A Partnership Between the Dioxin Receptor and a Basic Helix-Loop-Helix Protein

ERIC F. JOHNSON

THE DIOXIN RECEPTOR IS THE FOCUS OF CURRENT CONTROversy regarding safe exposure levels for dioxin (*1*). Although the mechanisms are unclear, many of the toxic effects of dioxin have been linked to its receptor (*2*), leading to suggestions that most if not all of the toxic and carcinogenic effects of dioxin may be mediated by the dioxin receptor. If this is so, exposures to concentrations of dioxin well below the dissociation constant of dioxin from the receptor are unlikely to lead to toxicity, and standard methods for the extrapolation of dioxin's toxic and carcinogenic effects to lower doses would result in overestimation of the risks of exposure (*1*).

Dioxin is one of a large number of chemicals that can induce enzymes, such as specific forms of cytochrome P450 that metabolize lipid-soluble, foreign compounds (xenobiotics). These enzymes generally provide a means for the rapid elimination of foreign chemicals, although P450 enzymes can also convert chemical carcinogens to more genotoxic products. As such, selective alterations of P450 expression can alter the balance between the activation and detoxication of carcinogens in a tissue (*3*).

The induction of xenobiotic metabolizing enzymes by dioxin is controlled by the dioxin or aryl hydrocarbon (Ah) receptor. Studies of cloned P450IA1 genes indicate that the induction of gene transcription by dioxin is mediated by multiple copies of an upstream regulatory element (*4, 5*), and a protein-dioxin complex has been shown to bind directly to these upstream regulatory sites (*6*). This mechanism of action is similar to that of the glucocorticoid receptor (*7*). This parallel extends further to include the association in cytosol of the dioxin receptor with the heat shock protein, hsp90 (*8*).

These similarities between the dioxin and glucocorticoid receptors have led to the idea that the dioxin receptor and other receptors that might regulate the expression of xenobiotic metabolizing enzymes are likely to be members of the superfamily of ligand-activated transcription factors that include the steroid hormone receptors. This hypothesis was reinforced by the characterization of a cDNA encoding a member of the steroid hormone receptor superfamily that is activated by compounds that cause the number of peroxisomes in rodent liver to increase (*9*). These compounds also induce a subset of P450 genes, but the peroxisome proliferator–activated receptor (PPAR) has not yet been shown to directly regulate the transcription of the P450 genes.

A report in this issue from Oliver Hankinson's laboratory (*10*) describes the cloning of a cDNA encoding a protein that is required for the induction of the cytochrome P450IA1 gene by the dioxin receptor. The sequence of this protein suggests that it may be related to the family of DNA binding proteins that bear a basic helix-loop-helix motif (*11*). The identification and characterization of this protein provides new information regarding the dioxin receptor that should aid in its further characterization. It is also suggestive of differences between the dioxin receptor and members of the steroid receptor superfamily that have been characterized.

In earlier work, Hankinson and his colleagues described a mutant hepatoma cell line in which the induction of P450IA1 by dioxin did not occur (*12*). The cytosol of these cells appeared to contain a functional dioxin receptor, but the receptor did not accumulate in the nucleus after the administration of dioxin. The nuclear translocation negative mutants of the Ah receptor ($arnt^-$) were found to complement another class of mutants that did not express normal amounts of the dioxin binding protein in cytosol, suggesting that the Arnt protein was required for the function of the dioxin receptor. As reported here (*10*), the *arnt* gene was cloned by transfecting the $arnt^-$ cell line with human DNA and selecting for cells in which the induction of P450IA1 is restored. When the protein encoded by the corresponding Arnt cDNA is expressed in the $arnt^-$ cell line in the presence of dioxin, the receptor-dioxin complex is found in the nucleus, and P450IA1 is induced.

Although the way in which Arnt accomplishes this is not clear, Arnt may aid in the dissociation of the receptor from hsp90, which blocks the binding of the receptor to DNA (*13*). The binding of dioxin to the receptor promotes the dissociation of hsp90 from the complex. However, the complex between hsp90 and receptor-dioxin complex is relatively stable in vitro (*13*), and this might reflect a need for an accessory protein to effect this dissociation in vivo. Alternatively, the Arnt protein might facilitate the transport of the receptor into the nucleus. The glucocorticoid receptor must undergo both dissociation from hsp90 and translocation to the nucleus as well. However, this process is not affected in the $arnt^-$ cell line, suggesting that if Arnt functions in this manner it does not affect either of these processes for the glucocorticoid receptor.

It seems more likely that the Arnt protein may be a subunit of the dioxin receptor. The Arnt protein contains a region of high sequence similarity with Myc proteins that corresponds to a basic helix-loop-helix (bHLH) domain. This motif has been described for a family of homodimeric and heterodimeric transcription factors, and it is thought to reflect a domain for both DNA binding and protein dimerization (*11*). The presence of this motif in Arnt suggests that it might form an oligomeric DNA binding protein. Protein-DNA cross-linking studies suggest that the DNA-bound form of the rat dioxin receptor is a heterodimer composed of two subunits of about 100 and 110 kD, where the smaller of the two appears to be the ligand binding subunit (*14*). The size predicted for Arnt is slightly smaller than that estimated for the two proteins detected in the cross-linking experiments, but this could reflect differences between species.

If heterodimer formation is required for function, then the dioxin-receptor differs from the steroid receptors which appear to form homodimers when they interact with their principal target genes (*15*). As noted (*14*), this would also explain why the receptor bound to DNA exhibits only one dioxin ligand per oligomeric receptor-DNA complex (*5*). In addition, the recognition sequence for the dioxin receptor lacks the two half-site symmetry seen for responsive elements recognized by the homodimeric, steroid hormone receptors (*7*).

In addition to providing a better understanding of the regulation of the genes encoding enzymes that metabolize foreign compounds, the characterization of the dioxin receptor could provide information regarding the mechanism by which dioxin may be carcinogenic. Unlike most of the chemical carcinogens that have been identified, dioxin does not appear to be directly genotoxic or to be converted to a genotoxic metabolite (*16*). The peroxisome proliferators are also nongenotoxic carcinogens in rodents (*17*). Genetic damage may

The author is a member in the Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic, La Jolla, CA 92037.

result from the latter because of their stimulation of the activity of peroxisomes, which elevates metabolism of oxygen to products that can damage DNA (*18*). In a similar manner, the long-term induction of P450IA1 by dioxin would alter the capacity of the tissue to activate other environmental carcinogens to genotoxic products. However, the incidental exposure of laboratory animals to such carcinogens would be expected to be low and may not be sufficient to account for the carcinogenicity of dioxin in laboratory testing.

Alternatively, nongenotoxic carcinogens that are also inducers of P450's could act via their activation of transcription factors; these factors may adversely affect the expression of proteins that regulate cell growth and differentiation and result in a transformed phenotype (*17*). The complete characterization of the dioxin receptor could reveal how, where, and when these ligand-activated transcription factors function. This will not only expand our understanding of how our capacity to metabolize foreign compounds is regulated, but may also identify critical mechanisms in environmental carcinogenesis.

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