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DRUG-METABOLIZING ENZYMES IN LIGAND-MODULATED TRANSCRIPTION

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Abstract—Genes encoding many of the so-called drug-metabolizing enzymes (DMEs) are present in both prokaryotes and eukaryotes, suggesting that these genes arose on this planet more than 3.5 billion years ago—long before animal–plant divergence (estimated to be about 1.2 billion years ago) and long before the use and commercial development of drugs. What, therefore, are the *real* functions of DMEs? Several years ago I proposed that DMEs are upstream in the regulatory cascade of numerous signal transduction pathways, i.e. necessary for maintaining physiologically "safe", or "acceptable", steady-state levels of all small non-protein endogenous ligands ($M_r = 250 \pm 200$) in each cell. Innumerable foreign chemicals and drugs mimic these small endogenous ligands, thus binding to a particular receptor and acting either as an agonist or antagonist in activating or inhibiting genes effecting growth, differentiation, apoptosis, homeostasis and neuroendocrine functions. Discussed in this review are additional examples consistent with this theory and not described in previous reviews, including: (i) insect–plant symbiosis; (ii) "cross-talk" amongst genes in the aromatic hydrocarbon-responsive [*Ah*] battery; (iii) signal transduction pathways involving the arachidonic acid cascade; and (iv) the explanation in carcinogen-screening studies as to why a maximum, or half maximum, tolerated dose (MTD, MTD_{50}) of many test compounds might cause cell division and tumorigenesis in experimental animals.

The subject of this Symposium, "genomic pharmacology", concerns drugs that influence gene expression, leading to long-term biologic responses such as cell growth and division, differentiation, cell survival and plasticity. Rather than interacting directly with DNA, drugs almost always interact with critically important intracellular proteins (e.g. membrane-bound receptors, cytosolic receptors, nuclear receptors, second messengers, third messengers and transcription factors that bind DNA) (reviewed in Ref. 1).

The subject of this paper extends my hypothesis [2, 3] that DMEs† may regulate the subcellular steady-state concentrations of ligands important in growth and development. I shall describe additional examples and recently published data that are consistent with this hypothesis. First, the existence of DMEs from an evolutionary standpoint will be discussed. Second, the relationship between DME gene expression and numerous critical life functions will be explored. Third, I will examine DME-mediated metabolism of numerous endogenous ligands, as well as many foreign ligands acting either as agonists or antagonists. Finally, several biologic observations will be described, for which this hypothesis provides the most likely explanation.

How many receptors exist, to which distinct endogenous small molecule ($M_r = 250 \pm 200$) *ligands bind?*

The number of ligand-responsive receptors that act as transcriptional factors for controlling growth and differentiation appears to be far greater than originally predicted. For example, there are more than three dozen genes cloned and identified as members of the nuclear hormone receptor superfamily, yet less than one-third of these receptors actually have an endogenous ligand that has been unequivocally identified [3, 4]. Various transcription factors appear to function at miniscule concentrations—in the range of a few dozen to, at most, several thousand molecules per cell. How the steady-state levels and availability of endogenous ligands that effect transcription are regulated would, of course, be extremely important to genomic pharmacology [1].

How should we define DMEs?

Enzymes that metabolize drugs, foreign chemicals, carcinogens, and other environmental pollutants have classically been divided into two broad categories, "Phase I" and "Phase II" [5]. Phase I reactions have also been termed "functionalization reactions"; these enzymes provide a functional group, such as hydroxyl, which can then be acted upon by Phase II enzymes. Phase II reactions have also been called "conjugation reactions", because they often add a conjugate (glutathione, glucuronide, methyl group, acyl group, etc.) to the functional

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† Abbreviations: DMEs, drug-metabolizing enzymes; Ah receptor, aromatic hydrocarbon-binding receptor; TCDD (or dioxin), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AhREs, aromatic hydrocarbon-responsive elements; EpRE, electrophile-responsive element; NRE, negative response element; PPAR, peroxisome proliferator-activated receptor; PLA_2, phospholipase A_2; MTD, maximum tolerated dose; MTD_{50}, half maximum tolerated dose.

