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The Regulation of Gene Expression by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin*

JAMES P. WHITLOCK, JR.

Department of Pharmacology, Stanford University School of Medicine, Stanford, California 94305

I. Background

THE SOCIOPOLITICAL climate in the U. S. during the 1960s helped draw attention to the chemical that we have come to know as "dioxin." In the first place, cognizance of the potential risks associated with environmental contamination was on the increase; for example, in 1962 the publication of *Silent Spring* by Rachel Carson (17) generated particular concern about the increasing use of pesticides and herbicides. At the same time, there was growing restiveness about the conduct of the Vietnam war; one particular tactic, chemical defoliation of the countryside (Operation Ranch Hand), again focused attention on the possible adverse effects of the herbicides used to kill crops and vegetation in Southeast Asia. One particular herbicide (Agent Orange) used in Vietnam was a 1:1 mixture of (the *n*-butyl esters of) 2,4-dichlorophenoxyacetic acid (2,4-D)† and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); both compounds were also widely used as weed killers in the U. S. In 1970, an article in the *New Yorker* by Thomas Whiteside (172) publicized the suspicion that 2,4,5-T might cause birth defects.

Against this background, the report (24) that 2,4,5-T was teratogenic in rodents understandably aroused considerable concern among the public, environmental groups, the chemical industry, U. S. regulatory agencies, and Congress and led to restrictions on the use of the herbicide. The results of subsequent studies (20, 23, 160) implied that the actual teratogen was probably 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a contaminant that forms during the commercial synthesis of 2,4,5-T (fig. 1). Public and scientific attention then shifted from 2,4,5-T to TCDD (often described simply as "dioxin") and its potential risk to human health. The remarkable potency of TCDD (in its acute lethality for guinea pigs), combined with the relative resistance of TCDD to chemical and biological degradation, contributed to the fear that soon was associated with the dioxin. Several industrial accidents, episodes of leakage or improper disposal of chemical waste, and lawsuits brought by veterans who might have been exposed to Agent Orange have tended to keep TCDD in the public eye ever since. Despite the scientific and lay scrutiny that dioxin has received, it has been difficult to document that TCDD poses a major health hazard for humans. Studies in animals reveal marked quantitative differences in their sensitivity to TCDD; for example, the acute oral median lethal dose (LD_{50}) of TCDD is about 5000-fold higher for the hamster than for the guinea pig. In addition, the qualitative spectrum of effects produced by chronic exposure to TCDD varies substantially among animal species (140). These observations make it unusually difficult to extrapolate the results of animal studies to man. Long-term follow-up of individuals exposed to TCDD in an industrial setting does not implicate the dioxin as a cause of excess mortality or serious morbidity for humans (110, 161, 180). However, the number of individuals followed has been relatively small.

Its teratogenic effects in rodents stimulated scientific interest in TCDD and related chlorinated hydrocarbons. By the early 1960s, TCDD had been implicated in the etiology of chloracne in humans (6, 90, 91) and chick edema disease (69), but its other effects and its mechanism of action were unknown. Today, we know that

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† Abbreviations used are: AHH, aryl hydrocarbon hydroxylase; δ-ALAS, δ-aminolevulinic acid synthetase; HAH, halogenated aromatic hydrocarbon; PAH, polycyclic aromatic hydrocarbon; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 3MC, 3-methylcholanthrene; βNF, β-naphthoflavone; CAT, chloramphenicol acetyltransferase; DRE, dioxin-responsive element; GRE, glucocorticoid-responsive element; HMG, high-mobility group; LD_{50}, median lethal dose; ED_{50}, median effective dose; BP, benzo(*a*)pyrene; QSAR, quantitative structure-activity relationship; DBBD, 2,2-dimethyl-5-*t*-butyl-1,3-benzodioxole.

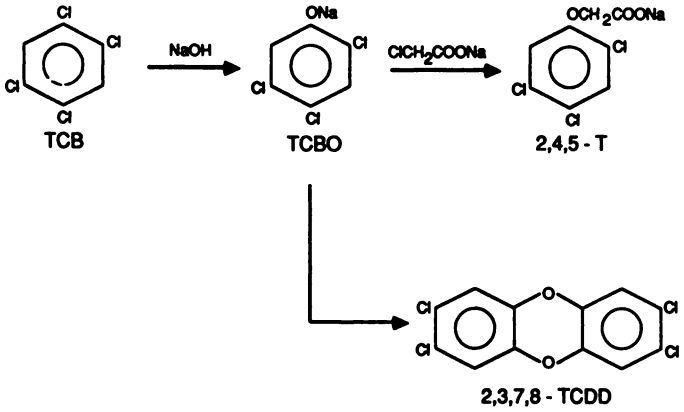

FIG. 1. Formation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (*2,3,7,8-TCDD*) during the synthesis of 2,4,5-trichlorophenoxyacetic acid (*2,4,5-T*). The first step in the industrial production of 2,4,5-T involves the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene (*TCB*) to form sodium 2,4,5-trichlorobenzeneoxide (*TCBO*). In the second step, TCBO reacts with chloroethanoate to form 2,4,5-T. If the temperature of the first step exceeds about 160°C, two molecules of TCBO can react in a double nucleophilic displacement to form 2,3,7,8-TCDD. Higher temperature and higher pH increase the formation of 2,3,7,8-TCDD. The side reaction is itself exothermic, possibly leading to even higher temperatures and uncontrolled reaction conditions (108, 143).

TCDD elicits a broad spectrum of biological effects, which vary according to the system in which the compound is tested. For example, in addition to its teratogenic effects, TCDD also produces several species- and tissue-dependent changes in epithelial tissues, immunological alterations, a wasting syndrome, tumor promotion, and the induction of several enzyme activities (139, 140). Therefore, models which seek to explain the mechanism of TCDD action must account for the diversity of effects that the compound produces. One reasonable hypothesis is that TCDD, acting by means of an intracellular receptor protein(s), alters the expression of a different set of genes in each TCDD-responsive cell type (54, 140). This particular model for TCDD action resembles that described for several steroid hormones, which also elicit diverse effects in receptor-dependent and tissue-specific fashion (146, 178). The evidence for and the molecular aspects of this model constitute the subject of this review.

The development of TCDD-responsive cell culture systems, combined with the use of recombinant DNA and gene transfer methods, has facilitated the analysis of TCDD action at the molecular level. We now know that TCDD can activate the rate of transcription of a gene that encodes a specific cytochrome P-450 isozyme (see below). In addition, exposure to TCDD produces phenotypic changes suggestive of altered differentiation in epidermal cells in culture (53, 75, 93, 122, 145) and in cultured thymic epithelium (25, 52). Furthermore, TCDD promotes the expression of a transformed phenotype in C3H10T½ cells (1). Although the mechanism(s) by which TCDD produces these altered phenotypes is not yet known, it seems quite likely that changes in the expression of specific genes are involved. We also know that other halogenated aromatic hydrocarbons (HAHs) that are related structurally to TCDD (e.g., dibenzo-*p*-dioxins, dibenzofurans, biphenyls, biphenylenes, naphthalenes, and azoxybenzenes) produce similar patterns of toxicity, although the compounds differ greatly in potency. Therefore, we assume that these HAHs share a common mechanism of action. Because it is the most potent, TCDD is the prototype, and it has been studied much more intensively than the other HAHs.

Early studies (15, 55, 56) revealed that TCDD induces hepatic, drug-metabolizing enzyme activities that are catalyzed by cytochrome P-450 isozymes. This class of microsomal hemoproteins oxygenates lipophilic substrates and contributes to many different biological processes, ranging from steroid biosynthesis to chemical carcinogenesis (44, 99, 168). At the time when TCDD was beginning to undergo intensive study, certain chemicals were already known to induce one (or more) of the various cytochrome P-450 isozymes. The effect of TCDD was similar to that of 3-methylcholanthrene (3MC), a polycyclic aromatic hydrocarbon (PAH) that preferentially induces a specific form of cytochrome P-450 (designated cytochrome P-450c in the rat and cytochrome P_1-450 in the mouse) (15, 55, 56). This particular cytochrome P-450 isozyme catalyzes aryl hydrocarbon hydroxylase (AHH) activity, which is present in many tissues and which is assayed using a simple and sensitive fluorescence technique (112). Therefore, measurement of AHH induction became a convenient way to determine if a particular tissue or cell type can respond to TCDD. (Note: the failure of TCDD to induce AHH activity in a particular cell type does not necessarily mean that the cell cannot exhibit some other response to the dioxin.) Given the long-established relationship between TCDD action and AHH induction, together with more recent achievements in the purification of cytochrome P-450 isozymes and the cloning of cytochrome P-450 genes (3, 173), it is not surprising that the most detailed knowledge of the mechanism of TCDD action has come from the study of TCDD-responsive cytochrome P-450 genes. We assume that TCDD influences the activity of other genes (i.e., those responsible for other phenotypic changes induced by the dioxin) by similar mechanisms. In retrospect, it is interesting that what began as a toxicological evaluation of a potent environmental contaminant has had unanticipated benefits. Analyses of TCDD action at the cellular and molecular levels have revealed a pathway by which an extracellular chemical signal can be transduced to the cell nucleus to activate the transcription of a specific gene. Further study of this TCDD-responsive signalling system in the future has the potential to reveal novel aspects of the mechanisms that control mammalian gene expression.

II. Evidence for a TCDD Receptor

The unusual potency of TCDD was the first clue that the dioxin might act through a specific receptor(s). For

example, Poland and Glover observed that TCDD was orders of magnitude more powerful than other compounds in inducing both AHH activity and δ-aminolevulinic acid synthetase (δ-ALAS) activity in the chick embryo (131, 132). In addition, studies of TCDD congeners revealed a relationship between dioxin structure and potency as an inducer (132). On the basis of these data, Poland and Glover postulated that TCDD acts by means of an "induction receptor" to elevate AHH and δ-ALAS activities. They also suggested that the hypothetical receptor might mediate other effects of TCDD, because the potencies of the halogenated dibenzo-*p*-dioxins as enzyme inducers paralleled their toxic potencies. Subsequent studies of AHH induction in rat liver confirmed the potency of TCDD; the dioxin was 30,000 times more potent than the prototypical AHH inducer, 3MC (133).

The foregoing results naturally led to the study of TCDD-inducible AHH activity in inbred strains of mice that were known to respond differentially to 3MC. Two groups had shown that, in certain mouse strains (typified by C57BL/6), 3MC induced hepatic AHH activity; however, in other strains (typified by DBA/2), it did not. In crosses between these strains, AHH inducibility segregated as an autosomal dominant trait (133, 164). Furthermore, other responses to PAHs exhibited a similar segregation pattern (152). Therefore, the genetic locus that conferred these phenotypes was thought to be regulatory and was designated *Ah* (for *a*romatic *h*ydrocarbon responsiveness). Mouse strains in which 3MC induced hepatic AHH activity were considered "responsive," a dominant trait governed by the Ah^b allele. Mouse strains in which 3MC did not induce hepatic AHH activity were considered "nonresponsive," a recessive trait governed by the Ah^d allele (50). Against this background, it was notable when Poland et al. reported that TCDD induced AHH activity to equally high levels in both C57BL/6 and DBA/2 mice (137). This observation indicated that the so-called nonresponsive DBA/2 strain could, in fact, exhibit a responsive phenotype if TCDD was the inducer instead of 3MC. This finding raised the possibility that the DBA/2 strain might contain an altered regulatory protein (i.e., receptor) to which 3MC bound poorly and, therefore, failed to elicit a response. However, the properties of TCDD might be such that it could still bind tightly enough to the altered receptor to induce AHH activity. The finding that the median effective dose (ED_{50}) for AHH induction by TCDD was about 20-fold higher in DBA/2 mice than in C57BL/6 mice was consistent with the idea that the DBA/2 strain contained a receptor with a lower binding affinity for the inducer (134). Subsequently, Poland et al., using [^{3}H]TCDD and a charcoal/dextran binding assay, identified in C57BL/6 hepatic cytosol a protein which bound the dioxin saturably and with high affinity, thus providing biochemical evidence for the existence of a TCDD receptor. DBA/2 hepatic cytosol did not contain a detectable protein that bound TCDD with a similar high affinity. Furthermore, competition studies with TCDD congeners revealed that their binding affinities paralleled their induction potencies, suggesting a functional role for the receptor in the mechanism of AHH induction (136). These biochemical observations complemented the genetic evidence for the existence of a TCDD receptor. Because it is (presumably) encoded by the *Ah* locus, the TCDD receptor is also known as the Ah receptor.

