2,3,7,8-Tetrachlorodibenzo-p-dioxin Receptors in Wild Type and Variant Mouse Hepatoma Cells

NUCLEAR LOCATION AND STRENGTH OF NUCLEAR BINDING*

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The volume of aqueous solvent present during subcellular fractionation of mouse hepatoma (Hepa 1c1c7) cells influences the distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) receptors between the nuclear and cytosolic fractions. When the effects of dilution are minimized, at least 80% of the receptors associate with nuclei. The receptors bind relatively strongly to nuclei, as measured by their release by KCl. TCDD-receptor complexes bind more strongly to nuclei than do unoccupied receptors. A temperature-dependent event further enhances the binding of TCDD-receptor complexes to nuclei. A class of variant cells contains receptors which bind relatively weakly to nuclei; this defect accounts for the variant phenotype. We conclude that, in the intact cell, TCDD receptors are located within the nucleus and that the temperature-dependent event in the induction of cytochrome P450 gene expression is one which strengthens the binding of the TCDD-receptor complex to chromatin.

Halogenated aromatic hydrocarbons are important toxic environmental contaminants which produce a variety of responses in numerous species and tissues (1, 2). One interesting property of these compounds is their ability to induce the activity of microsomal enzyme systems, in particular, aryl hydrocarbon hydroxylase. This cytochrome P-450-containing enzyme system metabolizes polycyclic aromatic hydrocarbons, such as the major environmental carcinogen benzo(a)pyrene (3). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (the prototypical halogenated aromatic hydrocarbon) is the most potent known inducer of the aryl hydrocarbon hydroxylase system. The mechanism of hydroxylase induction involves the binding of TCDD1 to an intracellular receptor protein and the accumulation of TCDD-receptor complexes within cell nuclei; this is accompanied by the accumulation of cytochrome P450-specific mRNA (2, 4–7). The current model postulates a two-step mechanism through which TCDD receptors control the expression of the cytochrome P450 gene: an binding of the receptor to an intracellular receptor protein, followed by a temperature-dependent “translocation” of the TCDD-receptor complex from the cytoplasm to the nucleus (2, 4).

We have been studying the mechanism by which TCDD induces aryl hydrocarbon hydroxylase activity, using (wild type) mouse hepatoma (Hepa 1c1c7) cells and variant cells which have defective induction mechanisms (7–10). One class of variant cells (designated here as Class I variants) contains receptors with altered TCDD-binding properties; nevertheless, the TCDD-receptor complexes are able to accumulate within nuclei in these cells. A second class of variant cells (designated here as Class II variants) contains receptors with normal TCDD-binding properties; however, the TCDD-receptor complexes apparently are unable to accumulate within nuclei in these cells. Cell fusion experiments indicate that both variant phenotypes are recessive and that the lesions in the different variants are in different genes (10).

Here we describe studies which address two issues: 1) the location of TCDD receptors in intact cells and 2) the defect in the Class II variants, which fail to accumulate TCDD-receptor complexes within the nucleus. Our results imply that 1) TCDD receptors are located primarily within the nucleus of wild type cells and 2) the Class II variant cells contain receptors with a decreased ability to bind to a nuclear component(s), presumably chromatin. These findings lead us to propose a mechanism for TCDD action which is different from those described previously.

EXPERIMENTAL PROCEDURES

Materials—Cell culture media was from Gibco Laboratories (Grand Island, NY); fetal bovine serum from Reheis Chemical Co. (Phoenix, AZ); plasticware from Falcon Plastics (Oxand, CA); and salts, buffers, sucrose, etc. were reagent grade, obtained from standard sources. Tritiated TCDD (39 Ci/mmol) was a gift from Dr. A. Poland.

