Functional Architecture of the Dioxin Receptor

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The intracellular dioxin receptor shows specific high affinity binding of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) and structurally related environmental pollutants. The receptor mediates signal transduction by dioxin and functions as a ligand-dependent, nuclear transcriptional regulator by binding to cognate xenobiotic response elements (XREs) of target genes[1-11]. The receptor contains a basic helix-loop-helix (bHLH) DNA binding/dimerization motif contiguous with a region that shows similarity to the Drosophila regulatory factors Per and Sim and the human factor Arnt (i.e. the PAS domain). Upon exposure to dioxin the receptor binds DNA as a stable heterodimer with the structurally related bHLH/PAS factor Arnt (reviewed in ref. 12). Neither the receptor nor the Arnt partner factor recognize XRE sequence motifs individually, nor do they recognize E box motifs[5,8], the well characterized target of other bHLH transcriptiopn factors, including, for instance, the protooncogene c-myc and the muscle-differentiating factors myogenin and MyoD[12].

Our laboratory is concerned with elucidating the structure and function of the dioxin receptor. Our current understanding of the functional architecture of both the receptor and its partner factor Arnt is summarized in Figure 1. Moreover, we are trying to understand the molecular mechanisms underlying the ligand-induced activation of human and rodent dioxin receptor function. In extracts from non-stimulated rodent and human cells, the receptor is recovered in an inducible cytoplasmic form associated with the molecular chaperone hsp90[1,12]. Hetrodimerization between the receptor and the Arnt partner factor occurs in solution but is strictly regulated by dioxin. While the bHLH motif of the receptor is critical for dimerization[5,8], the C-terminal half of the PAS domain constitutes the dioxin binding structure of the receptor[7]. In addition, this region of the receptor (but not the corresponding region of Arnt) mediates stable interaction with hsp90, and confers conditional regulation on heterologous transcription factors. We provide evidence for two distinct chaperone functions of hsp90: (i) folding of a high affinity dioxin binding conformation of the ligand binding domain of the receptor; and (ii) folding of a DNA binding conformation. In the absence of ligand, hsp90 seems to be directly involved in maintaining the dioxin receptor in a repressed state by interaction with the ligand binding domain and interference with Arnt-receptor dimerization[7]. In line with these data, deletion of the PAS domain of the receptor results in constitutive dimerization with Arnt. In contrast, this receptor mutant shows very low levels of XRE binding activity, indicating a possible novel function of the PAS domain: determination of DNA binding specificity of this subfamily of bHLH factors. What is the role of the partner factor Arnt in signal transduction by the receptor? In addition to determining target gene specificity, Arnt confers a potent transcriptional activation function to the receptor. Finally, preliminary evidence in vivo and in vitro indicates that Arnt (and/or associated factors), in fact,

may play an important role in promoting derepression of the dioxin receptor, i.e. release of hsp90[13]. These results suggest that it may under certain conditions be possible to bypass ligand-dependency for activation of the receptor simply by modulating cellular levels of Arnt or the pool of Arnt that is available for dimerization with the receptor. This alternative pathway for regulation of receptor function may provide a clue with regard to the physiological function of the dioxin receptor (This work was supported by grants from the Swedish Cancer Society).

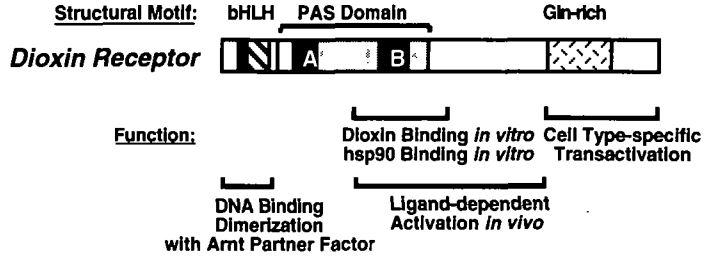

Figure 1. **Schematic Representation of Functional Domains of the Dioxin Receptor.** *bHLH*, basic helix-loop-helix motif; *PAS*, Per-Arnt-Sim homology region; *Black Boxes*, conserved hydrophobic repeats (denoted A and B) within the PAS domain. See text for details.

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