Mechanisms of Toxicity: New Insights on the Ah Receptor

REGULATION OF DIOXIN RECEPTOR FUNCTION

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Introduction

The ubiquitous, intracellular dioxin (aryl hydrocarbon) receptor 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 (TCDD) commonly known as dioxin (1, 2, 3). The dioxin receptor (DR) functions as a ligand activated transcription factor which, upon exposure to ligand, recognises specific dioxin or xenobiotic response elements (XRE's) upstream of promoters in target genes, as a heterodimeric complex with the structurally related partner factor Arnt (Ah receptor nuclear translocator, 4). Individually, neither the dioxin receptor nor Arnt exhibit any affinity for the XRE motif (5, 6, 7, 8).

Both the dioxin receptor and Arnt are members of a rapidly growing subclass of the basic helix-loop helix (bHLH) family of gene regulatory proteins termed bHLH/PAS. Contiguous to the bHLH motif, members of this subclass harbour an additional 250-300 amino acid region termed the PAS domain (for Per-Arnt-Sim homology) that has been shown to function as a dimerisation interface. More recently, the PAS domain has been postulated to play a role in partner factor selection during bHLH/PAS heterodimer formation and also in conferring target gene specificity on bHLH/PAS heterodimers (9, 10). In addition, in the case of the dioxin receptor, the PAS domain has been shown to harbour the ligand binding activity of the receptor and also to mediate association with the molecular chaperone heat shock protein hsp90 (11, 12). Association with hsp90 has been shown to be required for folding of a high affinity ligand binding conformation of the receptor *in vitro* (13) and for ligand responsiveness *in vivo* (14, 15). Novel members of the bHLH/PAS family are rapidly being identified and include the Hypoxia-Inducible-Factor, Hif-1α

(16) and the related Hif-Like-Factor/Endothelial PAS factor (HLF/EPAS; 17, 18), Sim and Trachealess, critical neuronal and tracheal developmental factors in Drosophila, respectively (19, 20), and the Clock and Per proteins involved in the regulation of circadian rhythmicity (21, 22, 23, 24), thereby indicating multiple diverse biological roles for these factors. In addition, a number of transcriptional co-activators have been identified as members of this subclass including SRC-1 (25), TIF-2 (26) and ACTR (27).

Results and Discussion.

Ligand dependent activation of dioxin receptor function appears to be a multi-step process, the mechanisms of which however, are still poorly understood. In the absence of ligand, the dioxin receptor exists in the cytoplasm as a latent, non-DNA binding heteromeric complex with hsp90. By comparison, the active DNA binding species exists in the nucleus as a heterodimer with Arnt (2, 3). We have been interested in elucidating further the stepwise conversion of the dioxin recepotor from the latent inactive non-DNA binding cytoplasmic form to the active nuclear DNA binding species. The classical model for conversion of the receptor to the high affinity DNA binding species suggests that ligand binding is required for the release of hsp90 followed by nuclear translocation and dimerisation with Arnt (2, 3). We have previously demonstrated however, that ligand alone is insufficient derepress dioxin receptor function but rather requires the concommittant recruitment of Arnt (28), thus adding a further level of complexity. Hence, changes in the cellular levels of Arnt or the pool of Arnt that is available for dimerisation with the receptor could represent a critical component in the regulation of receptor function. This observation has led us to explore the possibility that the dioxin receptor may be the target of multiple regulatory strategies depending on the availabilty of the Arnt cofactor. In addition, using mutants of the dioxin receptor that function as dominant positive activators of transcription, we have developped model systems with which to address the potential physiological role of the dioxin receptor.

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