The high affinity of TCDD for the TCDD receptor contributes to the high potency of the dioxin. In addition, TCDD's resistance to degradation means that the biological half-life of the compound is relatively long (9, 111, 123, 130). Thus, TCDD may produce sustained effects upon the cell, in comparison to those of other ligands for the receptor [e.g., 3MC or β-naphthoflavone (βNF)], whose biological half-lives are much shorter because the compounds are readily metabolized. It is not yet clear whether TCDD's ability to produce a prolonged biological response contributes substantially to the toxicity of the dioxin (54, 140). In addition, it is unclear why we have a receptor for TCDD at all. It is possible that the dioxin is only mimicking the binding of a "physiological" ligand to the receptor. However, the existence and properties of this hypothetical ligand (i.e., is it exogenous or endogenous; are its effects transitory or prolonged?) remain completely speculative (54, 140).

Studies of cells in culture have provided additional details about the involvement of a receptor in the response to TCDD. Hankinson exploited the observation of Gelboin et al. (45) that the PAH benzo(*a*)pyrene (BP) is toxic to cells that oxygenate the compound by means of the AHH system. Thus, he was able to select for AHH-defective cells by growth in the presence of BP (61). Miller and Whitlock took advantage of the fluorescence properties of BP and utilized the fluorescence-activated cell sorter to isolate cells that exhibit low (or no) AHH activity (107). Both groups identified two classes of receptor-defective mouse hepatoma cells. In one class, relatively few (i.e., 5 to 10% of wild-type) TCDD-receptor complexes form; however, those complexes that do form interact normally with a component(s) of the cell nucleus. These variants respond poorly to TCDD, as measured by AHH induction. In the other class, the formation of TCDD-receptor complexes appears normal. However, the complexes fail to interact normally at the nuclear level, and the variants fail to respond to TCDD at all. These results imply that AHH induction requires not only the formation of the TCDD-receptor complex but also a particular interaction between the complex and a component of the cell nucleus (97, 106). Cell fusion studies indicate that both variant phenotypes are recessive with respect to wild-type and that the variants belong to different complementation groups (62, 106). Thus, receptor function requires the contribution of (at least) two genes. The complementation analyses are open

to several interpretations. One possibility is that the TCDD receptor has distinct subunits; perhaps, one gene encodes a TCDD-binding subunit, and a second gene encodes a chromatin-binding subunit. Another possibility is that the TCDD-receptor complex requires enzymatic modification to convert it to a chromatin-binding species; in this scenario, one gene encodes the receptor, and a second gene encodes the modifying enzyme. The available data do not allow us to distinguish between these and other possibilities. Progress in receptor purification and characterization should allow the testing of these hypotheses in the future.

Okey et al. (119) have analyzed the TCDD receptor in a clone of C3H/10T½ mouse fibroblasts in which some PAHs (and TCDD) induce AHH activity, but 3MC does not. [^{3}H]3MC can bind to the receptor in cell extracts; however, it apparently is unable to do so in the intact cell. The basis for this interesting and unusual phenotype is unknown. More detailed studies of these cells (e.g., the dominant/recessive nature of the trait, structure-activity analyses of ligand binding) have the potential to reveal novel aspects of receptor structure and function in the future.

Genetic evidence for the *Ah* locus exists only in mice; phenotypes analogous to the responsive and nonresponsive mouse strains have not been observed in other species. However, other species, including humans, do contain a TCDD-binding protein(s) whose biochemical properties are similar to those of the mouse receptor (43, 68, 100). Therefore, the equivalent of the *Ah* locus presumably also exists in other species. In addition, crosses other than the prototypical C57BL/6 × DBA/2 mating imply that the mouse *Ah* regulatory system may be quite complicated. In some crosses (e.g., C3H/He × DBA/2), the induction of AHH activity by 3MC segregates as a codominant trait; this finding may indicate the existence of a third *Ah* allele (163). Furthermore, there is a single report that, in the C57BL/6N × AKR/N mating, the dominance is reversed, and the nonresponsive phenotype segregates as an autosomal dominant trait (148). This unusual observation, if confirmed, remains to be explained. Therefore, additional studies of these inbred mouse strains and their progeny seem worthwhile, in order to determine if the genetic findings are associated with differences in TCDD receptor structure or function.

The chromosomal location, organization, and structure of the *Ah* locus are unknown. Studies of somatic cell hybrids suggest that mouse chromosome 17 contains a gene that regulates AHH inducibility; however, there is no direct evidence that it encodes the receptor protein (98). Furthermore, the number of alleles at the *Ah* locus and the number of proteins encoded by the locus are unknown. Success in cloning the gene(s) for the TCDD receptor presumably will allow these issues to be addressed in the future.

III. Biochemical Properties of the TCDD Receptor

Assays of the TCDD receptor require measuring the specific binding of a radiolabelled ligand to a protein that is a minor component of a crude cell extract. The major problem is distinguishing between specific and nonspecific binding. The limited aqueous solubility of TCDD tends to increase nonspecific binding and compounds the difficulty of the assay. The potential usefulness of more hydrophilic ligands, such as 3MC or βNF, is negated by their substantially lower affinity for the receptor. Several investigators have utilized different techniques to improve upon the original assay, which employed dextran-coated charcoal to remove unbound [^{3}H]TCDD (136). Either adsorption of ligand-receptor complexes to hydroxylapatite (41, 127) or precipitation of ligand-receptor complexes with protamine sulfate (26) is a convenient, simple, and rapid method for assaying large numbers of samples. However, compared to more complicated techniques, these procedures tend to lack specificity, because they do not reveal any properties of the molecules to which TCDD is bound. In contrast, assays which involve centrifugation of TCDD-labelled material through sucrose gradients (116, 166) can verify that the TCDD-binding species has the appropriate sedimentation coefficient; however, such techniques are time-consuming, expensive, and relatively impractical for large numbers of samples. Other assays, such as isoelectric focusing in polyacrylamide gels (16) and gel permeation chromatography (42), have similar limitations. In practice, a combined approach seems reasonable. Impurities in the radiolabelled TCDD (28), contamination of the cell or tissue extract with serum proteins (129), and the presence of other PAH-binding proteins in the cell extract (18, 65, 165, 181) can introduce substantial artifacts into studies of the TCDD receptor. These factors need to be considered when interpreting the experimental data.

Several investigators (28, 43, 65, 101, 129) have compared the properties of the TCDD receptor from various animal species and/or tissues to find differences that might account for the diversity of TCDD's effects. In general, the results reveal that the hydrodynamic properties and the ligand-binding properties of the TCDD receptor are similar, but not identical, in various systems. In solution, the receptor behaves as a larger species (apparent M_r~250,000) in 0.1 M KCl and as a smaller species (apparent M_r~120,000) at 0.4 M KCl. This behavior may reflect the dissociation of an oligomeric species as the ionic strength is raised. If so, we do not yet know whether the receptor is homomeric or heteromeric. Both the faster and slower sedimenting species behave as asymmetric molecules, with axial ratios in the range of 11 to 12. Dissociation constants for TCDD fall in the range of 0.1 to 2 nM, and, in liver tissue, the number of TCDD binding sites is in the range of 30 to 60 fmol/mg

protein. Hydrodynamic differences among various TCDD receptors appear to be relatively small. For example, Denison et al. (28) found about a 10% difference between Sprague-Dawley rats and C57BL/6N mice in the relative molecular mass of the hepatic TCDD receptor. In addition, the rat receptor readily changes to the smaller, more slowly sedimenting form in 0.4 M KCl whereas the mouse receptor is relatively resistant to this salt effect. Furthermore, the rat and mouse receptors differ somewhat in their ligand-binding preferences. We do not know whether these biochemical variations are associated with meaningful differences in receptor function. Therefore, based on our present knowledge, differences in the properties of the TCDD receptor among animal species and/or tissues do not easily account for the qualitative and quantitative differences in TCDD's effects in various experimental systems.

Several investigators (18, 73, 165, 181) have characterized in rats and mice another intracellular protein(s) that binds PAHs (e.g., 3MC) with higher affinity than TCDD. The hydrodynamic and ligand-binding properties of this protein distinguish it from the TCDD receptor (18, 74). In addition, the production of the protein does not segregate with the *Ah* locus in inbred mouse strains (118). The function of this protein remains unknown; it might be involved in the regulation of the rat cytochrome P-450c gene (74). However, this remains to be demonstrated rigorously. The recent purification of the mouse protein (19) should allow the preparation of antibodies, which will be helpful in studying its structure and regulation in greater detail. If this PAH-binding protein does, in fact, influence gene expression, it will be interesting to compare its mechanism of action with that of the TCDD receptor.

Both the hydrodynamic properties of the TCDD receptor and its apparent mechanism of signal transduction are analogous to those of several steroid receptors (146, 178). These similarities have led several investigators to compare the properties of steroid and TCDD receptors in detail (178). The ligand-binding properties of the receptors are quite different; steroids do not exhibit high affinity for the TCDD receptor, and vice-versa. On the other hand, the TCDD receptor and the glucocorticoid receptor are similar with respect to their chromatographic behavior on DNA-cellulose and heparin-Sepharose (175). Studies involving limited proteolysis reveal that, like steroid receptors, the TCDD receptor has a ligand-binding domain that is distinct from a DNA-binding domain (64, 175). Molybdate stabilizes the higher molecular weight, ligand-binding form of steroid receptors; the compound has less effect on the TCDD receptor (29). The significance of this observation is not clear. Overall, the results of biochemical studies reveal some relatively crude structural similarities between the TCDD receptor and steroid receptors. In addition, both the TCDD receptor and steroid receptors transduce their respective chemical signals by mechanisms that appear similar at our relatively superficial level of knowledge (see below). These structural and functional similarities suggest that both types of receptor might belong to a family of proteins which evolved from a common ancestor. On the other hand, variant cells that contain defective TCDD receptors fall into several complementation groups, whereas, in the glucocorticoid-responsive system, the analogous receptor variants are all in the same complementation group (179). These genetic findings might reflect important structural or functional differences between the TCDD receptor and steroid receptors and could mean that the biochemical similarities between them do not reflect their evolution from a common ancestor. Purification and characterization of the TCDD receptor in the future will permit a more meaningful comparison with steroid receptors and a more rigorous evaluation of their possible evolutionary relatedness.

Several groups have characterized the ligand-binding site of the TCDD receptor using structure-activity analyses. Initial studies, involving several series of HAHs, revealed that the ligands with the highest binding affinity were essentially planar and would fit into a rectangle approximately 3 × 10 A, with halogen atoms at each corner (138, 140). However, this particular view of the binding site cannot easily account for the efficacy of ligands like 3MC or βNF, which are substantially different in structure from the HAHs. More recently, studies of a series of indoles (including βNF) suggested that viewing the binding site as a rectangle of 6.8 × 13.7 A could more easily account for all of the data (47).

