THE DIOXIN RECEPTOR: A SIGNAL-DEPENDENT GENE REGULATORY PROTEIN.

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Abstract. The dioxin receptor is a gene regulatory protein which, in response to dioxin, activates transcription from the cytochrome P-450IA1 gene by sequence-specific interaction with dioxin-responsive target sequences located at a distance from the transcription initiation site. Here we have reconstituted under cell-free conditions dioxin-induced conversion of the receptor from a cryptic to a DNA binding form. We show that the cryptic receptor form contains an inhibitory protein, the release of which is necessary to unmask functional activities of the receptor protein.

Introduction. The dioxin receptor regulates transcription of the cytochrome P-450IA1 gene in response to the environmental contaminant dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) or related compounds. This process is initiated by the binding of dioxin with high affinity to a single, presumably cytosolic receptor protein. Upon ligand binding the dioxin-receptor complex becomes activated undergoing a poorly understood structural alteration to a form which is retained on DNA cellulose *in vitro* or which apparently translocates from the cytosolic to the nuclear compartment *in vivo*. More recently, the *in vivo* activated dioxin receptor has been implicated in recognition of specific DNA sequences termed xenobiotic response elements (XREs) which modulate the activity of linked promoters thereby serving as dioxin-inducible enhancers (Fujisawa-Sehara *et al.*, 1987; 1988; Hapgood *et al.*, 1989). Thus, the mechanism of action of dioxin is similar to that of steroid hormones in that an intracellular soluble receptor protein transduces the extracellular signal to the transcriptional unit it regulates.

Results and Discussion. We have used the dioxin receptor system to investigate:
(i) *The role of an inhibitory protein (the 90 kD heat shock protein) in modulating functional activities of the receptor by protein-protein interaction.* In the absence of ligand, the dioxin receptor is found in the cytoplasm in a form unable to bind to DNA. The cytoplasmic form is bound to an inhibitory protein, the 90 kD heat shock protein (hsp 90), from which it can be released following exposure to receptor ligands *in vitro* or *in vivo* (Nemoto *et al.*, 1990; Wilhelmsson *et al.*, 1990). Treatment of target cells with dioxin *in vivo* leads to nuclear accumulation of the dioxin receptor and a concomitant unmasking of the previously cryptic DNA binding activity toward XRE target sequences (Hapgood *et*

al., 1989 and references therein). Hsp 90 has been shown to exhibit a similar inhibitory function on the DNA binding activity of the glucocorticoid receptor (Denis *et al.*, 1988 and references therein). In the case of the dioxin receptor, it is possible to resolve two forms of dioxin receptor within nuclear extract prepared from treated cells: one DNA binding (and presumably functional) form; and one non-DNA-binding form which contains hsp 90 and which is biochemically indistiguishable from the cryptic, cytosolic receptor form found in untreated cells (Wilhelmsson *et al.*, 1990). Thus, it appears as if inhibition of DNA binding may also occur within the nuceus, and that the nuclear translocation process of the *in vivo* dioxin-activated receptor can be dissociated from repression of receptor function by hsp 90.

To elucidate the mechanism of signal transfer from the cytoplasm to the nucleus, we have developed an *in vitro* system in which addition of ligand to a partially purified latent form of the receptor leads to the activation of the XRE-specific DNA-binding activity of the receptor. Our results indicate that the latent form of the receptor contains hsp 90; that hsp 90 is required for efficient binding of ligand; and that dissociation of hsp 90 is sufficient to unmask the cryptic XRE-specific DNA binding activity of the ligand-binding receptor subunit. Importantly, there exists a perfect correlation between the rank order of different dioxin receptor ligands to induce specific DNA binding activity *in vitro*, their affinities for the receptor protein, and their potencies to induce cytochrome P-450IA1gene transcription *in vivo*. Finally, our cell-free reconstitution experiments indicate that ligand (such as dioxin) is necessary but not sufficient to activate the cryptic, hsp 90-containing receptor complex. In addition, biochemical manipulation (*i.e.*: exposure to an elevated temperature or increased ionic strength) of the still latent ligand-receptor complex to efficiently dissociate hsp 90 *in vitro* and unmask functional activity. We

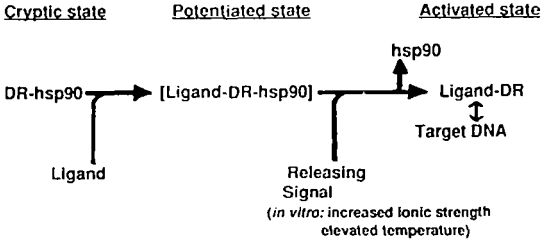

FIG. 1. ***Model for Signal-Induced Activation of the Dioxin Receptor.*** Schematic illustration of functional steps during activation of the dioxin receptor (*DR*) by a ligand (such as dioxin). The activated receptor represents a functional species which can activate target genes by interaction with genomic dioxin-responsive elements.

interpret these results as if dioxin induces the receptor into a potentiated state, from which it can be converted into a functional state by an hsp 90-releasing signal. The nature of this signal *in vivo* is still unkonwn. A schematic illustration of this model is shown in Fig. 1.

(ii) *The chromatin structure at the dioxin response elements XRE1 and 2 found upstream of the cytochrome P-450IA1 promoter.* Regulatory processes that lead to induced or differential gene expression are often reflected in local changes in chromatin structure. We have analyzed the chromatin structure of the XRE1 and 2 elements found at about -1,000 upstream of the transcription start site of the rat P-450IA1 gene. A DNase1 hypersensitive site at about -1,000 of the P-450IA1 gene is found in untreated, control rat liver nuclei. Upon dioxin treatment this hypersensitive site is unaltered, and no additional changes in chromatin structure are found in this region of the gene during gene activation. The presence of such a constitutive hypersensitive site correlates with the presence of a constitutive XRE-specific factor, distinct from the dioxin receptor. These data suggest that a specific DNA-protein architecture may be maintained at the XREs, regardless of changes in the transcriptional state of the gene. A similar constitutive occupation of a genomic response element has recently been described for the serum regulatory element of c-*fos*, the protein-DNA contacts of which (as assessed by *in vivo* foot printing) are unaltered by growth factor induction. Thus, such established structures could be important generally in rapid transcriptional responses to extracellular signals. Moreover, it remains to be established whether the constitutive XRE binding activity is displaced by the ligand-activated dioxin receptor *in vivo*, and if this process is accompanied by derepression of cytochrome P-450IA1 target gene transcription resulting in dioxin-induced high levels of P-450IA1 gene expression.

Conclusion. The dioxin receptor represents a gene regulatory protein residing in target cells in two functional states: (i) a latent; and (ii) a ligand-activated state. Latent forms can be recovered both in cytosolic and nuclear extracts of dioxin-treated cells, whereas the activated form is only detectable in nuclear extracts. We have reconstituted dioxin-induced activation of the dioxin receptor under cell-free conditions and shown that, in the latent state, receptor function is repressed by protein-protein interaction with an inhibitory protein, hsp 90. Thus, the dioxin receptor system may serve as a model for signal-dependent modulation and regualtion of eukaryotic transcription factor function. Finally, the possible interplay between the ligand-activated dioxin receptor and a constitutive factor binding to genomic dioxin response elements indicates that the rapid and dramatic induction of cytochrome P-450 IA1 gene transcription by dioxin may be the result of a derepression phenomenon of the gene.

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