MECHANISM OF LIGAND-DEPENDENT ACTIVATION OF THE DIOXIN RECEPTOR

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The intracellular dioxin (aryl hydrocarbon) receptor functions as a ligand-activated DNA binding transcription factor that mediates the metabolic, toxic and possibly carcinogenic effects of polycyclic aromatic hydrocarbons and halogenated aromatic hydrocarbons, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). The dioxin receptor contains a basic helix-loop-helix (bHLH) motif contiguous with a region, PAS, that is conserved in the Drosophila circadian rythm regulator Period, the mammalian transcription factor Arnt, and the Drosophila neurodevelopmental factor Single-minded. Upon exposure to ligand, the dioxin receptor dimerizes with the structurally related partner factor Arnt, a process that enables the dioxin-activated Arnt-receptor complex to recognize dioxin (xenobiotic) response elements of target genes, e.g. cytochrome P4501A1 and glutathion-S-transferase target genes (Whitelaw et al., 1993b; Berghard et al., 1993). Neither the receptor nor Arnt recognize the target DNA sequence motifs individually. In extracts from non-stimulated cells, the receptor is recovered in an inducible cytoplasmic form associated with the molecular chaperone hsp90. Hsp90 has dual functions: chaperoning of a DNA and ligand binding receptor conformation, and repression of receptor activity in the absence of ligand (Coumailleau et al., 1995; Whitelaw et al., 1995, and references therein).

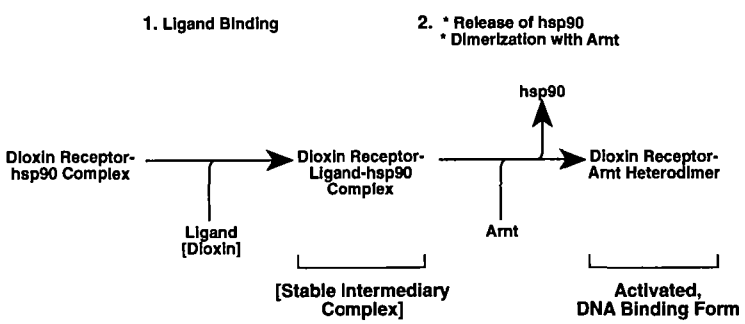

FIGURE 1. Two critical steps in the dioxin receptor activation process. In the presence of ligand, the Arnt partner factor facilitates release of the hsp90 chaperone and thus promotes derepression of dioxin receptor function.

We have mapped the functional architecture of both the dioxin receptor and the Arnt partner factor (Whitelaw et al., 1993a; 1994). Functional domains include transactivation domains, dimerization interfaces, and the ligand binding domain of the receptor. Release of hsp90 is necessary for derepression (Gradin et al., 1994), i.e. unmasking of DNA binding and dimerization activities of the dioxin receptor (Whitelaw et al., 1993b; McGuire et al., 1994). Interestingly, the ligand binding structure of the dioxin receptor is co-localized with the minimal region mediating interaction with hsp90 and transcriptional repression (Whitelaw et al., 1993a). We demonstrate that the process of ligand binding is not sufficient for derepression of dioxin receptor function. In fact, as outlined in Figure 1, concomitant recruitment of Arnt is required for facilitating release of hsp90 and receptor activation (McGuire et al., 1994; McGuire et al., 1995). The very complex architecture of the conserved PAS motif is intriguing. In addition to harboring the ligand binding (and hsp90 binding) domain of the dioxin receptor, the C-terminal half of both the dioxin receptor and Arnt also functions as an auxiliary dimization interface (Lindebro et al., 1995). In contrast, the N-terminal half of the PAS domain dictates dimerization specificity of the potent primary bHLH dimerization (and DNA binding) domain. Our attempts to understand the structure and function of the PAS domain, and the mechanism of repression and derepression of PAS domain function within the dioxin receptor, will also be important for investigating the biology for related conditionally regulated bHLH-PAS factors. These factors include a recently identified hypoxia-inducible factor (HIF), a number of key developmental factors in Drosophila (Sim and trachealess), and a Sim-related human putative Down syndrome critical factor, all of which appear to require Arnt as a common partner factor for function. The complex role of the PAS domain in dioxin receptor function will be discussed, and compared to its role in signal transduction by other members of the bHLH-PAS transcription factors, a novel and growing family of putative "orphan receptors", i.e. intracellular receptors for as yet unknown classes of ligands.

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