Quantitative structure-activity relationship (QSAR) methods (10) have also been used to study the interactions between various ligands and the TCDD receptor. In this approach, one studies a series of structurally related ligands, whose physicochemical properties (e.g., hydrophobicity, electronegativity, hydrogen-bonding capacity, van der Waals volume) can be estimated in quantitative terms. Multiple linear regression analysis is used to determine which physicochemical property(s) correlates with the ligand's ability to produce the effect being studied (e.g., binding to the receptor). Safe and coworkers (150) found that the binding affinity of 33 chlorinated dibenzo-*p*-dioxins and dibenzofurans correlated with the hydrophobicity of the compounds (within limits imposed by the volume of the ligand). These observations imply that the ligand-binding site of the TCDD receptor is very hydrophobic (31, 32, 150). QSAR analysis of a series of halogenated biphenyls suggested that, for these ligands, hydrophobicity, electronegativity, and hydrogen bond-accepting ability all enhance ligand-receptor binding (5). One potential limitation of the QSAR approach is that the data may not be amenable to unambiguous interpretation. For example, McKinney and coworkers have interpreted the halogenated biphenyl binding data to mean that dispersive interactions are the primary forces that

stabilize the ligand-receptor complex (102, 103). A second, and perhaps more serious, limitation of the QSAR approach involves the potential artifacts associated with the study of very insoluble ligands. For example, in some cases, the dissociation constant calculated for receptor binding substantially exceeds the aqueous solubility of the ligand. This raises questions as to the biological significance of the binding data. In general, the QSAR analyses suggest that the interactions which stabilize ligand-receptor binding are primarily hydrophobic, but can vary to some extent, depending upon the properties of the ligand. The ligand-binding site appears to be a hydrophobic pocket of somewhat undefined volume. It is conceivable that the binding site is somewhat flexible; the receptor might undergo small changes in conformation so as to optimize the binding interactions for any given ligand. To put the QSAR data in some perspective, it is worth noting that a thermodynamic analysis of glucocorticoid-receptor interactions implies that the forces which stabilize the hormone-receptor complex in that system are also primarily hydrophobic (177).

In the future, the QSAR approach might provide clues about the function of the TCDD-receptor complex. For example, Denomme et al. observed that, for two series of chlorinated dibenzo-*p*-dioxins and dibenzofurans, the receptor binding affinity correlated with lipophilicity alone, whereas the ability to induce AHH activity correlated with lipophilicity plus a steric factor (31, 32). These findings suggest that the formation of a ligand-receptor complex does not by itself suffice to evoke a biological response. Denomme et al. (31, 32) infer that the ligand-receptor complex must undergo a subsequent biochemical change(s) (perhaps conformational) in order to become functional. This interpretation is consistent with other studies of the TCDD receptor, involving different experimental techniques (see below). Also, in studies of the estrogen receptor, Hanson and Gorski, using a thermodynamic analysis, have reached a similar conclusion (66). Overall, despite its limitations, the QSAR approach appears useful for studying TCDD receptor structure and function in the future, particularly if used in conjunction with other experimental approaches.

Despite its biochemical similarities to steroid receptors, the TCDD receptor has been refractory to substantial purification by techniques used successfully for steroid receptors. Several factors have contributed to the difficulty. First, the relatively low receptor concentration (of the order of 10^5 molecules/cell, assuming one TCDD-binding site per receptor) necessitates extensive purification. Second, like many proteins, the receptor tends to interact nonspecifically with other macromolecules during attempts at purification. Third, the extreme hydrophobicity of the ligand, combined with low receptor concentrations, aggravates the problem of nonspecific binding. Fourth, the noncovalent nature of ligand binding does not permit the use of denaturing procedures. To address the last factor, Poland et al. (135) have synthesized an ^{125}I-labeled, 2-azido-3-iodo-7,8-dibromodibenzo-*p*-dioxin as a photoaffinity reagent. They have used this compound to specifically covalently label in C57BL/6J mouse liver a protein that is likely to be the TCDD receptor (135). The protein migrates in denaturing polyacrylamide gels with an apparent molecular weight of about 95,000. The development of this reagent will permit a substantially greater degree of receptor purification (albeit in denatured form) than has previously been possible. In principle, this will lead to the generation of antibodies, which would be very powerful reagents for studying the structure and function of the TCDD receptor and could permit the cloning of its gene(s). Thus, studies during the next few years may produce substantial advances in our knowledge of the biochemical properties of the TCDD receptor.

IV. Function of the TCDD Receptor

Our understanding of the mechanism by which the TCDD receptor transduces a chemical signal into a cellular response is sketchy. Much of the current thinking is based on the apparent functional analogies between the TCDD receptor and steroid receptors, which have been studied more extensively. The hydrophobic ligand apparently enters the cell by passive diffusion; there is no evidence that active transport is required. The binding of TCDD to its receptor occurs inside the cell and apparently requires both ATP (58) and reduced sulfhydryl groups (30, 88). These findings may mean that the TCDD receptor undergoes cyclic phosphorylation/dephosphorylation during signal transduction and that the cell contains an enzyme system that can maintain the receptor in a reduced state. However, these hypotheses remain to be tested.

The location of the unoccupied receptor in the intact cell is open to question. In homogenates of untreated cells, the unoccupied receptor distributes primarily to the cytosolic fraction; conversely, in homogenates of TCDD-treated cells, the ligand-receptor complex distributes largely to the nuclear fraction (116, 117). One interpretation of these data is that, in the intact cell, the unoccupied receptor is in the cytoplasm and that ligand binding produces a "translocation" of the TCDD-receptor complex to the nucleus (116, 117). However, the TCDD receptor can redistribute between cytoplasm and nucleus during cell homogenization and fractionation (27, 174). Therefore, data from broken-cell experiments are difficult to interpret unambiguously. An alternative interpretation is that the unoccupied receptor is primarily nuclear and that the binding of TCDD increases the affinity of the ligand-receptor complex for a nuclear component (e.g., chromatin), thus reducing the tendency of the complex to redistribute into the cytosol during cell fractionation (174). Studies of the distribution of the TCDD receptor in cells enucleated by cytochalasin B are also difficult to interpret unambiguously because expo-

sure of cells to the antibiotic results in the loss of most TCDD-binding activity (59). Analogous studies of steroid receptors in cell homogenates are not particularly helpful either; for example, under aerobic conditions, the unoccupied estrogen receptor appears to reside in the nucleus (92, 171), whereas the unoccupied glucocorticoid receptor is apparently cytoplasmic (4). Interestingly, in ATP-depleted cells, the unoccupied glucocorticoid receptor appears to be nuclear (104). This may mean that the release of receptors from the nucleus is an energy-requiring event. Perhaps the simplest interpretation of the available data is that, in the intact cell, the unoccupied TCDD receptor is neither entirely cytoplasmic nor entirely nuclear but is in equilibrium between the two compartments.

Despite the uncertainty about the intracellular location of the unoccupied TCDD receptor, it seems clear that the biological response to TCDD requires an action of the inducer-receptor complex at the nuclear level. The most compelling evidence on this point stems from studies of receptor-defective cells. Two groups have isolated variant mouse hepatoma cells in which the TCDD-receptor complex apparently forms normally, but the complex binds weakly to a component of the nucleus (97, 106). This class of variant cells fails to transcribe the cytochrome P_1-450 gene in response to TCDD (63, 77, 78). These findings imply that, in order to evoke a response, the TCDD-receptor complex must interact with an element in the cell nucleus.

The phenotype of these variant cells implies that the binding of TCDD to its receptor is not sufficient to generate a functional inducer-receptor complex. This conclusion is consistent with the observation that, if the TCDD-receptor complex forms at 4°C (as opposed to 37°C), it fails to bind strongly to the nucleus (117, 174). Thus, the generation of a functional TCDD-receptor complex apparently requires a temperature-dependent "activation" event(s). The temperature-dependent step has the effect of increasing the affinity of the TCDD-receptor complex for nuclear binding sites, presumably on chromatin (116, 174). In addition, ligand binding enhances the affinity of the TCDD receptor for DNA-cellulose or DNA-Sepharose in vitro (40, 64). However, we know virtually nothing about the mechanism of activation. For example, the temperature dependence could reflect a conformational change in the TCDD-receptor complex or a dissociation of subunits (which could expose a chromatin-binding domain), an enzymatic modification of the complex (which could alter its affinity for a nuclear binding site), or a combination of such events. In fact, studies of estrogen and glucocorticoid receptors suggest that several steps occur during the activation of the steroid-receptor complex to its functional form (67, 153, 154, 158). More detailed biochemical analysis of the activation phenomenon (e.g., after antibodies for the TCDD receptor become available) seems to be a potentially fruitful area for future research. In addition, the isolation of variants in the activation pathway would permit genetic analyses of the event(s) involved.

The interaction of the activated TCDD-receptor complex with the nucleus can lead rapidly to a biological response. For example, the increase in cytochrome P_1-450 gene transcription is half-maximal about 15 min after exposure of mouse hepatoma cells to TCDD (78). Furthermore, the response occurs in the absence of ongoing protein synthesis (76). These findings imply that the TCDD-receptor complex can activate gene transcription directly, without a requirement for intervening biochemical events, such as the generation of "second messengers" or the induction of other proteins. Studies in XB mouse teratoma cells support this conclusion, in that no evidence for the participation of several second messengers in the response to TCDD could be demonstrated (95).

We know very little about the factors that regulate the concentration of the TCDD receptor within the cell. There is disagreement in the literature as to whether exposure to TCDD-like ligands alters the intracellular receptor concentration (33, 157). However, the experiments are inherently difficult to interpret, because the only way to measure the receptor is with a ligand-binding assay, and the hydrophobicity of the ligand makes the studies technically difficult. In the future, it will be interesting to determine if TCDD regulates the expression of the TCDD receptor gene by a feedback mechanism, as may occur in the glucocorticoid-responsive signalling system (121). Such studies await the development of antibody probes for the TCDD receptor and the cloning of the TCDD receptor gene. A priori, there is no obvious reason to think that other inducers of cytochrome P-450 enzyme activities should influence the level of the TCDD receptor within the cell. (See ref. 173 for a discussion of the cytochrome P-450 isozymes and the different types of cytochrome P-450 inducers.) Yet, several investigators have reported that compounds of the "phenobarbital type" produce a 2- to 3-fold increase in the concentration of the hepatic TCDD receptor in rats and mice (33, 120). We know neither the mechanism by which this effect occurs nor its functional significance. Other workers have reported that 2,2-dimethyl-5-*t*-butyl-1,3-benzodioxole (DBBD), which is an "isosafrole type" of cytochrome P-450 inducer, apparently produces about a 2-fold decrease in the hepatic TCDD receptor in Dub:ICR and C57BL/6 mice (22). Again, the mechanism by which this reduction occurs is unknown. Furthermore, DBBD-treated mice also exhibit decreased enzyme induction in response to 3MC (a "TCDD-type" ligand), suggesting that the decrease in the TCDD receptor is functionally significant. However, this result seems to conflict with findings in C57BL/6 × DBA/2 mice, which indicate that a 2-fold reduction in receptor concentration has no apparent effect on maximal AHH induction by

TCDD (101). Overall, it seems premature to draw firm conclusions about the regulation of the intracellular TCDD receptor concentration and the quantitative relationship between receptor concentration and a particular biological response. Development of antibody probes for the receptor will greatly facilitate the experimental analysis of these issues in the future.