Table 1. List of enzymes that are often included as drug-metabolizing enzymes

Phase I
P450s, flavin-containing monooxygenases (FMOs), hydroxylases, lipoxygenases, cyclooxygenases, peroxidases, oxidases, monoamine oxidases (MAOs), dioxygenases, reductases
Phase II
UDP glucuronosyl-, glutathione-, and sulfo-transferases
Transaminases, acetyltransferases, methyltransferases, acyltransferases
Quinone reductases, aldoketoreductases, carboxylesterases
NAD- and NADP-dependent alcohol, and steroid dehydrogenases
Glycosylases, glucuronidases, various hydrolases and esterases

Although some colleagues might not consider most reductases, dehydrogenases, epoxide hydrolases and esterases to be representative of Phase I or Phase II metabolism, for the purposes of the hypothesis set forth in this review we shall lump all DMEs into only the two categories.

group. Usually, Phase I plus Phase II metabolism ultimately results in the conversion of relatively hydrophobic drugs and other chemicals to hydrophilic intermediates and products that are more readily excreted.

DMEs have classically been regarded as a "hepatic detoxification system". Unfortunately, this concept has been persistently taught in graduate training and medical school courses for more than 40 years. This perception is only a small portion, however, of the total picture.

Table 1 lists most categories of enzymes that are capable of metabolizing drugs. There are several noteworthy features about these enzymes. (a) Each one of these enzymes without exception has also been found to metabolize endogenous substrates, i.e. no enzyme exists for the sole responsibility of drug metabolism. (b) Many of these classes of DMEs exist in prokaryotes, although the number of individual enzymes in each category might be larger in eukaryotes and the diversification of these enzymes is generally much greater in mammals. (c) Interestingly, in response to numerous endogenous and foreign signals (e.g. castration, dietary differences, partial hepatectomy, stress, exposure to dioxin, insecticides, certain prescribed drugs, oxidative stress, differentiation, tumorigenesis, etc.), the levels of particular subsets of DMEs rise and fall, suggesting up- and down-regulation of the corresponding genes. (d) There are examples of coordinate regulation of *trans*-acting DME genes, in response to many of these aforementioned signals. As the reader will conclude after reading this paper, a better name for "drug-metabolizing enzymes" might be "effector ligand-metabolizing enzymes".

Evolutionary divergence of DMEs and their substrates in eukaryotes from those in prokaryotes

Very recent geologic [6] and molecular biologic [7] evidence suggests that eukaryotes and eocyte prokaryotes are immediate relatives (Fig. 1). Such an emerging concept is very exciting to me, because it would suggest that any gene occurring in both eubacteria and eukaryotes probably arose from a common ancestral gene more than 3.5 billion years ago [8]. Virtually all classes of DMEs listed in Table 1 are present in prokaryotes as well as eukaryotes. It therefore seems very likely that many DMEs originated during early evolution, in order to provide the organism with metabolism of important endogenous molecules, as well as the breakdown of environmental chemicals utilized for energy. Subsequently, the DMEs in eukaryotes probably diversified, in response to the adverse effects of dietary pressures and other environmental chemicals (reviewed in Refs. 3 and 9).

What would have been among the earliest DME substrates? A very early environmental chemical that comes to mind is CS_2, which is known to be metabolized by P450 to carbonyl sulfide and carbon dioxide [10]; once the early cell developed sufficient levels of intracellular pO_2 [8], it is likely that a P450-like enzyme appeared in order to break down CS_2 for energy use. In terms of endogenous substrates: purines, pyrimidines and biogenic amine-related compounds are believed to have originated very early in evolution [8]. For numerous cell surface and intracellular receptors, control of gene transcription, and other factors in signal transduction pathways, the important inter-relationships amongst, and the critical roles of, catecholamines and cyclic AMP [11, 12], ATP derivatives [13], GMP- and GTP-binding proteins [14, 15], and the GTPase superfamily [16], have been well characterized in both prokaryotes and eukaryotes.