Cells and Cell Culture—Wild type Hepa 1c1c7 mouse hepatoma cells are a subclone (11) of the Hepa 1 cell line (12). Variant cells (BP'c') were isolated by fluorescence-activated cell sorting (8), followed by growth in medium containing benzo(a)pyrene. The variant cells apparently are unable to accumulate TCDD-receptor complexes in the nucleus and contain no detectable basal or inducible cytochrome P450 mRNA or aryl hydrocarbon hydroxylase activity (7, 10). Cells were grown and subcultured using standard techniques, as previously described (10).

Receptor Analyses—These procedures are similar to those we have used previously (10); the major difference is that here we have homogenized cells in a minimal volume of buffer. Cells were incubated in 10 mM HEPES, pH 7.6 (0 °C, 30 min), collected in ice-cold MDH buffer (3 mM MgCl2, 1 mM dithiothreitol, 25 mM HEPES, pH 7.6) by scraping into a Dounce homogenizer, and centrifuged (4 °C, 30 min), collected in ice-cold MDH buffer (3 mM MgCl2, 1 mM dithiothreitol, 25 mM HEPES, pH 7.6) by scraping into a Dounce homogenizer, and centrifuged (4 °C, 30 min) and the supernatant buffer was removed. The cell pellet (1–2 × 107 cells/ml) was homogenized using 15 strokes of a tight fitting pestle; examination of the homogenate by light microscopy revealed virtually no intact cells. This homogenate was used for “dilution experiments” and “receptor release experiments,” described below.

Preparation of “cytosol” and “nuclear extract” by centrifugation (4 °C, 60 min, 105,000 g), removal of loosely bound [3H]TCDD with charcoal/dextran, and analysis of bound [3H]TCDD by sedimentation through sucrose gradients, followed by fractionation and scintillation.

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counting, were as previously described (10). The sucrose gradient analyses indicate that both wild type and variant cells contain a single peak of bound [3H]TCDD (10). Therefore, the amount of radioactivity in the peak on the sucrose gradient is directly proportional to the amount of radioactivity in the post-charcoal/dextran supernatant. Thus, in the experiments described below, we have usually used the radioactivity in the post-charcoal/dextran supernatant as biomarker of the amount of the TCDD-receptor complex. This approach yields results consistent with those obtained from sucrose gradient analyses. We have performed each type of experiment described below at least four times, with consistent results.

Dilution Experiments—The purpose of these experiments was to determine the effect of the volume of aqueous solvent on the distribution of TCDD receptors and TCDD-receptor complexes between the nuclear and cytosolic fractions during the analysis of broken cell preparations. The approach which we used is analogous to one described previously by others (13). We homogenized cells in minimal volume, as described above, and divided the homogenate into equal aliquots of known volume (30-60 μl). To each aliquot we added a different amount of MDH buffer with gentle mixing (0°C, 5 min). We then centrifuged the homogenate (4°C, 1 min, 15,000 × g) to separate nuclei (pellet) and cytoplasm (supernatant). We removed the supernatant, added MDH buffer to a final volume of 500 μl, and prepared cytosol by centrifugation, as described above. Following centrifugation, we added glycerol to both cytosol and nuclear extract to a final concentration of 60%, the cytosol and nuclear extract were treated with charcoal/dextran and analyzed by sedimentation through sucrose gradients, as previously described (10). If the experiment involved cells which had already been exposed to [3H]TCDD (1 nM, 60 min), the cytosol and nuclear extract were treated with charcoal/dextran and analyzed by sedimentation through sucrose gradients, as described above. The concentration of KCl (in the range 0.5-1 M) varied with different concentrations of KC1 (0-0.5 M) in the incubation mixture. The concentration of KC1 (in the range 0.5-1 M) in the incubation mixture varied with different concentrations of KC1 (0-0.5 M). Under these conditions, most (70-100%) of the receptors and complexes remain associated with the nuclei (see “Results”). To each of the nuclear pellets, we added 500 μl of KDH buffer, containing different concentrations of KCl (0-0.5 M), followed by centrifugation to prepare nuclear extract, as described above. We added glycerol to the nuclear extract to a final concentration of 60%. If the experiment involved cells which had already been exposed to [3H]TCDD, the nuclear extract was treated with charcoal/dextran and analyzed by sedimentation, as described above. If the experiment involved cells which had not been exposed to TCDD, each extract was brought to 0.5 M KCl, incubated with [3H]TCDD as described above, and then treated with charcoal/dextran and analyzed by centrifugation, as described above.