V. TCDD-responsive Genomic Elements

Studies in variant cells imply that the induction of cytochrome P-450 gene transcription requires an interaction(s) between the TCDD-receptor complex and an element in the cell nucleus (78). Furthermore, the TCDD-receptor complex is a DNA-binding protein (40, 64). These observations suggested that the inducer-receptor complex might act at a "genomic switch" that is located near the start site of transcription for the cytochrome P_1-450 gene. To test this idea, several groups have utilized a strategy (87) that involves (*a*) ligating the putative genomic switch to a heterologous "indicator" gene and (*b*) testing the hybrid gene for function by transfection (fig. 2). For example, in studies of mouse hepatoma cells, Jones et al. (83) isolated DNA from the region upstream of the cytochrome P_1-450 gene, ligated it to the bacterial chloramphenicol acetyltransferase (CAT) gene, and transfected the recombinant molecules into cells that contained a normal TCDD receptor. TCDD induced CAT activity in the transfected cells, implying that the hybrid gene contained a TCDD-responsive DNA element. Furthermore, CAT induction had the expected sensitivity (ED_{50}) to TCDD, and other ligands such as 3MC and βNF also induced CAT expression. Transfection of the hybrid gene into receptor-defective variant cells resulted in loss of TCDD responsiveness, indicating that the induction of CAT activity

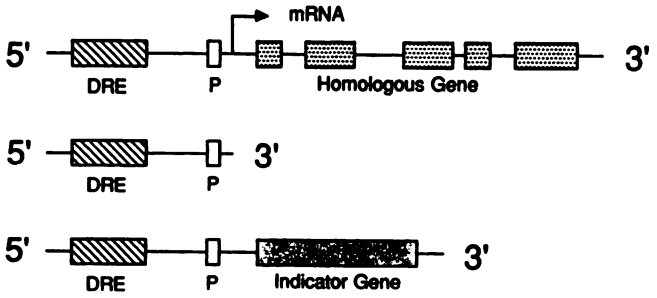

FIG. 2. Identification of dioxin-responsive elements. The *top diagram* depicts a TCDD-inducible gene (e.g., cytochrome P_1-450), containing a dioxin-responsive element (*DRE*) and a promoter (*P*), which are located upstream of the transcription start site (*arrow*). The *middle diagram* depicts the control region, which has been isolated from its homologous structural gene after cleaving the DNA with a restriction endonuclease(s). The *bottom diagram* depicts a hybrid gene, constructed by ligating the control region to a heterologous indicator gene, whose product (mRNA or protein) is convenient to assay. In the hybrid, the indicator gene (in principle) becomes responsive to TCDD. This hypothesis is tested by transfecting the hybrid gene into suitable (i.e., receptor-positive) cells, and determining if TCDD induces the product of the indicator gene. A positive result implies that the control region contains a DRE.

required a functional TCDD receptor. Together, these observations imply that the DNA that flanks the 5′-end of the cytochrome P_1-450 gene contains a domain(s) that functions as a dioxin-responsive element (DRE). Other workers have used a similar approach to find TCDD-responsive domains upstream of the cytochrome P_1-450 gene in C57BL/6 mice (49) and in the corresponding cytochrome P-450 genes in rats (39, 159) and humans (85). Thus, the current evidence suggests that TCDD acts by similar mechanisms in these different species. Furthermore, the functions of the TCDD receptor and its cognate DRE apparently have been conserved during evolution. For example, the DRE of the mouse responds to TCDD even when transfected into human cells (82), and the genomic elements of the rat (39, 159) and human (85) function when transfected into mouse cells. Thus, the TCDD receptor from one species apparently can recognize and act at a DRE from a heterologous species. These findings imply that the TCDD-responsive signalling system evolved prior to the divergence between mouse and man. Future studies in other species may substantiate this point more firmly. From an evolutionary standpoint, it is interesting that even some bacteria activate gene transcription by means of a receptor-dependent mechanism that responds to certain flavones as chemical signals (35, 125, 144). This (or a similar) system might represent the forerunner of the TCDD-responsive pathway present in eukaryotic cells.

The DNA that flanks the 5′-end of the cytochrome P_1-450 gene in mouse hepatoma cells contains other regulatory components in addition to the DRE. Jones et al. (83) used an exonuclease to produce progressively smaller DNA fragments, which were tested for function by transfection, after insertion into a CAT expression vector. These deletion analyses revealed an element that appears to function as a transcriptional promoter and confers constitutive expression upon the CAT gene. Still another functional domain is located at least 600 base pairs upstream of the promoter and acts to inhibit promoter function. Presumably, this inhibitory element interacts with a regulatory protein (i.e., a repressor), although this hypothesis remains to be tested. Furthermore, the mechanism by which inhibition occurs from such a distance is unknown; the situation is reminiscent of "silencer" elements in other systems (12, 96). The TCDD-responsive genomic domain is located upstream of the inhibitory element, at least 1500 base pairs away from the transcription start site. The ability to activate transcription from a distance is typical of "enhancer" control systems. This observation provided a clue that the TCDD-responsive element might function as a transcriptional enhancer (see below). Together, the deletion analyses indicate that the DNA which flanks the 5′-end of the cytochrome P_1-450 gene in mouse hepatoma cells contains a combination of (at least) three different genomic control elements, each of which presumably inter-

acts with specific regulatory proteins. Gonzalez and Nebert (49) have made similar observations in a C57BL/6 mouse liver system. Thus, in the case of the cytochrome P_1-450 gene, the TCDD-responsive system functions in a context that also includes inhibitory and constitutive regulatory components. This sort of combinatorial control of transcription may prove to be typical of many eukaryotic genes (14).

Transcriptional enhancers are DNA elements that bind specific proteins and thereby augment gene expression. In contrast to other types of regulatory components (e.g., promoters), enhancers function relatively independently of their distance and orientation with respect to the regulated gene (89, 156). The ability of the TCDD-responsive domain to function at a distance from the transcription start site suggested that the DRE might be an enhancer (83). To test this possibility, Jones et al. isolated the TCDD-responsive domain and inserted it into a CAT expression vector, which was designed to evaluate the enhancer properties of the insert. Analyses of the recombinants by transfection revealed that (*a*) the DRE can function independently of the inhibitory and constitutive regulatory components to which it is linked in vivo; (*b*) the DRE can activate transcription from a heterologous promoter; (*c*) the DRE functions relatively independently of its distance from the promoter; and (*d*) the DRE functions relatively independently of its orientation with respect to the promoter. These findings indicate that the DRE has properties characteristic of enhancers. Transfections into receptor-defective variant cells revealed that the DRE requires a functional TCDD receptor (82). Therefore, the DRE, together with the TCDD receptor, constitutes a dioxin-responsive enhancer system. Others have made similar observations using an analogous experimental approach (39, 115). The mechanism(s) by which enhancers activate transcription from a distance is unknown. For example, enhancers might (*a*) produce a change in chromatin structure that can be propagated and that converts the nucleoprotein to a "transcriptionally active" form, (*b*) provide a binding site for a factor(s) that then "slides" along the genome to the promoter and initiates transcription, or (*c*) produce "looping" of the genome and the formation of a stable nucleoprotein complex that is required for the activation of transcription (37, 142). The TCDD-responsive enhancer constitutes a system appropriate for testing these hypotheses in the future.

The fact that the dioxin-responsive signalling pathway can function relatively independently of the other control components (i.e., constitutive and inhibitory) to which it is linked would appear to increase the versatility of the system as a mechanism for regulating gene expression. In principle, the system could function in diverse regulatory contexts that generate different patterns of gene expression. To begin to test this concept, Jones et al. (82) inserted into a CAT expression vector both a DRE and a glucocorticoid-responsive element (GRE) in two different linear arrangements. When the DRE was positioned upstream of the GRE (i.e., the arrangement was 5′-DRE-GRE-promoter-CAT-3′), both TCDD and dexamethasone induced CAT activity independently, and CAT expression was additive in the presence of both inducers. Thus, in this context, both the dioxin-responsive system and the glucocorticoid-responsive system appear to function relatively independently of each other. In contrast, when the DRE was positioned downstream of the GRE (i.e., the arrangement was 5′-GRE-DRE-promoter-CAT-3′), TCDD by itself could induce CAT expression, but dexamethasone produced a response only if TCDD also was present. Thus, in this context, the dioxin-responsive system appears to exert a "permissive" effect on the glucocorticoid-responsive system. These findings suggest that two different inducible enhancer systems can become interdependent when linked and can exhibit altered responsiveness, depending upon the regulatory context in which they are placed. It is relatively easy to envision that, in other contexts, the response of a particular gene to TCDD may be a function not only of the TCDD-responsive system itself but also of the other control components with which it is linked. This might be a mechanism which could account for (at least some of) the species and tissue specificity that is characteristic of the biological responses to TCDD. The interaction of the *Ah* and *hr* loci (94, 141) is a possible example of how regulatory systems might act in combination to control gene expression. In receptor-positive (Ah^+) hairless (HRS/J) mice, TCDD produces epidermal hyperplasia and promotes skin papillomas only in homozygous animals (hr^-/hr^-) bearing a recessive mutation at the *hr* locus (94, 141). These observations may indicate the existence of a regulatory system that can block the response to TCDD. For example, suppose the *hr* locus encodes a regulatory protein that blocks gene expression by binding to a *cis*-acting genomic control element. Furthermore, suppose that the inhibitory (*hr*) system dominates the stimulatory (*Ah*) system when the two are linked. Then, a (hypothetical) keratinocyte gene that is under the control of both systems will not respond to TCDD unless the two *hr* alleles have been inactivated. This type of model might account for the responsiveness of hr^-/hr^- mouse skin to TCDD. The model makes predictions that are testable, in principle. However, the mechanisms by which control systems act in combination to regulate gene expression remain to be determined. Knowledge of the principles and mechanisms that govern combinatorial control of gene transcription appears fundamental to an understanding of major biological phenomena, such as differentiation or carcinogenesis. [See, for example, studies of the mouse alpha-fetoprotein gene (60)]. The TCDD-responsive system appears potentially useful for analyzing the mechanisms of combinatorial control in the future.

The number of DREs in a regulatory hierarchy may also influence the response of the linked gene to TCDD. Deletion analyses suggested the presence of multiple TCDD-responsive elements upstream of the cytochrome P_1-450 gene (83). To examine this possibility, Jones et al. performed a more detailed study of the TCDD-responsive region in mouse hepatoma cells. Their findings revealed the existence of (at least) two distinct, nonoverlapping DNA fragments, each of which functions as a TCDD-responsive element when inserted into a CAT expression vector and transfected into wild-type cells. Transfections into receptor-defective cells imply that each element requires the TCDD receptor for its function. Each element has the properties of a transcriptional enhancer, and each can function independently of the other. The combined effects of the two elements are (at least) additive (81). Sogawa et al. (159) have also reported findings that are consistent with the existence of multiple TCDD-responsive domains upstream of the cytochrome P-450c gene of the rat. The significance of these observations is unknown at present. It is possible that the association of multiple DREs with the cytochrome P-450 gene is atypical and that other TCDD-responsive genes are linked to a single DRE. A second possibility is that multiple DREs are typical of TCDD-responsive genes and are advantageous in some way. For example, linking DREs in tandem may allow the formation of additional protein-protein interactions (e.g., between adjacent TCDD-receptor complexes) that stabilize a productive transcriptional complex, thereby permitting more effective gene expression. If so, then increasing the number of linked DREs might have a synergistic effect on gene expression. In addition, the spacing between DREs (which could affect protein-protein interactions) might also influence the response of the linked gene to TCDD (see, for example, refs. 11 and 162). Experiments designed to test these ideas are feasible in principle and may reveal additional details of the mechanism of TCDD action in the future.

The activation of cytochrome P_1-450 gene expression requires both the TCDD receptor and the DRE. However, the fact that both components are required does not necessarily demand that they physically interact during the process of signal transduction. To address this issue, Durrin and Whitlock (36) utilized an assay which measures the accessibility of the DRE in situ (determined by its susceptibility to digestion by an exonuclease) as a function of exposure of the cell to TCDD. Their studies in mouse hepatoma cells revealed that (*a*) a specific DNA region upstream of the cytochrome P_1-450 gene is protected from exonuclease digestion in TCDD-induced cells, but not in uninduced cells; (*b*) protection does not occur in receptor-defective cells; (*c*) protection occurs within 1 h of exposure of the cell to TCDD; (*d*) protection occurs in the absence of ongoing protein synthesis; (*e*) the protected region is in a domain that functions as a DRE. These observations imply that both the DRE and the TCDD-receptor complex contribute to the formation of a stable nucleoprotein structure that is relatively resistant to exonuclease attack. These findings strongly imply that the TCDD-receptor complex and the DRE interact in vivo to activate the transcription of the cytochrome P_1-450 gene. Others have made similar observations in studies of the glucocorticoid-responsive system (8). The details of the protein-DNA interactions and the possible participation of other proteins in the activation of gene transcription are interesting issues requiring additional research.

The properties of the chromatin recognition site(s) for the TCDD-receptor complex remain to be determined in more detail. Sogawa et al. (159) have proposed that the inducer-receptor complex recognizes a specific "consensus" decanucleotide sequence that is present in multiple copies in the DNA that flanks the 5′-end of the rat cytochrome P-450c gene. Interestingly, they observed that a synthetic concatemer of one such decanucleotide augmented the response of a linked CAT gene to 3MC. On the other hand, it is not yet clear that this effect is dependent upon the TCDD receptor, because the construct was not tested in receptor-defective cells. Also, two copies of the putative recognition sequence are located in a DNA region that does not exhibit responsiveness to 3MC (159). Thus, the specific chromatin structure that the TCDD-receptor complex recognizes remains uncertain. While a specific DNA sequence may be a necessary constituent of the recognition site, it may not be sufficient. In other systems, the binding of a regulatory protein to a specific DNA sequence does not generate a response unless additional specific protein-protein interactions can also occur (13, 57, 71, 86). An analogous situation may also exist for the TCDD-responsive system. According to this view, the TCDD-receptor complex could bind to a specific DNA sequence; however, the binding will not produce a response unless the complex can also form additional interactions with other proteins that bind to adjacent regions of the genome. Thus, both DNA and protein would contribute to a functional recognition site for the TCDD-receptor complex.