"Late" DME genes need not carry out the same functions as "early" DME genes

It should be emphasized that genes encoding DMEs that existed 1 or 3.5 billion years ago will probably differ in function from those that have only existed for 10 or 50 million years [9]. It seems reasonable to presume that a "new" DME gene that has just appeared (due to gene duplication by unequal crossing-over, gene inactivations or terminal mutational events, replication slippage, insertions and gene conversions) during the past several dozen million years of evolution will not have the same likelihood of carrying out a critical life function as a DME gene that has existed for more than 1 or 3.5 billion years. This would explain the recently described, numerous human polymorphisms in many

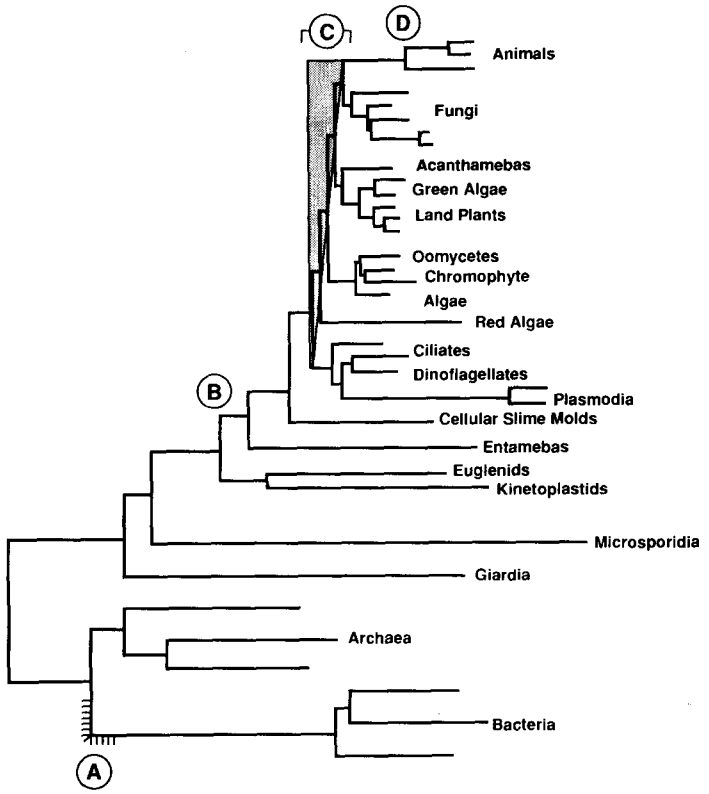

Fig. 1. Molecular phylogeny of eukaryotic organisms based on sequence comparisons of small subunit rRNA and constructed by distance matrix methods. The hachured lines denote the position of the tree root, as inferred from analysis of protein sequences. (A) The diversification point of the major domains, 3.5 billion years ago or earlier. (B) The acquisition of mitochondria, inferred from the geochemical record to be between 2.8 and 2.4 billion years ago. (C) The major radiation of phenotypic diversity of eukaryotes, suggested on the basis of fossils to be 1.5 to 1.0 billion years ago. (D) The radiation of coelomate animal phyla, about 560 to 540 million years ago. (Modified and reproduced, with permission from Knoll, 1992; Ref. 6.)

DME genes, which may or may not be important in terms of cell division or cell death, differentiation, homeostasis or neuroendocrine functions [9].

