyces cerevisiae* and *Saccharomyces pombe*. The yeast may be genetically transformed with plasmids expressing the Ah receptor, the Ah receptor nuclear translocator, and a reporter gene driven by the dioxin responsive element. Additionally, the yeast may be transformed with a plasmid expressing a chimeric Ah receptor and a plasmid expressing a reporter gene driven by a suitable operator. The chimeric Ah receptor is constructed by replacing the binding and dimerization region of the Ah receptor with an analogous domain from a protein capable of binding DNA sequences. The operator sequence contains the binding sites from the binding domain of the protein used to replace the binding and dimerization domain of the Ah receptor.

[0011] The second type of cells that can be transformed are mammalian cells, such as COS-1 cells. As with the yeast cells, the mammalian cells can be transformed with a