IDENTIFICATION OF A HUMAN DIOXIN-RESPONSIVE cDNA (CLONE 1) AS A NEW MEMBER OF THE CYTOCHROME P450 SUPERFAMILY

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is among the most toxic pollutants known, and it is a prototype for a large class of halogenated aromatic hydrocarbons that include members of the dibenzofurans, biphenyls and other chemicals of environmental concern. In general, there is a good correlation between the ligand binding affinity for the Ah receptor (AhR) and the potency of a ligand to elicit biological response[1,2].

TCDD is carcinogenic[3] and teratogenic[4] in rodents, but effects of exposure to TCDD in humans are less well understood. In humans the most common adverse response to TCDD is chloracne[5]. The pathogenesis of this disorder is characterized by altered patterns of proliferation and differentiation in the skin that result in thickening of the epidermis, hyperkeratosis, and squamous metaplasia of the epithelial lining of the sebaceous gland[6].

These effects and others associated with exposure to TCDD in both humans and in other animals are believed to occur through the high-affinity binding of TCDD to the AhR and subsequent changes of gene expression in responsive cells[1,7]. The most extensively studied TCDD-responsive gene is CYP1A1 (cytochrome P_1-450). Increased transcription of CYP1A1 requires accumulation of the TCDD-AhR complex in the nucleus and interaction of the complex with specific DNA response elements[2]. Other genes are believed to be similarly regulated by the AhR. It is hypothesized that the altered expression of these genes is linked causally to the pleiotropic toxic effects of TCDD and other AhR agonists[1,7] (Figure 1).

To elucidate the events occurring in human cells in response to treatment with TCDD, we recently reported the use of differential hybridization to isolate five TCDD-responsive cDNA clones from a human keratinocyte cell line. Two of the isolated clones represented known human genes that encode proteins important in several growth regulatory processes of cellular homeostasis. Two other TCDD-responsive cDNA clones, designated clone 1 and clone 141, represent previously unidentified human genes[8].

Our initial focus has been to characterize the clone 1 cDNA. We have previously shown that levels of clone 1 mRNA are increased nearly 50-fold by treatment with 10 nM

TCDD, and that this increase in amounts of RNA reflects, in part, increased rates of transcription[8]. Preliminary DNA sequence analysis indicates that clone 1 is likely to encode a new member of the cytochrome P450 superfamily.