Changes in DMEs associated with numerous cellular functions

During the past three decades it has frequently been observed in pharmacologic research that, when many different drugs and other foreign chemicals are administered to a laboratory animal, certain DMEs are increased with concomitant metabolism of the incoming signal (Fig. 2). These studies have been termed enzyme induction, or DME induction [17]. In addition, large doses of numerous drugs and other foreign chemicals appear to cause increases in cell type-specific cell division (e.g. proliferation, tumor promotion), apoptosis (e.g. thymic involution, death of colon epithelial cells), altered differentiation (e.g. cleft palate, polydactyly), and changes in homeostasis (e.g. vasoconstriction, alteration in electrolyte balance) and in neuroendocrine functions

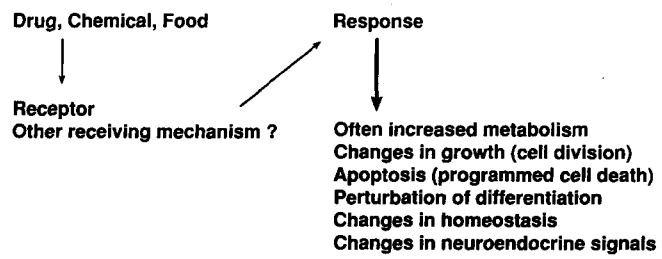

Fig. 2. Simplified diagram of the diversity of cell type-specific or organ-specific responses in an organism, following a challenge with innumerable environmental chemicals.

Table 2. Types of stimuli/substances associated with changes in drug-metabolizing enzymes*

Mammalian
Glucocorticoids, sex steroids
Dioxin, polycyclic hydrocarbons
Phenobarbital, numerous other drugs
Pyrazole, imidazole, acetone, ethanol
Peroxisome proliferators
Griseofulvin, erythromycin, other macrolide antibiotics
Retinoic acid-induced differentiation
Cholestyramine, bilirubin, bile acids
Virtually all chemicals in *The Merck Index*
ACTH, IGF, LH, FSH, GH, IL-1, IL-6, gastrin, thyroxine
Non-mammalian
Ecdysone and phenobarbital in insects
Energy substrate utilization in cockroach
Gonadotrophic hormone regulation in pond snail
Sporulation in yeast
Virtually all chemicals in *The Merck Index*
Wounding, ripening, M^{++}, flower color, phytoalexin production in plants
Virulence of (fungal and bacterial) pathogens
Energy substrate utilization: alkanes in yeast; camphor/phenobarbital/sulfonylureas in bacteria
Other curious changes in mammalian growth, homeostasis and differentiation
Increases or decreases in DMEs during embryogenesis, differentiation, partial hepatectomy, preneoplastic nodule formation
Appreciation for presence of multiple DMEs in brain
Changes in contractility: sphincters of ductus venosus, ductus artiosus in mammalian heart
Wounding of cornea
Spontaneous hypertension
Ion transport in renal medulla
Na^+ depletion; K^+ overload
Changes in oxidative stress, hormones, temperature, circadian and circannual rhythms

* Detailed in Refs. 3, 18.

(e.g. chemical-induced somnolence, amnesia) (reviewed in Ref. 3). Table 2 lists the growing number of experimental observations in which changes in these cellular functions (under the direction of endogenous signals as well as drugs and other foreign chemicals) are associated with increases or decreases in particular subsets of DMEs. These data clearly support the concept that DMEs have other roles in addition to hepatic detoxification of drugs.

DME-mediated metabolism of endogenous ligands and effector molecules

Without exception, the synthesis and degradation of all known endogenous ligands occur via DME-mediated metabolism (reviewed in Ref. 3). Table 3 shows a partial list of representative cases in which DMEs synthesize and degrade endogenous ligands. For example, an alcohol dehydrogenase forms retinal from the substrate retinol, and an aldehyde dehydrogenase metabolizes the substrate retinal to

Table 3. Representative examples of DME-mediated metabolism of growth effector molecules