RESULTS

Wild Type Cells

Dilution Experiments—In these experiments, we asked whether the conditions we used for isolating subcellular components influenced the nuclear-cyttoplasmic distribution of TCDD receptors. The results in Fig. 1A show that dilution of the homogenate prior to centrifugation markedly influences the distribution of bound [3H]TCDD between the nuclear and cytosolic fractions. In undiluted homogenate, about 80% of the bound [3H]TCDD is associated with the nuclear fraction; following an 8-fold dilution, about 80% is associated with the cytosolic fraction. Intermediate dilutions yield intermediate distributions. We observe analogous changes in receptor distribution if we perform the homogenizations at different cell dilutions: thus, the sequence of the homogenization and dilution steps does not substantially influence our results (data not shown). These results indicate that the [3H]TCDD-binding species in these cells is subject to redistribution among subcellular fractions during homogenization and centrifugation. Thus, the appearance of TCDD receptors in cytosolic fractions may be a consequence of redistribution and may not reflect their location in the intact cell. Our finding that, in undiluted homogenate, 80% of the bound [3H]TCDD associates with nuclei suggests that, in intact cells, the TCDD-binding species may have a nuclear location.

The sucrose gradient analyses shown in Fig. 1B indicate that the nuclear TCDD-binding species has a sedimentation velocity (about 8 S) similar to that previously described for the cytosolic TCDD receptor. (The small difference in sedimentation velocity between the nuclear and cytosolic species probably is related to the greater protein concentration of the cytosolic fraction (14)). In addition, these studies again reveal the marked effect of dilution on the distribution of TCDD receptors and demonstrate the reciprocal relationship between the amounts of bound [3H]TCDD in the nuclear and cytosolic fractions. At intermediate dilutions, the sucrose gradient analyses show the expected intermediate distributions of receptors (data not shown).

Competition experiments indicate that the nuclear [3H]TCDD-binding species has the specificity expected for TCDD receptors (Table I). Thus, nonradioactive TCDD, 3-methylcholanthrene, benzo(a)pyrene, and β-naphthoflavone, known ligands of the TCDD receptor, compete with [3H]TCDD for the nuclear binding sites; in contrast, dexamethasone, known not to be a ligand of the TCDD receptor, does not compete (4). In addition, Scatchard analyses of the binding indicate that the nuclear and cytosolic species both have an apparent dissociation constant for [3H]TCDD of about 1 nM (data not shown). These results indicate that the nuclear [3H]TCDD-binding species has properties expected for TCDD receptors.

We performed similar dilution experiments after incubating intact cells with [3H]TCDD. The results (Fig. 2) indicate that, in cells incubated at 0°C with [3H]TCDD, dilution of the homogenate again has a marked effect on the amount of TCDD-receptor complex associated with nuclei. In undiluted homogenate, most (60-80%) of the inducer-receptor complexes are nuclear; after an 8-fold dilution of the homogenate, almost none of the complexes are nuclear. Sucrose gradient profiles indicate that the bound [3H]TCDD has the expected sedimentation velocity and reveal the reciprocal relationship between the amounts of the complex in the nuclear and cytosolic fractions (data not shown). These results indicate that TCDD-receptor complexes undergo redistribution during homogenization and centrifugation and that redistribution can account for their appearance in cytosolic fractions. Our observation that, in undiluted homogenate, most of the complexes are nuclear suggests that, in intact cells, the TCDD-receptor complexes have a nuclear location, even if the complexes have formed at 0°C.

