Mechanism of Conditional Regulation of the bHLH/PAS Dioxin Receptor

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Introduction

The intracellular dioxin (aryl hydrocarbon) receptor mediates the wide spectrum of toxic and possibly carcinogenic effects attributed to the environmental pollutant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) commonly known as dioxin, and structurally related compounds (1, 2). The dioxin receptor also regulates the expression of a number of xenobiotic metabolising enzymes including cytochrome P4501A1, glutathione-S-transferase Ya, aldehyde dehydrogenase and quinone oxidoreductase. Although the physiological function of the dioxin receptor and the nature of a possible physiological ligand or activator molecule are presently not known, targeted disruption of the mouse dioxin receptor gene has indicated that the the receptor may play a role in hepatic (3, 4, 5, 6) and perhaps also lymphoid development (4).

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, 7). Individually, neither the dioxin receptor nor Arnt exhibit any affinity for the XRE motif (8, 9, 10, 11). Both the dioxin receptor and Arnt are members of a distinct subclass of the basic helix- loop helix (bHLH) family of gene regulatory proteins termed bHLH/PAS. Juxtaposed to their bHLH domains,

these factors contain a second region of homology termed the PAS domain common to the *Drosophila* circadian rhythm factor Per and the midline developmental factor Sim. Additional members of this distinct subclass are rapidly being identified, including the Hypoxia Inducible Factor-1α (HIF-1α, 12) and the related HIF-Like-Factor/Endothelial PAS factor (HLF/EPAS, 13, 14), the *Drosophila* tracheal developmental factor Trachealess (15, 16), the mammalian circadian rhythm proteins Clock (17, 18) and Per (19, 20,) and a putative Down's syndrome critical factor (21) indicating multiple distinct biological roles for these factors.

Results and Discussion.

In the absence of ligand, the dioxin receptor is recovered in a latent, non-DNA binding heteromeric complex with the molecular chaperone, hsp90. Association with hsp90 has been shown to be required for folding of a high affinity ligand binding conformation of the receptor *in vitro* (22) and for ligand responsiveness *in vivo* (23, 24). Conversion of the dioxin receptor to a high affinity DNA binding species appears to be a multi-step process involving ligand binding, release of hsp90 and dimerisation with Arnt . Despite intensive efforts however, the mechanism(s) by which these steps occur is still poorly understood.. We have thus been interested in elucidating further this complex activation process of the dioxin receptor. Our results indicate that ligand alone is insufficient to activate the dioxin receptor but rather requires the concommitant recruitment of Arnt, thereby adding a further level of complexity. 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.

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