MECHANISM OF SIGNAL TRANSDUCTION BY THE DIOXIN (AH) RECEPTOR

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Introduction

The intracellular dioxin (aryl hydrocarbon) receptor functions as a ligand-activated transcription factor and mediates induction of transcription of a battery of target genes most notably encoding drug metabolizing enzymes (e.g. cytochrome P4501A1). The receptor belongs to a rapidly growing family of transcription factors factors (bHLH/PAS) including hypoxia-inducible transcription factors and (possibly light-inducible) circadian rhythmicity regulators. It is thus possible that this family of gene regulatory protein constitutes a novel group of environmental stress sensors.

Results and Discussion

In the absence of ligand the dioxin receptor is present in a latent conformation in the cytoplasmic compartment of the cell associated with the molecular chaperone hsp90. Hsp90 is required both for maintaining the dioxin receptor in a latent non-DNA binding state and a ligand binding conformation. Expression of the dioxin

receptor in mutant yeast cells containing reduced levels of hsp90 abolishes ligand responsiveness demonstrating the critical importance of hsp90 for dioxin receptor function. The nuclear form of the dioxin receptor interacts with Arnt and does no longer posses the ability to bind ligand and does not interact with the molecular chaperone hsp90. This form of the receptor specifically binds to enhancer elements known as XREs (Xenobiotic Response Elements) of a number of genes encoding drug metabolizing enzymes. Release of hsp90 from the latent form of the dioxin receptor is therefore a critical step in the activation process of the dioxin receptor.

We have previously observed that ligand-dependent release of hsp90 in vitro requires the interaction of the dioxin receptor with additional cellular factors including Arnt. Interestingly, in the present study, fractionation of cellular extracts through sucrose density gradients yielded an hsp90-associated form of the dioxin receptor which did not require ligand to generate the DNA binding complex with Arnt. This loss of ligand-dependency correlated with dissociation of p23 from the dioxin receptor-hsp90 complex. It was possible to reconstitute ligand dependency in receptor activation by addition of molybdate, an agent which has been shown stabilize the interaction between hsp90 and p23. Thus, these results indicate a role of p23 in modulating ligand-responsiveness in receptor activation.

Repression/derepression of both the dioxin receptor is intimately correlated with inducible nuclear import prior to conditionally regulated recruitment of transcriptional coactivators which harbor histone acetyl transferase activity. In fact, the dioxin receptor interacts with the same battery of coactivators that are recruited in a ligand-dependent manner to the ligand binding domain of members of the steroid hormone receptor gene family. We have investigated the role of the PAS domain in this process and identified domains that are critical for conditional recruitment of coactivators, and for repression and derepression of protein function. Although there is no apparent conservation of primary structural motifs between the dioxin receptor and members of the steroid hormone receptor family, the functional architecture of the bHLH-PAS factors is showing interesting similarities to the functional organization of the ligand and corepressor/coactivator binding domain of steroid hormone receptors, indicating common strategies of conditional regulation.

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