Retinol → retinal → retinoic acid
Hydroxylation of retinoic acid
Glucuronidation of retinoic acid
Hydroxylation, dehalogenation of thyroxine, thyroid hormone
20-Hydroxylation of ecdysone
1α-Hydroxylation of 25-HO-D_3 (cholecalciferol, vitamin D)
24-Hydroxylation of 1α,25-di-HO-D_3
7α-Hydroxylation of cholesterol
Numerous steroid hydroxylations
Glucuronide, GSH, and SO_4 conjugations of steroids
Epinephrine → norepinephrine → isoproterenol
Tryptamine → serotonin (5HT) → melatonin

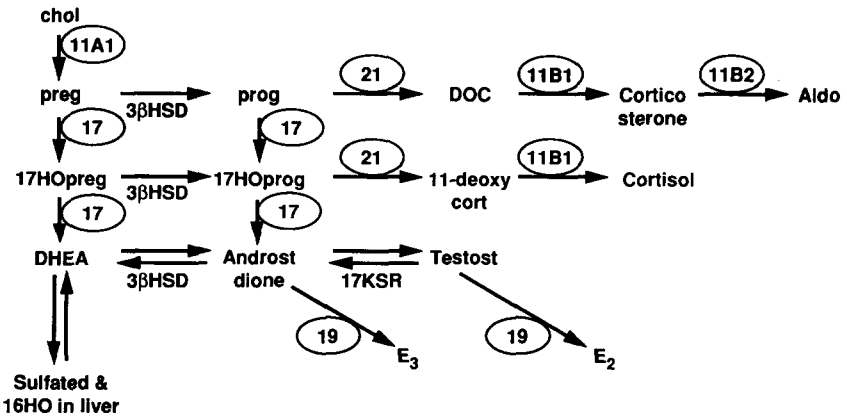

Fig. 3. Metabolic pathway of steroidogenesis. Numbers and letters in the ellipses denote six cytochromes P450: *CYP11A1* = cholesterol side-chain cleavage; *11B1* = 11β-hydroxylase; *11B2* = aldosterone synthase; *17* = 17α-hydroxylase; *21* = 21-hydroxylase; *19* = aromatase. Other abbreviations: chol, cholesterol; preg, pregnenolone; prog, progesterone; DOC, deoxycorticosterone; Aldo, aldosterone; DHEA, dehydroepiandrosterone; 16HO, 16-hydroxylation; 3βHSD, 3β-hydroxysteroid dehydrogenase; 17HOprog, 17-hydroxyprogesterone; 11-deoxycort, 11-deoxycortisol; Androst dione, androstenedione; 17KSR, 17-ketosteroid reductase; Testost, testosterone; E_3, estriol; E_2, estradiol.

retinoic acid, the active ligand for the retinoic acid receptors (RAR, RXR). Hydroxylation and glucuronidation of retinoic acid are means by which the activated ligand, retinoic acid, can be rendered inactive. The 1α-hydroxylation of 25-hydroxyvitamin D_3 forms the active ligand for the vitamin D receptor; 24-hydroxylation of 1α,25-dihydroxy-D_3 causes the ligand to become inactive. Similar pathways, for the formation of an active ligand, as well as degradation of the active effector to an inactive ligand, can be described for each of the other examples in Table 3, and the concept is further underscored if one appreciates the steroidogenesis pathway in Fig. 3.

The steroidogenesis pathway is a particularly well known example in which the further metabolism of a particular substrate will produce an inactive ligand for one receptor and the formation of an active ligand for another receptor. For example (Fig. 3), aromatization of testosterone will lead to a decrease in testosterone (i.e. the number of ligand molecules available for the androgen receptor) and an increase in β-estradiol (i.e. the number of ligand molecules available for the estrogen receptor).