VI. Future Prospects

The purification and characterization of TCDD receptors remain important areas of research for the future. Major advances in this area may occur during the next several years. For example, the ability to covalently label the receptor with an affinity reagent will allow the use of denaturing conditions during the isolation of the TCDD-binding protein; this will lead to a much greater degree of purification than has been possible previously. Antibodies raised against the purified protein (either in its denatured form or, possibly, after renaturation) should be useful reagents for studying the structural and functional domains of the receptor and for its isolation

using immunoaffinity techniques. Anti-receptor antibodies should permit more detailed analyses of receptor heterogeneity, receptor modification, receptor synthesis and degradation, and the temperature-dependent activation event that occurs during transduction of the TCDD signal. In addition, sequence analysis of the denatured protein should permit the synthesis of an oligonucleotide(s) that might be used to isolate the corresponding gene.

Other approaches to receptor purification may also be useful. For example, the functional similarities between TCDD receptors and steroid receptors suggest that structural similarities may also exist. Therefore, it may be possible to find antibodies, raised against purified steroid receptors, that cross-react with TCDD receptors. Such antibodies could be used in receptor purification. In addition, we may find that the TCDD-receptor complex recognizes a specific DNA sequence. If so, oligonucleotides that contain this sequence may be useful affinity reagents for the purification of the TCDD-receptor complex (see, for example, refs. 84 and 149).

Anti-receptor antibodies presumably could be used to clone the gene(s) for the TCDD receptor. An alternative approach might be to insert either genomic DNA or cDNA into an appropriate expression vector and to use the recombinant to complement the lesion in receptor-defective variant cells, with the selection procedure developed by van Gurp and Hankinson (167). Cloning and characterization of the TCDD receptor gene(s) will permit studies of its expression and lead to a better understanding of the factors which regulate the intracellular concentration of the receptor.

The TCDD receptor presumably consists of multiple functional domains, including a ligand-binding domain, a DNA (chromatin)-binding domain, and possibly, a domain(s) that interacts with other transcription factors. Cloning and expression of cDNA for the TCDD receptor, when combined with mutagenesis and gene transfer methodologies, should permit a detailed analysis of its functional domains (see, for example, refs. 46, 48, and 105). Furthermore, given the similarities between the TCDD receptor and steroid receptors described above, it will be intriguing to learn whether the TCDD receptor is a member of the hormone receptor family that is related to the viral *erb A* oncogene (51).

Variant cells have been very useful in characterizing the TCDD-responsive system to date; the study of additional variants would seem to be worthwhile in the future. For example, Hankinson and coworkers (62) have already identified by complementation analysis cells which presumably contain defects at other steps in the signal transduction pathway. In the future, the isolation of temperature-sensitive variants would allow us to analyze the reversibility of particular steps in signal transduction and to study the requirements for the maintenance of TCDD-induced changes in gene expression. Selection of cells that overproduce TCDD receptors might be useful for purifying the receptor and for cloning its gene, as well as for studying quantitative aspects of signal transduction.

A great deal remains to be learned about the mechanism by which the dioxin-responsive element, together with the TCDD-receptor complex, functions as a transcriptional enhancer. Mutagenesis and gene transfer techniques can be used to define the functional boundaries of various DREs. DNA sequence analyses should reveal whether each DRE contains a specific sequence that forms part of the recognition site for the TCDD-receptor complex. The development of an enhancer-dependent in vitro transcription system (see, for example, ref. 151) would facilitate the functional analysis of the dioxin-responsive pathway. In view of what is known about other enhancer systems (126, 155), it seems likely that the DRE will be found to interact with several other proteins, in addition to the TCDD-receptor complex. If so, the task of understanding the mechanism by which the inducer-receptor complex activates transcription will become substantially more complicated.

The chromatin structure (124, 169, 170) of TCDD-responsive genes is an interesting area for future study. For example, we know very little about the nucleoprotein organization of the DRE and other linked regulatory components (38). Are these elements associated with histones or other chromosomal [e.g., high-mobility group (HMG)] proteins? Are they organized into nucleosomes in vivo? If so, how do these structural features influence the function of the regulatory elements? If (as seems more likely) the DRE does not assume a nucleosomal structure in vivo, why not? What determines the chromatin structure of the DRE? Does the nucleoprotein structure of the element change upon its interaction with the TCDD-receptor complex? If so, is the structural alteration local or does it propagate along the chromatin fiber? What is the mechanism by which a change in structure leads to activation of gene transcription? Future studies that address these issues may generate interesting information that is relevant to transcriptional enhancement in general. In addition, studies in other systems suggest that transcriptionally active regions of chromatin may be preferentially associated with the nuclear matrix (80, 114). The role that the nuclear matrix plays in the cellular response to TCDD may also be a productive area for future research.

We know that TCDD induces the activity of UDP-glucuronyltransferase and NADPH:quinone reductase, apparently by activating the transcription of the corresponding gene (79, 147, 176). However, we do not yet know whether the activation of these other genes occurs in the absence of ongoing protein synthesis (i.e., if induction reflects a primary response to the TCDD-receptor complex). For example, others have proposed that TCDD induces a protein that secondarily activates a

battery of other genes (54, 140). In fact, some glucocorticoid-responsive genes appear to display this type of regulation (2, 7). The study of additional (i.e., non-cytochrome P-450) TCDD-responsive genes might provide evidence for a protein(s) that mediates a TCDD-induced cascade of biological responses. The isolation and characterization of such a factor would be fundamental to our understanding of the mechanism by which TCDD elicits its diverse effects. The TCDD-responsive signalling system could also diminish the rate of transcription of some genes, either directly via the TCDD-receptor complex, or indirectly, via the synthesis of an inhibitory factor. This idea is testable, in principle. Also, the study of additional TCDD-responsive genes can increase our knowledge of how the dioxin-responsive enhancer system functions in other regulatory contexts, in combination with different promoters, silencers, and enhancers. Such information could make a valuable contribution to our understanding of the principles that govern the combinatorial control of gene transcription. Appropriate TCDD-responsive cell systems are available to begin the study of these problems (1, 25, 93, 122, 145).

The results of on-going epidemiological investigations suggest that exposure to TCDD poses less of a human health risk than was once feared, although the issue remains somewhat controversial (21, 70, 72, 109, 161). Most of us probably have accumulated some TCDD in our cells (123); however, it is not clear that this constitutes any measurable risk to the well-being of the general population. However, we cannot rule out the possibility that certain individuals are relatively susceptible to the effects of TCDD, either because of a genetic predisposition (34, 94, 141) and/or because of exposure to an additional environmental chemical(s). Future studies of TCDD action at the molecular level may ultimately help to clarify this issue and to resolve the uncertainty about the risk that dioxin poses to humans.

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REFERENCES

1. Abernethy, D. J., Greenlee, W. F., Huband, J. C., and Boreiko, C. J.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) promotes the transformation of C3H/10T½ cells. Carcinogenesis (Lond.) **6:** 651–653, 1985.
2. Addison, W. R., and Kurtz, D. T.: Nucleotide sequences required for the regulation of a rat α_{2u}-globulin gene by glucocorticoids. Mol. Cell. Biol. **6:** 2334–2346, 1986.
3. Adesnik, M., and Atchison, M.: Genes for cytochrome P-450 and their regulation. CRC Crit. Rev. Biochem. **19:** 247–305, 1985.
4. Antakly, T., and Eisen, H. J.: Immunocytochemical localization of glucocorticoid receptor in target cells. Endocrinology **115:** 1984–1989, 1984.
5. Bandiera, S., Sawyer, T., Campbell, M. A., Fujita, T., and Safe, S.: Competitive binding to the cytosolic 2,3,7,8-TCDD receptor: effects of structure on the affinities of substituted halogenated biphenyls—a QSAR approach. Biochem. Pharmacol. **32:** 3803–3813, 1983.
6. Bauer, H., Schulz, K. H., and Speigelberg, U.: Occupational intoxications in manufacturing chlorophenol compounds. Arch. Gewerbepathol. Gewerbehyg. **18:** 538–555, 1961.
7. Baumann, H., and Maquat, L. E.: Localization of DNA sequences involved in dexamethasone-dependent expression of the rat α_1-acid glycoprotein gene. Mol. Cell. Biol. **6:** 2551–2561, 1986.
8. Becker, P. B., Gloss, B., Schmid, W., Strahle, U., and Schultz, G.: In vivo protein-DNA interactions in a glucocorticoid response element require the presence of the hormone. Nature (Lond.) **324:** 686–688, 1986.
9. Birnbaum, L. S.: The role of structure in the disposition of halogenated aromatic xenobiotics. Environ. Health Perspect. **61:** 11–20, 1985.
10. Blankley, C. J.: Introduction: a review of QSAR methodology. *In* Quantitative Structure-Activity Relationships of Drugs, pp. 1–21, Academic Press, New York, 1983.
11. Brady, J., Loeken, M. R., and Khoury, G.: Interaction between two transcriptional control sequences required for tumor-antigen-mediated simian virus 40 late gene expression. Proc. Natl. Acad. Sci. USA **82:** 7299–7303, 1985.
12. Brand, A. H., Breeden, L., Abraham, J., Sternglanz, R., and Nasmyth, K.: Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41:** 41–48, 1985.
13. Brent, R., and Ptashne, M.: A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell **43:** 729–736, 1985.
14. Brown, D. D.: The role of stable complexes that repress and activate eukaryotic genes. Cell **37:** 359–365, 1984.
15. Buu-Hoi, N. P., Hien, D. P., Saint-Ruf, G., and Servoin-Sidoine, J.: Canceromimetic properties of tetrachloro-2,3,7,8-dibenzo-*p*-dioxin ("dioxin"). C. R. Acad. Sci. Paris **D272:** 1447–1450, 1971.
16. Carlstedt-Duke, J., Elfstrom, G., Snochowski, M., Hogberg, B., and Gustafsson, J. A.: Detection of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) receptor in rat liver by isoelectric focusing in polyacrylamide gels. Toxicol. Lett. **2:** 365–373, 1978.
17. Carson, R.: Silent Spring, 368 pp., Houghton Mifflin Co., Boston, MA, 1962.
18. Collins, S., and Marletta, M.: Carcinogen-binding proteins. High-affinity binding sites for benzo(*a*)pyrene in mouse liver distinct from the *Ah* receptor. Mol. Pharmacol. **26:** 353–359, 1984.
19. Collins, S., and Marletta, M. A.: Purification of a benzo(*a*)yrene binding protein by affinity chromatography and photoaffinity labeling. Biochemistry **25:** 4322–4329, 1986.
20. Collins, T. F. X., Williams, C. H., and Gray, G. C.: Teratogenic studies with 2,4,5-T and 2,4-D in the hamster. Bull. Environ. Contam. Toxicol. **6:** 559–567, 1971.
21. Colton, T.: Herbicide exposure and cancer. J. Am. Med. Assoc. **256:** 1176–1178, 1986.
22. Cook, J. C., and Hodgson, E.: Cytochrome P-450 induction by 3-methylcholanthrene and its antagonism by 2,2-dimethyl-5-*t*-butyl-1,3-benzodioxole. Biochem. Pharmacol. **35:** 167–176, 1986.
23. Courtney, C. D., and Moore, J. A.: Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. **20:** 396–403, 1971.
24. Courtney, K. D., Gaylor, D. W., Hogan, M. D., Falk, H. L., Bates, R. R., and Mitchell, I.: Teratogenic evaluation of 2,4,5-T. Science (Wash. DC) **168:** 864–866, 1970.
25. Dencker, L., Hassoun, E., d'Argy, R., and Alm, G.: Fetal thymus organ culture as an in vitro model for the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and its congeners. Mol. Pharmacol. **27:** 133–140, 1985.
26. Denison, M. S., Fine, J., and Wilkinson, C. F.: Protamine sulfate precipitation: a new assay for the *Ah* receptor. Anal. Biochem. **142:** 28–36, 1984.
27. Denison, M. S., Harper, P. A., and Okey, A. B.: *Ah* receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Codistribution of unoccupied receptor with cytosolic marker enzymes during fractionation of mouse liver, rat liver, and cultured Hepa-1c1 cells. Eur. J. Biochem. **155:** 223–229, 1986.
28. Denison, M. S., Vella, L. M., and Okey, A. B.: Structure and function of the *Ah* receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Species differences in molecular properties of the receptors from mouse and rat hepatic cytosols. J. Biol. Chem. **261:** 3987–3995, 1986.
29. Denison, M. S., Vella, L. M., and Okey, A. B.: Hepatic *Ah* receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: partial stabilization by molybdate. J. Biol. Chem. **261:** 10189–10195, 1986.
30. Denison, M. S., Vella, L. M., and Okey, A. B.: Structure and function of the *Ah* receptor: sulfhydryl groups required for binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to cytosolic receptor from rodent livers. Arch. Biochem. Biophys. **252:** 388–395, 1987.
31. Denomme, M. A., Homonko, K., Fujita, T., Sawyer, T., and Safe, S.: Effects of substituents on the cytosolic receptor-binding avidities and aryl hydrocarbon hydroxylase induction potencies of 7-substituted 2,3-dichlorodibenzo-*p*-dioxins. A QSAR analysis. Mol. Pharmacol. *27:* 656–661, 1985.
32. Denomme, M. A., Homonko, K., Fujita, T., Sawyer, T., and Safe, S.: Substituted polychlorinated dibenzofuran receptor binding affinities and aryl hydrocarbon hydroxylase induction potencies—a QSAR analysis. Chem.-Biol. Interact. **57:** 175–187, 1986.
33. Denomme, M. A., Leece, B., Li, A., Towner, R., and Safe, S.: Elevation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) binding by polychlorinated biphenyls. Biochem. Pharmacol. **35:** 277–282, 1986.
34. Doss, M., Sauer, H., von Tiepermann, R., and Colombi, A. M.: Development of chronic hepatic porphyria (porphyria cutanea tarda) with inherited uroporphyrinogen decarboxylase deficiency under exposure to dioxin. Int. J. Biochem. **16:** 369–373, 1984.