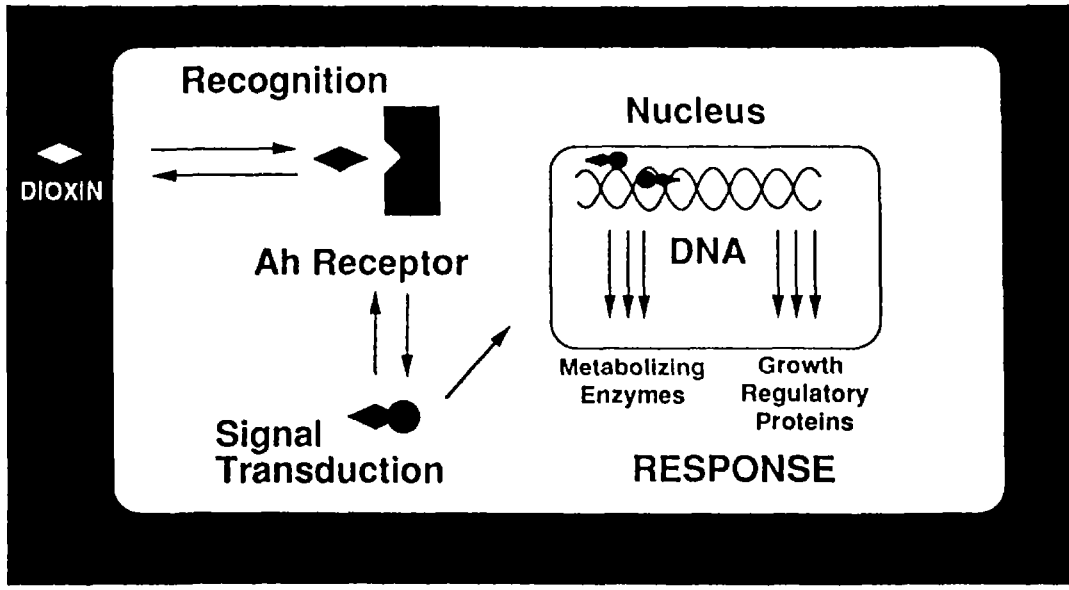

Figure 1. Proposed mechanism of action of TCDD and other AhR agonists. It is known that TCDD enters the cell and binds with high affinity to the Ah receptor. Activation and nuclear translocation of the TCDD-AhR complex must occur prior to binding to DNA (Ligand Recognition). The AhR, and at least two additional proteins are known to be involved in this process. Once in the nucleus, this activated complex binds to specific DNA response elements and begins the process to activate gene transcription (Signal Transduction). This process results in increased levels of specific gene products that include metabolic enzymes and growth regulatory proteins (Primary Biological Response). It is also possible that this process could result in repressed gene expression, but no such examples are known. It is hypothesized that the toxic effects of TCDD are caused by the altered expression of specific subsets of genes that are regulated by the AhR.

RESULTS AND DISCUSSION

During the original library screening, five plasmids representing clone 1 were isolated. Restriction endonuclease digestion and gel electrophoresis showed that plasmid T01-57 contained the largest clone 1 cDNA insert, 1.55 kb[8]. This cDNA insert was isolated and used to screen 30,000 members of the original cDNA library by colony

hybridization. From 283 initial positives, 126 plasmids were isolated and analyzed. The largest insert isolated, plasmid T01-57-247, contained approximately 47 percent of a full length clone 1 cDNA insert. Since oligo(dT) was used to prime the first strand cDNA synthesis[8], the majority of the isolated plasmids represent the 3-prime portion of the clone 1 cDNA. The organization of the clone 1 cDNA, inferred from the plasmids isolated to date, is shown (Figure 2).

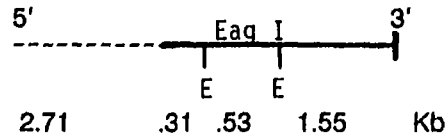

Figure 2. The current understanding of the organization of the clone 1 cDNA based on restriction endonuclease digestion and analysis by gel electrophoresis of clone 1 containing plasmids. The isolated plasmids (see text) were digested with Eag I (E), a restriction enzyme that cuts on both sides of the multiple cloning site of the vector (pcDNA II) used to construct the cDNA library[8]. The 5-prime (5'; amino-terminal) to 3-prime (3'; carboxy-terminal) orientation of the clone 1 cDNA is shown. The solid line indicates the sequence contained in the plasmid T01-57-247. The restriction map location and respective fragment sizes in kilobase-pairs (kb) is indicated at the bottom of the figure.

While additional screening for a full-length clone 1 cDNA was in progress, we determined the nucleotide sequence of clone T01-57-247 by the dideoxy chain-termination method as previously described[8] (T.R. Sutter, data not shown). By comparing the predicted endonuclease restriction map of this sequence with the map previously generated (Figure 2), we were able to identify the 5-prime to 3-prime orientation of the clone 1 cDNA sequence. Moreover, this sequence predicted only a single open reading frame of 528 amino acids, and a 3-prime non-translated region of approximately 0.8 kb.

Using the FASTA comparison program[9], the deduced amino acid sequence of this single open reading frame was compared to the protein sequences present in the most recent releases of the GenBank, EMBL, SWISS-PROT, and GenPept databases. More than 100 highly significant similarities [z values > 100, where z value = (similarity score - mean of random scores) / (standard deviation of random scores)[10]] were identified between the clone 1 deduced amino acid sequence and sequences present in these databases. Inspection of the 100 best scores indicated that each sequence was a

cytochrome P450[11]. For example, under the initial search conditions (ktup: 2 and fact: 8) the best score was obtained for the sequence MUSCYP145X, the mouse mRNA for cytochrome P1-450. This comparison of the deduced clone 1 amino acid to the MUSCYP145X sequence resulted in an optimal similarity score of 894. Alignment of these sequences reveals 40.1 percent identity in a 441 amino acid overlap.

The two groups of cytochrome P450 sequences most similar to the clone 1 sequence were the CYP1A1 and CYP1A2 sequences, respectively. However, significant similarity was also evident for several CYP subfamilies. A more detailed analysis will be required to determine if the clone 1 sequence represents a new member of the CYP1A subfamily, i.e. CYP1A3, or a new CYP subfamily. However, since the most similar CYP subfamilies did contain their corresponding human sequences, it is clear that the clone 1 sequence does represent a unique member of the cytochrome P450 superfamily, and not simply the human homolog of a previously identified gene.

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