DME subsets rise and fall in response to particular signals

Particular drugs, or classes of chemicals, not only cause increases or decreases in DMEs, but particular subsets of DMEs respond uniquely to a particular class of DME inducer (reviewed in Refs. 3, 17–19). For example, TCDD or polycyclic hydrocarbon treatment of laboratory animals is associated with increases in particular subsets of cytochrome P450, UDP glucuronosyltransferase, glutathione transferase, quinone reductase, aldehyde dehydrogenase and esterase (Table 4). Phenobarbital treatment is correlated with increases in particular subsets of mammalian P450, epoxide hydrolase, NADPH-P450 oxidoreductase, UDP glucuronosyltransferase, glutathione transferase, quinone reductase and aldehyde dehydrogenase. Interestingly, treatment with polycyclic hydrocarbon plus phenobarbital is additive, causing increases in both subsets with no apparent overlap. Treatment with polycyclic hydrocarbon plus phenobarbital plus a peroxisome proliferator is further additive, causing increases in all three subsets of DMEs without any

Table 4. Foreign chemicals, presumably mimicking endogenous growth effectors, activate particular subsets of DME genes

Tetrachlorodibenzo-*p*-dioxin
P450, quinone reductase, UDP glucuronosyltransferase, glutathione transferase, aldehyde dehydrogenase, esterase
Phenobarbital
P450, quinone reductase, UDP glucuronosyltransferase, NADPH-P450 oxidoreductase, epoxide hydrolase, glutathione transferase, aldehyde dehydrogenase
Peroxisome proliferators
P450, UDP glucuronosyltransferase, epoxide hydrolase, glutathione transferase, aldehyde dehydrogenase
Glucocorticoids, estrogens, androgens, etc.

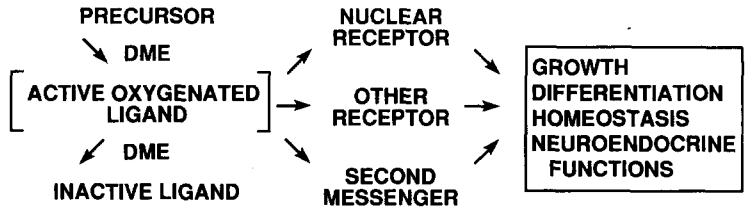

Fig. 4. Illustrated summary of the probable relationship between DMEs and signal transduction pathways effecting critical functions of the cell. Examples of the steroid-thyroid hormone nuclear receptor superfamily would be the glucocorticoid receptor and the vitamin D_3 receptor [4]. Examples of "other receptors" would include the recently discovered Per/Ah receptor/ARNT/Sim (PAAS) superfamily of transcription factors [21] and the odorant receptor superfamily that appears to be restricted to the olfactory epithelium [22]. The function of arachidonic acid metabolites as a second messenger system [23] is described in the text. (Modified and reproduced, with permission from Nebert, 1991; Ref. 3.)

apparent overlap [19]. The same can be said for DME subsets in response to certain endogenous steroid hormones (Table 4). In each of these instances, the class of DME inducers operates by way of a well known endogenous receptor, with the exception of phenobarbital for which no receptor has been identified yet. As discussed later, an endogenous ligand had not originally been identified for the peroxisome proliferator-activated receptor [2, 3, 20].

Proposed role of DMEs in controlling steady-state levels of endogenous ligands

I have postulated [2, 3] that DMEs are responsible for the regulation of steady-state levels of ligands involved in ligand-modulated transcriptional regulatory effectors of growth and differentiation (Fig. 4). This hypothesis was based on the following observations. (i) Non-protein endogenous ligands for receptors involved in growth-regulation are small molecules ($M_r = 250 \pm 200$). (ii) The synthesis and degradation of these molecules always involves DME-mediated metabolism. (iii) Binding of the natural ligand to the receptor often appears to be associated with developmental- or cell type-specific increases in particular DME subsets. (iv) Foreign chemicals that induce these subsets of DMEs always appear to bind to endogenous receptors, acting as either agonists or antagonists of receptor function. In the remainder of this paper, I will provide further biologic observations and summarize recent studies consistent with the pathways depicted in the Fig. 4 hypothesis.