35. DOWNIE, J. A., AND JOHNSTON, A. W. B.: Nodulation of legumes by Rhizobium: the recognized root? Cell **47:** 153–154, 1986.
36. DURRIN, L. K., AND WHITLOCK, J. P., JR.: In situ protein—DNA interactions at a dioxin-responsive enhancer associated with the cytochrome P_1-450 gene. Mol. Cell. Biol. in press, 1980.
37. ECHOLS, H.: Multiple DNA-protein interactions governing high-precision DNA transactions. Science (Wash. DC) **233:** 1050–1056, 1986.
38. ENICK, L., FAGAN, J., AND BUSTIN, M.: Chromatin structure of the cytochrome P450c gene changes following induction. Biochemistry **25:** 7062–7068, 1986.
39. FUJISAWA-SEHARA, A., SOGAWA, K., NISHI, C., AND FUJII-KURIYAMA, Y.: Regulatory DNA elements localized remotely upstream from the drug-metabolizing cytochrome P-450c gene. Nucl. Acids Res. **14:** 1465–1477, 1986.
40. GASIEWICZ, T. A., AND BAUMAN, P. A.: Heterogeneity of the rat hepatic *Ah* receptor and evidence for transformation in vitro and in vivo. J. Biol. Chem. **262:** 2116–2120, 1987.
41. GASIEWICZ, T. A., AND NEAL, R. A.: The examination and quantitation of tissue cytosolic receptors for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using hydroxylapatite. Anal. Biochem. **124:** 1–11, 1982.
42. GASIEWICZ, T. A., AND RUCCI, G.: Examination and rapid analysis of hepatic cytosolic receptors for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using gel-permeation high performance liquid chromatography. Biochim. Biophys. Acta **798:** 37–45, 1984.
43. GASIEWICZ, T. A., AND RUCCI, G.: Cytosolic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Evidence for a homologous nature among various mammalian species. Mol. Pharmacol. **26:** 90–98, 1984.
44. GELBOIN, H. V.: Benzo(*a*)pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. Physiol. Rev. **60:** 1107–1166, 1980.
45. GELBOIN, H. V., HUBERMAN, E., AND SACHS, L.: Enzymatic hydroxylation of benzopyrene and its relationship to cytotoxicity. Proc. Natl. Acad. Sci. USA **64:** 1188–1194, 1976.
46. GIGUERE, V., HOLLENBERG, S. M., ROSENFELD, M. G., AND EVANS, R. M.: Functional domains of the human glucocorticoid receptor. Cell **46:** 645–652, 1986.
47. GILLNER, M., BERGMAN, J., CAMBILLAN, C., FERNSTROM, B., AND GUSTAFSSON, J. A.: Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. Mol. Pharmacol. **28:** 357–363, 1985.
48. GODOWSKI, P. J., RUSCONI, S., MIESFELD, R., AND YAMAMOTO, K. R.: Glucocorticoid receptor mutants that are constitutive activators of transcriptional enhancement. Nature (Lond.) **325:** 365–368, 1987.
49. GONZALEZ, F. J., AND NEBERT, D. W.: Autoregulation plus upstream positive and negative control regions associated with transcriptional activation of the mouse P_1-450 gene. Nucl. Acids Res. **13:** 7269–7288, 1985.
50. GREEN, M. C.: Guidelines for nomenclature of genetically determined biochemical variants in the house mouse, *Mus musculus*. Biochem. Genet. **9:** 369–374, 1973.
51. GREEN, S., AND CHAMBON, P.: A superfamily of potentially oncogenic hormone receptors. Nature (Lond.) **324:** 615–617, 1986.
52. GREENLEE, W. F., DOLD, K. M., IRONS, R. D., AND OSBORNE, R.: Evidence for direct action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on thymic epithelium. Toxicol. Appl. Pharmacol. **79:** 112–120, 1985.
53. GREENLEE, W. F., DOLD, K. M., AND OSBORNE, R.: Actions of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on human epidermal keratinocytes in culture. In Vitro Cell. Dev. Biol. **21:** 509–512, 1985.
54. GREENLEE, W. F., AND NEAL, R. A.: The *Ah* receptor: a biochemical and biological perspective. *In* The Receptors, ed. by M. Conn, Vol. 2, pp. 89–129, Academic Press, New York, 1985.
55. GRIEG, J. B.: Effect of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin on drug metabolism in the rat. Biochem. Pharmacol. **21:** 3196–3198, 1972.
56. GRIEG, J. B., AND DEMATTEIS, F.: Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on drug metabolism and hepatic microsomes of rats and mice. Environ. Health Perspect. (no. 5): 211–219, 1973.
57. GRIFFITH, J., HOCHSCHILD, A., AND PTASHNE, M.: DNA loops induced by cooperative binding of λ repressor. Nature (Lond.) **322:** 750–752, 1986.
58. GUDAS, J. M., AND HANKINSON, O.: Reversible inactivation of the *Ah* receptor associated with changes in intracellular ATP levels. J. Cell. Physiol. **128:** 449–456, 1986.
59. GUDAS, J. M., KARENLAMPI, S. O., AND HANKINSON, O.: Intracellular location of the *Ah* receptor. J. Cell. Physiol. **128:** 441–448, 1986.
60. HAMMER, R. E., KRUMLAUF, R., CAMPER, S. A., BRINSTER, R. L., AND TILGHMAN, S. M.: Diversity of alpha-fetoprotein gene expression in mice is generated by a combination of separate enhancer elements. Science (Wash. DC) **235:** 53–58, 1987.
61. HANKINSON, O.: Single-step selection of clones of a mouse hepatoma line deficient in aryl hydrocarbon hydroxylase. Proc. Natl. Acad. Sci. USA **76:** 373–376, 1979.
62. HANKINSON, O.: Dominant and recessive aryl hydrocarbon hydroxylase-deficient mutants of the mouse hepatoma line, Hepa-1, and assignment of the recessive mutants to three complementation groups. Somatic Cell Genet. **9:** 497–514, 1983.
63. HANKINSON, O., ANDERSON, R. D., BIRREN, B. W., SANDER, F., NEGISHI, M., AND NEBERT, D. W.: Mutations affecting the regulation of transcription of the cytochrome P_1-450 gene in the mouse Hepa-1 cell line. J. Biol. Chem. **260:** 1790–1795, 1985.
64. HANNAH, R. R., LUND, J., POELLINGER, L., GILLNER, M., AND GUSTAFSSON, J. A.: Characterization of the DNA-binding properties of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Eur. J. Biochem. **156:** 237–242, 1986.
65. HANNAH, R. R., NEBERT, D. W., AND EISEN, H. J.: Regulatory gene product of the *Ah* complex: comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3-methylcholanthrene binding to several moieties in mouse liver cytosol. J. Biol. Chem. **256:** 4584–4590, 1981.
66. HANSEN, J. C., AND GORSKI, J.: Conformational and electrostatic properties of unoccupied and liganded estrogen receptors determined by aqueous two-phase partitioning. Biochemistry **24:** 6078–6085, 1985.
67. HANSEN, J. C., AND GORSKI, J.: Conformational transitions of the estrogen receptor monomer. Effects of estrogens, antiestrogens, and temperature. J. Biol. Chem. **261:** 13990–13996, 1986.
68. HARPER, P. A., GOLAS, C. L., AND OKEY, A. B.: Characterization of the Ah receptor and aryl hydrocarbon hydroxylase induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benz(*a*)anthracene in the human A431 squamous cell carcinoma line. Submitted for publication.
69. HIGGINBOTHAM, G. R., HUANG, A., FIRESTONE, D., VERRETT, J., RESS, J., AND CAMPBELL, A. D.: Chemical and toxicological evaluations of isolated and synthetic chloro derivatives of dibenzo-*p*-dioxin. Nature (Lond.) **220:** 702–703, 1968.
70. HOAR, S. K., BLAIR, A., HOLMES, F. F., BOYSEN, C. D., ROBEL, F. J., HOOVER, R., AND FRAUMENI, J. F., JR.: Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. J. Am. Med. Assoc. **256:** 1141–1147, 1986.
71. HOCHSCHILD, A., AND PTASHNE, M.: Cooperative binding of λ-repressors to sites separated by integral turns of the DNA helix. Cell **44:** 681–687, 1986.
72. HOFFMAN, R. E., STEHR-GREEN, P. A., WEBB, K. B., EVANS, R. G., KNUTSEN, A. P., SCHRAMM, W. F., STAAKE, J. L., GIBSON, B. B., AND STEINBERG, K. K.: Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J. Am. Med. Assoc. **255:** 2031–2038, 1986.
73. HOLDER, G. M., TIERNEY, B., AND BRESNICK, E.: Nuclear uptake and subsequent nuclear metabolism of benzo(*a*)pyrene complexed to cytosolic proteins. Cancer Res. **41:** 4408–4414, 1981.
74. HOUSER, W. H., HINES, R. N., AND BRESNICK, E.: Implication of the "4S" polycyclic aromatic hydrocarbon binding protein in the transregulation of rat cytochrome P-450c expression. Biochemistry **24:** 7839–7845, 1985.
75. HUDSON, L. G., TOSCANO, W. A., JR., AND GREENLEE, W. F.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) modulates epidermal growth factor (EGF) binding to basal cells from a human keratinocyte cell line. Toxicol. Appl. Pharmacol. **82:** 481–492, 1986.
76. ISRAEL, D. I., ESTOLANO, M. G., GALEAZZI, D. R., AND WHITLOCK, J. P., JR.: Superinduction of cytochrome P_1-450 gene transcription by inhibition of protein synthesis in wild type and variant mouse hepatoma cells. J. Biol. Chem. **260:** 5648–5653, 1985.
77. ISRAEL, D. I., AND WHITLOCK, J. P., JR.: Induction of mRNA specific for cytochrome P_1-450 in wild type and variant mouse hepatoma cells. J. Biol. Chem. **258:** 10390–10394, 1983.
78. ISRAEL, D. I., AND WHITLOCK, J. P., JR.: Regulation of cytochrome P_1-450 gene transcription by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in wild type and variant mouse hepatoma cells. J. Biol. Chem. **259:** 5400–5402, 1984.
79. IYANAI, T., HANIU, M., SOGAWA, K., FUJII-KURIYAMA, Y., WATANABE, S., SHIVELY, J. E., AND ANAN, K. F.: Cloning and characterization of cDNA encoding 3-methylcholanthrene inducible rat mRNA for UDP-glucuronosyltransferase. J. Biol. Chem. **261:** 15607–15614, 1986.
80. JACKSON, D. A., AND COOK, P. R.: Transcription occurs at a nucleoskeleton. EMBO (Eur. Mol. Biol. Organ.) J. **4:** 919–925, 1985.
81. JONES, P. B. C., DURRIN, L. K., FISHER, J. M., AND WHITLOCK, J. P., JR.: Control of gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: multiple dioxin-responsive domains 5′-ward of the cytochrome P_1-450 gene. J. Biol. Chem. **261:** 6647–6650, 1986.
82. JONES, P. B. C., DURRIN, L. K., GALEAZZI, D. R., AND WHITLOCK, J. P., JR.: Control of cytochrome P_1-450 gene expression: analysis of a dioxin-responsive enhancer system. Proc. Natl. Acad. Sci. USA **83:** 2802–2806, 1986.
83. JONES, P. B. C., GALEAZZI, D. R., FISHER, J. M., AND WHITLOCK, J. P., JR.: Control of cytochrome P_1-450 gene expression by dioxin. Science (Wash. DC) **227:** 1499–1502, 1985.
84. KADONAGA, J. T., AND TJIAN, R.: Affinity purification of sequence-specific DNA binding proteins. Proc. Natl. Acad. Sci. USA **83:** 5889–5893, 1986.
85. KAWAJIRI, K., WATANABE, J., GOTOH, O., TAGASHIRA, Y., SOGAWA, Z., AND FUJII-KURIYAMA, Y.: Structure and drug inducibility of the human cytochrome P-450c gene. Eur. J. Biochem. **159:** 219–225, 1986.
86. KEEGAN, L., GILL, G., AND PTASHNE, M.: Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein. Science (Wash. DC) **231:** 699–704, 1986.
87. KELLY, J. H., AND DARLINGTON, G. J.: Hybrid genes: molecular approaches to tissue-specific gene regulation. Annu. Rev. Genet. **19:** 273–296, 1985.
88. KESTER, J. E., AND GASIEWICZ, T. A.: Characterization of the in vitro stability of the rat hepatic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Arch. Biochem. Biophys. **252:** 606–625, 1987.