The results shown in Fig. 2 also indicate that the effect of dilution is less in cells incubated with [3H]TCDD at 37°C, compared to the effect of dilution in cells incubated at 0°C. After an 8-fold dilution of the homogenate of cells incubated with [3H]TCDD at 37°C, about 80% of the inducer-receptor complexes still remain associated with nuclei, compared to about 10% in cells incubated with [3H]TCDD at 0°C. These results indicate again that dilution of the homogenate influences the distribution of the TCDD-receptor complexes between nuclei and cytosol; however, dilution has a much
cells incubated with TCDD at 0 °C. These findings suggested to us that the binding of TCDD-receptor complexes to nuclei might be stronger in cells incubated with TCDD at 37 °C than in cells incubated with TCDD at 0 °C; if so, the complexes formed at 37 °C might be less susceptible to redistribution caused by dilution of the homogenate. To test this hypothesis, we performed the experiments described below.

**Receptor Release Experiments**—We studied the strength of the interaction between TCDD receptors (or TCDD-receptor complexes) and nuclei by determining the concentration of KCl required to release the receptors (or complexes) from nuclei. The results shown in Fig. 3 indicate that, in untreated cells, low concentrations of KCl (0–0.1 M) release 40–50% of the receptors from nuclei; the remaining receptors require higher KCl concentrations (up to 0.3 M) to be released. Thus, in the untreated cell, at least 50% of the TCDD receptors bind relatively strongly to nuclei. The release of receptors at low concentrations of KCl could reflect either 1) relatively weak, nonspecific binding to nuclei by a subpopulation of receptors or 2) the effect of dilution and the redistribution of receptors as described above. For reasons described below, we think that the latter explanation is correct.

We performed similar experiments after incubating intact cells with [3H]TCDD. Fig. 3 shows that, in cells incubated at 0 °C with TCDD, low concentrations of KCl (0–0.1 M) release 25–35% of the inducer-receptor complexes from nuclei. The remaining complexes require higher KCl concentrations (0.2–

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**Table 1**

Selectivity of [3H]TCDD binding in nuclei of wild type cells

| Competitor          | [3H]TCDD bound % of control |
|---------------------|-----------------------------|
| None                | 100                         |
| TCDD                | 0                           |
| Dexamethasone       | 10                          |
| 3-Methylcholanthrene| 0                           |
| Benzo[a]pyrene      | 6                           |
| β-Naphthoflavone    | 1                           |

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**Fig. 1**. Effect of homogenate dilution on the distribution of unoccupied TCDD receptors between cytosol and nucleus in wild type cells. A, we diluted aliquots of homogenate, prepared cytosolic and nuclear fractions from each aliquot, and assayed each fraction for TCDD receptors, as described under "Experimental Procedures." ○, cytosolic receptors; ●, nuclear receptors. Brackets indicate the range of values. B, we performed a dilution experiment as described for A. We assayed the bound [3H]TCDD by velocity sedimentation through sucrose gradients, followed by fractionation and scintillation counting, as described under "Experimental Procedures." ○, cytosolic receptors; ●, nuclear receptors. Left, undiluted homogenate; right, 8-fold dilution of homogenate.

**Fig. 2**. Effect of homogenate dilution on the amount of nuclear TCDD-receptor complexes (I-R complexes) in wild type cells exposed to TCDD at 0 °C or at 37 °C. We performed dilution experiments using cells which had been exposed to [3H]TCDD at 0 °C or at 37 °C, as described under "Experimental Procedures." At zero dilution 60–80% (cells incubated at 0 °C) and 85–100% (cells incubated at 37 °C) of the complexes associated with the nuclear fraction. ○, cells exposed to [3H]TCDD at 0 °C; ●, cells exposed to [3H]TCDD at 37 °C. Brackets indicate the range of values.