"Secondary metabolites" in microbes

Microbial secondary metabolites, such as A-factor and B-factor and other hormones in actinomycetes strains, are both synthesized and degraded by DMEs [24, 24]. These metabolites are well known as essential chemical signals for induction of cell division and differentiation in yeast and filamentous fungi. The possible involvement of these divergent secondary metabolites in a symbiotic relationship in the ecosystem has recently been reviewed [24]. It has also been proposed [25] that gene transfer between organisms might have been an important factor in the evolution of secondary metabolism in some of these organisms.

Animal–plant interactions affecting growth and differentiation

Evolution of diverse growth effectors during animal–plant interactions is well known (Fig. 5). Plants often depend upon animals for the propagation of plants (e.g. pollination and dispersal of seeds). There are numerous examples in which the plant controls the rate of animal growth and development (e.g. pupation) by the production of metabolites that presumably act as agonists or antagonists in binding to endogenous receptors in the animal [26, 27]. The co-evolutionary synergism of the black swallowtail butterfly *Papilio polyxenes* and plants of the Umbelliferae and Rutaceae family is a recent example that has become appreciated at the molecular level; whereas the level of furanocoumarins in these plants is often lethal to most insects, the swallowtail caterpillar is able to feed exclusively on these plants due to the high levels of a particular P450 in the midgut that is capable of detoxifying these xanthotoxins [28].

Another example involves a *Drosophila*–cactus interaction. Each of four *Drosophila* species endemic to the Sonoran Desert use the necrotic tissue from a different, particular cactus species as breeding substrates, and each cactus species is known to have a different array of allelochemicals having detrimental

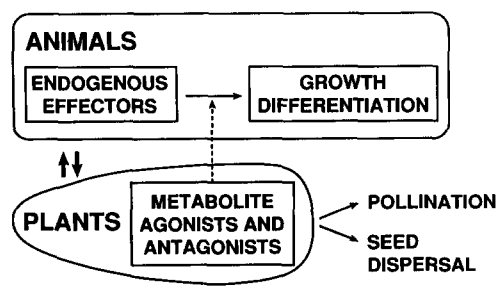

Fig. 5. Illustration of "animal–plant interactions" controlled by drug-metabolizing enzymes. The life cycle of animals can be governed by plant metabolites acting as agonists or antagonists of ligand-modulated transcription of genes involved in growth, homeostasis and differentiation. Animals, in turn, are able to help the reproductive cycle of plants.

effects on non-resident fly species. The involvement of P450 enzymes was shown to play an important role in host-plant utilization by each Sonoran Desert *Drosophila* species [29].

Aldosterone-induced homeostasis

Aldosterone, formed by CYP11B2 (Fig. 3) and the active ligand for the mineralocorticoid receptor, is one example of DME-mediated metabolism of an effector ligand that will alter homeostasis (e.g. ion transport). The amiloride-blockable Na^+ channel, expressed in *Xenopus* oocytes injected with total RNA from the toad urinary bladder, has been used to investigate mechanisms that mediate the natriferic action of aldosterone. Aldosterone was shown to enhance the apical Na^+ permeability of tight epithelia via short-term activation of pre-existing channels in the oocyte, followed by chronic induction of a new channel protein [30].

Mechanisms for lowering the estrogen response

Lowered estrogen responsiveness is, at least in part, due to lower levels of active ligand available to bind to the estrogen receptor. TCDD, polycyclic hydrocarbons and cigarette smoking are known to enhance dramatically the 2-hydroxylation of β-estradiol, a process that would decrease the levels of the active ligand estrogen [31, 32]. Exposure to these agents would thus lead to fewer functional estrogen molecules available to take part in ligand-modulated transcription. These data would suggest that there might be less estrogen response in a cigarette-smoking woman.