89. KHOURY, G., AND GRUSS, P.: Enhancer elements. Cell 33: 313–314, 1983.
90. KIMMIG, J., AND SCHULZ, K. H.: Chlorinated aromatic cyclic ethers as a cause of the so-called chloracne. Naturwissenschaften 44: 337–338, 1957.
91. KIMMIG, J., AND SCHULZ, K. H.: Occupational acne (so-called chloracne) due to chlorinated aromatic cyclic ethers. Dermatologia 115: 540–546, 1957.
92. KING, W. S., AND GREENE, G. L.: Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature (Lond.) 307: 745–747, 1984.
93. KNUTSON, J. C., AND POLAND, A.: Keratinization of mouse teratoma cell line XB produced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: an in vitro model of toxicity. Cell 22: 27–36, 1980.
94. KNUTSON, J. C., AND POLAND, A.: Response of murine epidermis to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: interaction of the *Ah* and *hr* loci. Cell 30: 225–234, 1982.
95. KNUTSON, J. C., AND POLAND, A.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: examination of biochemical effects involved in the proliferation and differentiation of XB cells. J. Cell. Physiol. 121: 143–151, 1984.
96. LAIMINS, L., HOLMGREN-KONIG, M., AND KHOURY, G.: Transcriptional "silencer" element in rat repetitive sequences associated with the rat insulin 1 gene locus. Proc. Natl. Acad. Sci. USA 83: 3151–3155, 1986.
97. LEGRAVEREND, C., HANNAH, R. R., EISEN, H. J., OWENS, I. S., NEBERT, D. W., AND HANKINSON, O.: Regulatory gene product of the *Ah* locus. Characterization of receptor mutants among mouse hepatoma clones. J. Biol. Chem. 257: 6402–6407, 1982.
98. LEGRAVEREND, C., KARENLAMPI, S. O., BIGELOW, S. W., LALLEY, P. A., KOZAK, C. A., WOMACK, J. E., AND NEBERT, D. W.: Aryl hydrocarbon hydroxylase induction by benzo(*a*)anthracene: regulatory gene localized to the distal portion of mouse chromosome 17. Genetics 107: 447–461, 1984.
99. LU, A. Y. H., AND WEST, S. B.: Multiplicity of mammalian microsomal cytochromes P-450. Pharmacol. Rev. 31: 277–295, 1979.
100. MANCHESTER, D. K., GORDON, S. K., GOLAS, C. L., ROBERTS, E. A., AND OKEY, A. B.: *Ah* receptor in human placenta: stabilization by molybdate and characterization of binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 3-methylcholanthrene, and benzo(*a*)pyrene. Cancer Res. in press, 1987.
101. MASON, M. E., AND OKEY, A. B.: Cytosolic and nuclear binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to the *Ah* receptor in extra-hepatic tissues of rats and mice. Eur. J. Biochem. 123: 209–215, 1982.
102. MCKINNEY, J. D., DARDEN, T., LYERLY, M. A., AND PEDERSEN, L. G.: PCB and related compound binding to the *Ah* receptor(s). Theoretical model based on molecular parameters and molecular mechanics. Quant. Struct.-Act. Relat. 4: 166–172, 1985.
103. MCKINNEY, J. D., LONG, G. A., AND PEDERSON, L.: PCB and dioxin binding to cytosol receptors: a theoretical model based on molecular parameters. Quant. Struct.-Act. Relat. 3: 99–105, 1984.
104. MENDEL, D. B., BODWELL, J. E., AND MUNCK, A.: Glucocorticoid receptors lacking hormone-binding activity are bound in nuclei of ATP-depleted cells. Nature (Lond.) 324: 478–480, 1986.
105. MIESFELD, R., RUSCONI, S., GODOWSKI, P. J., MALER, B. A., OKRET, S., WIKSTROM, A. C., GUSTAFSSON, J. A., AND YAMAMOTO, K. R.: Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA. Cell 46: 389–399, 1986.
106. MILLER, A. G., ISRAEL, D. I., AND WHITLOCK, J. P., JR.: Biochemical and genetic analysis of variant mouse hepatoma cells defective in the induction of benzo(*a*)pyrene-metabolizing enzyme activity. J. Biol. Chem. 258: 3523–3527, 1983.
107. MILLER, A. G., AND WHITLOCK, J. P., JR.: Novel variants in benzo(*a*)pyrene metabolism. Isolation by fluorescence-activated cell sorting. J. Biol. Chem. 256: 2433–2437, 1981.
108. MILNES, M. H.: Formation of 2,3,7,8-tetrachlorodibenzodioxin by thermal decomposition of sodium 2,4,5-trichlorophenate. Nature (Lond.) 232: 395–396, 1971.
109. MOCARELLI, P., MAROCCHI, A., BRAMBILLA, P., GERTHOUX, P., YOUNG, D. S., AND MANTEL, N.: Clinical laboratory manifestations of exposure to dioxin in children: a six-year study of the effects of an environmental disaster near Seveso, Italy. J. Am. Med. Assoc. 256: 2687–2695, 1986.
110. MOSES, M., LILIS, R., CROW, K. D., THORNTON, J., FISCHBEIN, A., ANDERSON, H. A., AND SELIKOFF, I. J.: Health status of workers with past exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the manufacture of 2,4,5-trichlorophenoxyacetic acid: comparison of findings with and without chloracne. Am. J. Indust. Med. 5: 161–182, 1984.
111. NEAL, R. A., OLSON, J. R., GASIEWICZ, T. A., AND GEIGER, L. E.: The toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian systems. Drug Metab. Rev. 13: 355–385, 1982.
112. NEBERT, D. W., AND GELBOIN, H. V.: Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. Assay and properties of induced enzyme. J. Biol. Chem. 243: 6242–6249, 1968.
113. NEBERT, D. W., GOUJAN, F. M., AND GIELEN, J. E.: Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: simple autosomal dominant trait in the mouse. Nature New Biol. 236: 107–110, 1972.
114. NELSON, W. G., PIENTA, K. J., BARRACK, E. R., AND COFFEY, D. S.: The role of the nuclear matrix in the organization and function of DNA. Annu. Rev. Biophys. Biophys. Chem. 15: 457–475, 1986.
115. NEUHOLD, L. A., GONZALEZ, F. J., JAISWAL, A. K., AND NEBERT, D. W.: Dioxin-inducible enhancer region upstream from the mouse P_1-450 gene and interaction with a heterologous promoter. DNA (NY) 5: 403–411, 1986.
116. OKEY, A. B., BONDY, G. P., MASON, M. E., KAHL, G. S., EISEN, H. J., GUENTHNER, T. M., AND NEBERT, D. W.: Regulatory gene product of the *Ah* locus. Characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. J. Biol. Chem. 254: 11636–11648, 1979.
117. OKEY, A. B., BONDY, G. P., MASON, M. E., NEBERT, D. W., FORSTER-GIBSON, C. J., MUNCAN, J., AND DUFRESNE, M. J.: Temperature-dependent cytosol-to-nucleus translocation of the *Ah* receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in continuous cell culture lines. J. Biol. Chem. 255: 11415–11422, 1980.
118. OKEY, A. B., DUBÉ, A. W., AND VELLA, L. M.: Binding of benzo(*a*)pyrene and dibenz(*a,h*)anthracene to the *Ah* receptor in mouse and rat hepatic cytosols. Cancer Res. 44: 1426–1432, 1984.
119. OKEY, A. B., MASON, M. E., GEHLY, E. B., HEIDELBERGER, C., MUNCAN, J., AND DUFRESNE, M. J.: Defective binding of 3-methylcholanthrene to the *Ah* receptor within C3H/10T½ clone 8 mouse fibroblasts in culture. Eur. J. Biochem. 132: 219–227, 1983.
120. OKEY, A. B., AND VELLA, L. M.: Elevated binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3-methylcholanthrene to the *Ah* receptor in hepatic cytosols from phenobarbital-treated rats and mice. Biochem. Pharmacol. 33: 531–538, 1984.
121. OKRET, S., POELLINGER, L., DONG, Y., AND GUSTAFSSON, J. A.: Down-regulation of glucocorticoid receptor in RNA by glucocorticoid hormones and recognition by the receptor of a specific binding sequence within a receptor cDNA clone. Proc. Natl. Acad. Sci. USA 83: 5899–5903, 1986.
122. OSBORNE, R., AND GREENLEE, W. F.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) enhances terminal differentiation of cultured human epidermal cells. Toxicol. Appl. Pharmacol. 77: 434–443, 1985.
123. PATTERSON, D. G., HOFFMAN, R. E., NEEDHAM, L. L., ROBERTS, D. W., BAGBY, J. R., PIRCKLE, J. L., FALK, H., SAMPSON, E. J., AND HOUK, V. N.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin levels in adipose tissue of exposed and control persons in Missouri: an interim report. J. Am. Med. Assoc. 256: 2683–2686, 1986.
124. PEDERSON, D. S., THOMA, F., AND SIMPSON, R. T.: Core particle, fiber, and transcriptionally-active chromatin structure. Annu. Rev. Cell Biol. 2: 117–147, 1986.
125. PETERS, N. K., FROST, J. W., AND LONG, S. R.: A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation. Science (Wash. DC) 233: 977–980, 1986.
126. PETERSON, C. L., ORTH, K., AND CALAME, K. L.: Binding in vitro of multiple cellular proteins to immunoglobulin heavy-chain enhancer DNA. Mol. Cell. Biol. 6: 4168–4178, 1986.
127. POELLINGER, L., LUND, J., DAHLBERG, E., AND GUSTAFSSON, J. A.: A hydroxylapatite microassay for receptor binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3-methylcholanthrene in various target tissues. Anal. Biochem. 144: 371–384, 1985.
128. POELLINGER, L., LUND, J., GILLNER, M., AND GUSTAFSSON, J. A.: The receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: similarities and dissimilarities with steroid hormone receptors. *In* Molecular Mechanism of Steroid Hormone Action, ed. by V. K. Moudgil, pp. 755–789, Walter de Gruyter and Co., Berlin, 1985.
129. POELLINGER, L., LUND, J., GILLNER, M., HANSSON, L. A., AND GUSTAFSSON, J. A.: Physicochemical characterization of specific and nonspecific polyaromatic hydrocarbon binders in rat and mouse liver cytosol. J. Biol. Chem. 258: 13535–13542, 1983.
130. POIGER, H., AND SCHLATTER, C.: Pharmacokinetics of 2,3,7,8-TCDD in man. Chemosphere 15: 1489–1494, 1986.
131. POLAND, A., AND GLOVER, E.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: a potent inducer of δ-aminolevulinic acid synthetase. Science (Wash. DC) 179: 476–477, 1973.
132. POLAND, A., AND GLOVER, E.: Chlorinated dibenzo-*p*-dioxins: potent inducers of δ-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase. Mol. Pharmacol. 9: 736–747, 1973.
133. POLAND, A., AND GLOVER, E.: Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. Mol. Pharmacol. 10: 349–359, 1974.
134. POLAND, A., AND GLOVER, E.: Genetic expression of aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: evidence for a receptor mutation in genetically non-responsive mice. Mol. Pharmacol. 11: 389–398, 1975.
135. POLAND, A., GLOVER, E., EBETINO, F. H., AND KENDE, A. S.: Photoaffinity labelling of the *Ah* receptor. J. Biol. Chem. 261: 6352–6365, 1986.
136. POLAND, A., GLOVER, E., AND KENDE, A. S.: Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol. J. Biol. Chem. 251: 4936–4946, 1976.
137. POLAND, A. P., GLOVER, E., ROBINSON, J. R., AND NEBERT, D. W.: Genetic expression of aryl hydrocarbon hydroxylase activity. Induction of monooxygenase activities and cytochrome P_1-450 formation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mice genetically "nonresponsive" to other aromatic hydrocarbons. J. Biol. Chem. 249: 5599–5606, 1974.
138. POLAND, A., GREENLEE, W. F., AND KENDE, A. S.: Studies on the mechanism of action of the chlorinated dibenzo-*p*-dioxins and related compounds. Ann. NY Acad. Sci. 320: 214–230, 1979.