**Fig. 3**. Release of TCDD receptors and TCDD-receptor complexes (I-R complexes) from nuclei of wild type cells. We performed receptor release experiments using nuclei from untreated cells, from cells exposed to [3H]TCDD at 0 °C, and from cells exposed to [3H]TCDD at 37 °C, as described under "Experimental Procedures." ○, TCDD receptors released from untreated cells; ●, TCDD-receptor complexes released from cells exposed to [3H]TCDD at 0 °C; ○, TCDD-receptor complexes released from cells exposed to [3H]TCDD at 37 °C. Brackets indicate the range of values.
0.4 M) to be released. Furthermore, any given concentration of KCl below 0.4 M is less effective at releasing TCDD-receptor complexes from nuclei than it is at releasing unoccupied receptors. This result implies that TCDD-receptor complexes bind more strongly to nuclei than do unoccupied receptors. This interpretation predicts that the TCDD-receptor complexes should be less susceptible than the unoccupied receptors to the effect of dilution; this prediction is verified by our observation that, in solvent containing no KCl (where the effects reflect dilution alone), the fraction of TCDD-receptor complexes released from nuclei is about one-half the fraction of unoccupied receptors released (Fig. 3).

Fig. 3 also indicates that, in cells incubated at 37 °C with TCDD, low concentrations of KCl (0-0.1 M) release none of the TCDD-receptor complexes from nuclei; all the complexes require relatively high concentrations of KCl (0.2-0.4 M) to be released. Furthermore, any given concentration of KCl below 0.4 M releases fewer TCDD-receptor complexes from nuclei of cells exposed at 37 °C than from nuclei of cells exposed at 0 °C, indicating that the complexes bind more tightly to nuclei of cells exposed to TCDD at 37 °C. These results imply that the TCDD-receptor complexes undergo a temperature-dependent event which strengthens their binding to nuclei. Again, this interpretation predicts that the TCDD-receptor complexes in cells exposed to TCDD at 37 °C should be less susceptible to the effects of dilution than are the complexes in cells exposed at 0 °C; this prediction is verified, since solvent containing no KCl releases fewer TCDD-receptor complexes from nuclei of cells exposed to TCDD at 37 °C than from nuclei of cells exposed at 0 °C (Fig. 3).

Variant Cells

Dilution Experiments—We have used the same experimental approaches to analyze the Class II variant cells, which bind TCDD normally but which are apparently unable to accumulate TCDD-receptor complexes within nuclei (10). According to the current model for induction, these variants seem to contain a defect in the temperature-dependent translocation of the TCDD-receptor complexes from cytoplasm to nucleus (2, 4, 10). The results in Fig. 4 indicate that, as we observed for wild type cells, dilution of homogenate from variant cells prior to separation of the nuclear and cytoplasmic fractions greatly alters the distribution of unoccupied receptors between the two fractions. In the undiluted homogenate, about 50% of the receptors associate with nuclei; following an 8-fold dilution, almost all of the receptors appear in the cytosolic fraction. Sucrose gradient profiles indicate that the [3H]TCDD-binding species sediments with the same velocity as the wild type receptors and demonstrate the reciprocal relationship between the amounts of receptor in the nuclear and cytosolic fractions (data not shown). These results indicate that TCDD receptors in the variant cells are susceptible to redistribution during homogenization and centrifugation and that, when the effects of dilution are minimized, a substantial fraction of the receptors associates with nuclei. However, in the variant cells, the fraction of receptors associated with nuclei is smaller than the fraction in wild type cells measured under the same conditions (compare Fig. 1A and Fig. 4).

We performed analogous dilution experiments after incubating intact variant cells with [3H]TCDD. The results in Fig. 5 indicate that, in cells incubated with TCDD at 0 °C, dilution of the homogenate has a large effect on the amount of TCDD-receptor complexes which associate with nuclei. In undiluted homogenate, 70-80% of the complexes associate with nuclei; after an 8-fold dilution of the homogenate, less than 10% of the complexes remain associated with nuclei. These observations are similar to those made previously in wild type cells (Fig. 2). However, when we performed dilution experiments using variant cells incubated with TCDD at 37 °C, our results were very different from those in wild type cells. In variant cells incubated with TCDD at 37 °C, we observed a substantial effect of dilution on the distribution of inducer-receptor complexes; following an 8-fold dilution of the homogenate, only 10-20% of the TCDD-receptor complexes remained associated with nuclei (Fig. 5). Thus, in contrast with the situation in wild type cells (Fig. 2), incubation of variant cells with TCDD at 37 °C generates TCDD-receptor complexes which remain highly susceptible to redistribution following dilution. These findings suggested to us that the binding of the receptors and complexes to nuclei is weaker in the variant cells than in wild type cells. We performed receptor release experiments to test this hypothesis.

Receptor Release Experiments—The results shown in Fig. 6 indicate that low concentrations of KCl (0-0.1 M) release a substantially greater fraction of receptors from variant nuclei than is released from wild type nuclei under the same conditions (Fig. 3). These findings are consistent with the results of the dilution experiments and imply that, in variant cells,
plexes to nuclei in the variants is consistent with their binding to nuclei in variant cells than in wild type cells. The magnitude of the change (measured as a shift of about 0.06 units on the abscissa) is the same as that which occurs in wild type cells (Fig. 3); this suggests that the temperature-dependent event is normal in the variant cells. Under all of our experimental conditions, the binding of the TCDD-receptor complexes to nuclei in variant cells is substantially weaker than the binding in wild type cells (compare Fig. 3 and Fig. 6). This suggests that the Class II variant cells contain a defect in a receptor domain responsible for the binding to chromatin. This type of lesion can account for the increased susceptibility of the TCDD-receptor complexes in the variant cells to redistribution during dilution. In addition, our present findings account for our previous inability to detect TCDD-receptor complexes in the nuclei of the variant cells, since we performed our earlier experiments under conditions where the effects of dilution are large (10).

DISCUSSION

The results of our dilution experiments indicate that, in broken cells, both unoccupied TCDD receptors and TCDD-receptor complexes redistribute between the nuclear and cytoplasmic fractions and that redistribution can account for the appearance of the receptors (and complexes) in cytosolic preparations. When we minimize the effects of dilution, we find that 80-100% of the receptors associate with the nuclear fraction, implying that, in the intact cell, the receptors are located in the nucleus. Earlier reports, both from our lab and other labs, which describe the receptors as cytosolic are probably misleading, due to redistribution artifacts which occur during subcellular fractionation. The nuclear location of TCDD receptors is consistent with the results of our receptor release experiments, which show that, in wild type cells, both unoccupied TCDD receptors and TCDD-receptor complexes bind relatively strongly to nuclei. We assume that the binding is primarily to chromatin, because 1) the accumulation of nuclear TCDD-receptor complexes is associated with transcription of the cytochrome P450 gene (6, 7) and 2) the TCDD-receptor complexes bind to DNA-cellulose (15). We also assume that we are measuring the binding of the receptors (or complexes) to chromatin in general, since our techniques are presumably not sensitive enough to detect binding to specific regulatory regions within the nucleoprotein (16).

Dilution and/or ionic strength influence the nuclear-cytoplasmic distribution of estrogen receptors (13, 17), progesterone receptors (18), 1,25-dihydroxyvitamin D3 receptors (19-21), and ecdysteroid receptors (22, 23) in broken cell preparations. Thus, the appearance of these receptors in cytosolic fractions may not reflect their location in the intact cell. Likewise, DNA polymerase α, a nuclear enzyme (24-26), may appear to be cytoplasmic, because it is subject to redistribution artifacts during homogenization and centrifugation in aqueous buffers (27). A similar situation may exist for DNA ligase (28).