139. POLAND, A., AND KIMBROUGH, R. D. (EDS.): Biological Mechanisms of Dioxin Action, 500 pp., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984.
140. POLAND, A., AND KNUTSON, J. C.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. **22:** 517–554, 1982.
141. POLAND, A., PALEN, D., AND GLOVER, E.: Tumor promotion by TCDD in skin of HRS/J hairless mice. Nature (Lond.) **300:** 271–273, 1982.
142. PTASHNE, M.: Gene regulation by proteins acting nearby and at a distance. Nature (Lond.) **322:** 697–701, 1986.
143. RAPPE, C.: Chemical background of the phenoxy acids and dioxins. Ecol. Bull. **27:** 28–30, 1978.
144. REDMOND, J. W., BATLEY, M., DJORDJEVIC, M. A., INNES, R. W., KUEMPEL, P. L., AND ROLFE, B. G.: Flavones induce expression of nodulation genes in *Rhizobium*. Nature (Lond.) **323:** 632–635, 1986.
145. RICE, R. H., AND CLINE, P. R.: Opposing effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hydrocortisone on growth and differentiation of cultured malignant human keratinocytes. Carcinogenesis (Lond.) **5:** 367–371, 1984.
146. RINGOLD, G. M.: Steroid hormone regulation of gene expression. Annu. Rev. Pharmacol. Toxicol. **25:** 529–566, 1985.
147. ROBERTSON, J. A., CHEN, H. C., AND NEBERT, D. W.: NAD(P)H:menadione oxidoreductase: novel purification of enzyme, cDNA and complete amino acid sequence, and gene regulation. J. Biol. Chem. **261:** 15794–15799, 1986.
148. ROBINSON, J. R., CONSODINE, N., AND NEBERT, D. W.: Genetic expression of aryl hydrocarbon hydroxylase induction. Evidence for the involvement of other genetic loci. J. Biol. Chem. **249:** 5851–5859, 1974.
149. ROSENFELD, P. J., AND KELLY, T. J.: Purification of nuclear factor I by DNA recognition site affinity chromatography. J. Biol. Chem. **261:** 1398–1408, 1986.
150. SAFE, S. H.: Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Annu. Rev. Pharmacol. Toxicol. **26:** 371–399, 1986.
151. SASSONE-CORSI, P., DOUGHERTY, J. P., WASYLYK, B., AND CHAMBON, P.: Stimulation of in vitro transcription from heterologous promoters by the SV40 enhancer. Proc. Natl. Acad. Sci. USA **81:** 308–312, 1984.
152. SCHMID, F. A., ELMER, I., AND TARNOWSKI, G. S.: Genetic determination of differential inflammatory reactivity and subcutaneous tumor susceptibility of AKR/J and C57BL/6J mice to 7,12-dimethylbenz(*a*)anthracene. Cancer Res. **29:** 1585–1589, 1969.
153. SCHMIDT, T. J., DIEHL, E. E., DAVIDSON, C. J., PUK, M. J., WEBB, M. L., AND LITWACK, G.: Effects of pancreatic ribonuclease A, S protein, and S peptide on activation of purified rat hepatic glucocorticoid-receptor complexes. Biochemistry **25:** 5955–5961, 1986.
154. SCHMIDT, T. J., MILLER-DIENER, A., WEBB, M. L., AND LITWACK, G.: Thermal activation of the purified rat hepatic glucocorticoid receptor. Evidence for a two-step mechanism. J. Biol. Chem. **260:** 16255–16262, 1985.
155. SEN, R., AND BALTIMORE, D.: Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell **46:** 705–716, 1986.
156. SERFLING, E., JASIU, M., AND SCHAFFNER, W.: Enhancers and eukaryotic gene transcription. Trends Genet. **1:** 224–230, 1985.
157. SLOOP, T. C., AND LUCIER, G. W.: Dose-dependent elevation of *Ah* receptor binding by TCDD in rat liver. Toxicol. Appl. Pharmacol., in press, 1987.
158. SMITH, A. C., ELSASSER, M. S., AND HARMON, J. M.: Analysis of glucocorticoid receptor activation by high resolution two-dimensional electrophoresis of affinity-labeled receptor. J. Biol. Chem. **261:** 13285–13292, 1986.
159. SOGAWA, K., FUJISAWA-SEHARA, A., YAMANE, M., AND FUJII-KURIYAMA, Y.: Location of regulatory elements responsible for drug induction in the rat cytochrome P-450c gene. Proc. Natl. Acad. Sci. USA **83:** 8044–8048, 1986.
160. SPARSCHU, G. L., DUNN, F. L., AND ROWE, V. K.: Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. Food Cosmet. Toxicol. **9:** 405–412, 1971.
161. SUSKIND, R. R., AND HERTZBERG, V. S.: Human health effects of 2,4,5-T and its toxic contaminants. J. Am. Med. Assoc. **251:** 2372–2380, 1984.
162. TAKAHASHI, K., VIGNERON, M., MATTHES, H., WILDEMAN, A., ZENKE, M., AND CHAMBON, P.: Requirement of stereospecific alignments for initiation from the simian virus 40 early promoter. Nature (Lond.) **319:** 121–126, 1986.
163. THOMAS, P. E., AND HUTTON, J. J.: Genetics of aryl hydrocarbon hydroxylase induction: additive inheritance in crosses between C3H/HeJ and DBA/2J. Biochem. Genet. **8:** 249–257, 1973.
164. THOMAS, P. E., KOURI, R. E., AND HUTTON, J. J.: The genetics of aryl hydrocarbon hydroxylase induction in mice: a single gene difference between C57BL/6J and DBA/2J. Biochem. Genet. **6:** 157–168, 1972.
165. TIERNEY, B., WEAVER, D., HEINTZ, N. H., SCHAFFER, W. I., AND BRESNICK, E.: The identity and nuclear uptake of a cytosolic binding protein for 3-methylcholanthrene. Biochim. Biophys. Acta **200:** 513–523, 1980.
166. TSUI, H. W., AND OKEY, A. B.: Rapid vertical tube rotor gradient assay for binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to the *Ah* receptor. Can J Physiol. Pharmacol. **59:** 927–931, 1981.
167. VAN GURP, J. R., AND HANKINSON, O.: Isolation and characterization of revertants from four different classes of aryl hydrocarbon hydroxylase-deficient Hepa-1 mutants. Mol. Cell. Biol. **4:** 1597–1604, 1984.
168. WATERMAN, M. R., AND SIMPSON, E. R.: Regulation of the synthesis of cytochromes P-450 involved in steroid hormone synthesis. Mol. Cell. Endocrinol. **39:** 81–89, 1985.
169. WEINTRAUB, H.: Assembly and propagation of repressed and derepressed chromosomal states. Cell **42:** 705–711, 1985.
170. WEISBROD, S.: Active chromatin. Nature (Lond.) **297:** 289–295, 1982.
171. WELSHONS, W. B., LIEBERMAN, M. B., AND GORSKI, J.: Nuclear localization of unoccupied oestrogen receptors. Nature (Lond.) **307:** 747–749, 1984.
172. WHITESIDE, T.: Defoliation. The New Yorker **45:** 32–69, 1970 (Feb. 7).
173. WHITLOCK, J. P., JR.: The regulation of cytochrome P-450 gene expression. Annu. Rev. Pharmacol. Toxicol. **26:** 333–369, 1986.
174. WHITLOCK, J. P., JR., AND GALEAZZI, D. R.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin receptors in wild type and variant mouse hepatoma cells. Nuclear location and strength of nuclear binding. J. Biol. Chem. **259:** 980–985, 1984.
175. WILHELMSSON, A., WIKSTROM, A. C., AND POELLINGER, L.: Polyanionic-binding properties of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: a comparison with the glucocorticoid receptor. J. Biol. Chem. **261:** 13456–13463, 1986.
176. WILLIAMS, J. B., LU, A. Y. N., CAMERON, R. G., AND PICKETT, C. B.: Rat liver NAD(P)H:quinone reductase: construction of a quinone reductase cDNA clone and regulation of quinone reductase mRNA by 3-methylcholanthrene and in persistent hepatocyte nodules induced by chemical carcinogens. J. Biol. Chem. **261:** 5524–5528, 1986.
177. WOLFF, M. E., BAXTER, J. D., KOLLMAN, P. A., LEE, D. L., KUNTZ, K. D., BLOOM, E., MATULICH, D. T., AND MORRIS, J.: Nature of steroid-glucocorticoid receptor interactions: thermodynamic analysis of the binding reaction. Biochemistry **17:** 3201–3208, 1978.
178. YAMAMOTO, K. R.: Steroid receptor regulated transcription of specific genes and gene networks. Annu. Rev. Genet. **19:** 209–252, 1985.
179. YAMAMOTO, K. R., GEHRING, U., STAMPFER, M. R., AND SIBLEY, C. H.: Genetic approaches to steroid hormone action. Recent Prog. Horm. Res. **32:** 3–32, 1976.
180. ZACK, J. A., AND SUSKIND, R. R.: The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J. Occup. Med. **22:** 11–14, 1980.
181. ZYTKOVICZ, T. H.: Identification and characterization of a high-affinity saturable binding protein for the carcinogen benzo(*a*)pyrene. Cancer Res. **42:** 4387–4393, 1982.