We think that the association of the receptors with wild type nuclei in undiluted homogenate is not due to nonspecific binding because 1) it requires conditions of relatively high ionic strength to release most of the receptors from nuclei and 2) the amount of receptor associated with nuclei varies substantially, depending upon the physiological state of the receptor (i.e., unoccupied versus occupied, occupied at 0 °C versus occupied at 37 °C). For these same reasons, we do not think that our results reflect an artifactual shift of receptors into nuclei during homogenization, followed by an outward redistribution during dilution; however, our results do not formally rule out this possibility. Future studies using intact cells and other experimental approaches (e.g., immunocytochemical or autoradiographic) may address this possibility more directly.

Our results are compatible with a two-step model for the mechanism of TCDD action, analogous to models proposed for steroid hormone action (29, 30). One event is the binding of TCDD to its receptor; our results imply that this occurs primarily in the nucleus. The presumed nuclear location of this event imposes a constraint on the possible nature of the second, temperature-dependent event. For example, the second step is not a translocation of the TCDD-receptor complex from cytoplasm to nucleus, if the complex forms within the nucleus in the first place. Our findings imply that the temperature-dependent event further strengthens the binding of the TCDD-receptor complex to the nucleus. By analogy with steroid systems, the enhanced binding could reflect a conformational change, an enzymatic modification of the TCDD-receptor complex, an association (or dissociation) of receptor subunits, or some other process (30).

What type of defect in the Class II variant cells can account for the variant phenotype? One possibility is that the cells
contain a lesion in a gene coding for the TCDD receptor. We have shown previously that the Class II variant cells contain receptors which are like wild type with respect to their TCDD-binding properties (10). Thus, the receptors in these variants apparently contain a normal TCDD-binding domain. We have shown here that the TCDD-receptor complexes in the Class II variants undergo a temperature-dependent increase in their binding to nuclei; the magnitude of the increase is similar to that which we observe in wild type cells. Thus, both the formation of TCDD-receptor complexes and the temperature-dependent enhancement of their binding to nuclei apparently occur normally in the Class II variant cells. However, in these variant cells, the binding of the receptors and complexes to nuclei is much weaker than that which we observe in wild type cells under comparable conditions. Thus, one possibility which accounts for the variant phenotype is that the Class II variants contain a defect in a domain of the receptor involved in its binding to nuclei (chromatin).

Others have reported that digestion of TCDD receptors with trypsin has little effect on TCDD binding, but destroys the ability of the TCDD-receptor complexes to bind to DNA-cellulose (15). These findings support the idea that TCDD receptors have a ligand-binding domain and a chromatin-binding domain which are biochemically distinct. We have previously described other variant cells (Class I) in which a lesion in the ligand-binding domain of the TCDD receptor can account for the variant phenotype; cell fusions indicate that the Class I variants are in a different complementation group than the Class II variants (10). Therefore, if the Class I variants contain a lesion in the ligand-binding domain of the receptor and if the Class II variants contain a lesion in the receptor's chromatin-binding domain, then our genetic analyses imply that TCDD receptors are composed of (at least) two proteins, one containing a TCDD-binding site and the other containing a chromatin-binding site.

A second possibility is that a lesion in a non-receptor gene accounts for the phenotype of the Class II variant cells. For example, these cells might be defective in a nuclear protein (e.g. an enzyme which modifies the TCDD-receptor complex) required for the correct binding of the inducer-receptor complexes to chromatin. If this possibility is correct, then our results imply that there is a third event (in addition to the formation of TCDD-receptor complexes and the temperature-dependent enhancement of their binding to chromatin) in the mechanism by which TCDD receptors control the expression of the cytochrome P-450 gene. We think that this is less likely than the first possibility, since a lesion in a non-receptor gene cannot account as easily for our observation that the binding in the variant cells is, under all of our experimental conditions, weaker than that in wild type cells.

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REFERENCES

1. Nicholson, W. J., and Moore, J. S. (eds) (1979) Ann. N. Y. Acad. Sci. 320, 1–730
2. Poland, A., and Knutson, J. C. (1982) Annu. Rev. Pharmacol. Toxicol. 22, 517–554
3. Gelboin, H. V. (1980) Physiol. Rev. 60, 1107–1166
4. Nebert, D. W., Eisen, H. J., Negishi, M., Lang, M. A., Hjelmeland, L. M., and Okey, A. B. (1981) Annu. Rev. Pharmacol. Toxicol. 21, 431–462
5. Tukey, R. H., Nebert, D. W., and Negishi, M. (1981) J. Biol. Chem. 256, 6969–6974
6. Tukey, R. H., Hannah, R. R., Negishi, M., Nebert, D. W., and Eisen, H. J. (1982) Cell 31, 275–284
7. Israel, D., and Whitlock, J. P., Jr. (1983) J. Biol. Chem. 258, 10390–10394
8. Miller, A. G., and Whitlock, J. P., Jr. (1981) J. Biol. Chem. 256, 2433–2437
9. Miller, A. G., and Whitlock, J. P., Jr. (1982) Mol. Cell. Biol. 2, 625–632
10. Miller, A. G., Israel, D., and Whitlock, J. P., Jr. (1983) J. Biol. Chem. 258, 3523–3527
11. Hankinson, O. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 373–376
12. Bernhard, H. P., Darlington, G. J., and Ruddle, F. H. (1973) Dev. Biol. 35, 83–96
13. Sheridan, P. J., Buchanan, J. M., Anselmo, V. C., and Martin, P. M. (1979) Nature (Lond.) 282, 579–582
14. Okey, A. B., Bondy, G. P., Mason, M. E., Kahl, G. F., Eisen, H. J., Guenther, T. M., and Neber, D. W. (1979) J. Biol. Chem. 254, 11636–11648
15. Carlstedt-Duke, J. M. B., Harnemo, U.-B., Hogberg, B., and Gustafsson, J. A. (1981) Biochim. Biophys. Acta 672, 131–141
16. Yamamoto, K. R., and Alberts, B. M. (1975) Cell 4, 301–310
17. Martin, P. M., and Sheridan, P. J. (1982) J. Steroid Biochem. 16, 215–229
18. Sheridan, P. J., Buchanan, J. M., Anselmo, V. C., and Sheridan, P. M. (1981) Endocrinology 108, 1533–1537
19. Walters, M. R., Hunziker, W., and Norman, A. W. (1980) J. Biol. Chem. 255, 6793–6805
20. Walters, M. R., Hunziker, W., and Norman, A. W. (1980) J. Recept. Res. 1, 313–327
21. Walters, M. R., Hunziker, W., and Norman, A. W. (1981) Biochim. Biophys. Res. Commun. 98, 990–996
22. Yund, M. A., King, D. S., and Fristrom, J. W. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 6039–6045
23. Sage, B. A., Tanis, M. A., and O'Connor, J. D. (1982) J. Biol. Chem. 257, 6373–6379
24. Herrick, G., Spear, B. B., and Veomett, G. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1136–1139
25. Foster, D. N., and Gurney, T. Jr. (1976) J. Biol. Chem. 251, 7893–7898
26. Bensch, K. G., Tanaka, S., Hu, S.-Z., Wang, T. S.-F., and Korn, D. (1982) J. Biol. Chem. 257, 8391–8396
27. Lynch, W. E., Surrey, S., and Lieberman, I. (1975) J. Biol. Chem. 250, 8179–8183
28. Soderhall, S., and Lindahl, T. (1976) FEBS Lett. 67, 1–8
29. Jensen, E. V., Greene, G. L., Closs, L. E., DeSombrer, E. R., and Nadji, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 1–8
30. Grody, W. W., Schrader, W. T., and O'Malley, B. W. (1982) Endocr. Rev. 3, 141–163
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