Mammalian estrogen sulfotransferase sulfurylates the hydroxyl group of estrogenic steroids; this is another mechanism that will decrease the amount of estrogen available to its receptor. Increases in hepatic androgen sensitivity in young adult male or androgen-treated female rats were found to be associated with elevated levels of estrogen sulfotransferase [33]. These results are consistent with the concept that target cell sensitivity for steroid hormones is regulated by both intracellular hormone receptor concentration and DME-mediated modulation of the receptor-active steroids.

Retinoic acid activates its own metabolism via its receptor

Topical application of a cream containing 0.1% retinoic acid to adult human skin has been reported to increase DME-mediated retinoic acid metabolism. Activation of DME gene expression was shown to occur via both classes of retinoic acid receptors, RAR and RXR-α [34], and cellular retinoic acid-binding protein (CRABP-II > CRABP-1) was demonstrated to be an important tissue-specific and differentiation-related down-regulator of retinoic acid action in human skin [35]. Furthermore, several forms of P450 have been shown to metabolize retinoic acid, retinol and retinal to multiple products [36].

Nitric oxide and carbon monoxide involved in neurotransmission

Both nitric oxide and carbon monoxide are formed in the mammalian cell by nitric oxide synthase and heme oxygenase, respectively. Why would such noxious gases be synthesized by endogenous enzymes? (In fact, concerning carbon monoxide, I can recall having asked my lecturer that question in 1960 during medical school biochemistry class.) Nitric oxide has been shown to be a second messenger involved in the regulation of blood vessel tone, the tumoricidal and bactericidal actions of macrophages, and the putative neurotransmitter in the central and peripheral nervous system [37]. Now it appears that carbon monoxide, like nitric oxide, might be a physiologic regulator of cyclic GMP [38]. As previously discussed [3], both nitric oxide and carbon monoxide can block P450 enzymic activity by preventing atmospheric oxygen from binding to heme in the active-site [39]. It is therefore tempting to speculate that both nitric oxide and carbon monoxide formation, in the microenvironment of a nerve terminus, for example, might prolong the action of a neurotransmitter molecule by inhibiting the particular P450 responsible for degrading the neuroactive ligand.

Biogenic amines as stimulators of cell growth

Isoproterenol is an interesting example of a neuroactive biogenic amine that can stimulate differentiation and cell division; this biogenic amine has been well studied in maxillary gland cell cultures [40]. Reserpine is a commonly prescribed drug used to treat hypertension. Reserpine is known to increase cell proliferation in the adrenal medulla [41] and to produce adrenal medullary tumors in male rats [42]. This response appears to be secondary to the neurogenic effects of reserpine. Reserpine-induced cell division can be prevented by unilateral denervation of the adrenal gland, indicating that cell proliferation is a neurologically-mediated reflexive response to catecholamine depletion [43]. These data suggest that DME-mediated metabolism is able to alter certain neuroendocrine functions, including neurogenic effects on cell division.

The aromatic hydrocarbon-responsive (Ah) battery as an example of coordinate gene regulation

The most well characterized example of coordinately regulated DME genes is the murine [*Ah*] battery. The reader is referred to Refs. 19, 44, 45 for many of the details not covered here. This battery contains at least six genes that are coordinately induced by dioxin and polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene (Fig. 6). In addition to two P450 genes (*Cyp1a1* and *Cyp1a2*) this

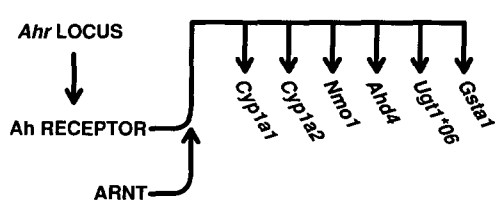

Fig. 6. The six genes defined as members of the murine [*Ah*] battery [44]. All six appear to be transcriptionally activated in a positive manner by the Ah receptor complex, which includes the Ah receptor nuclear translocator (ARNT) DNA-binding moiety.

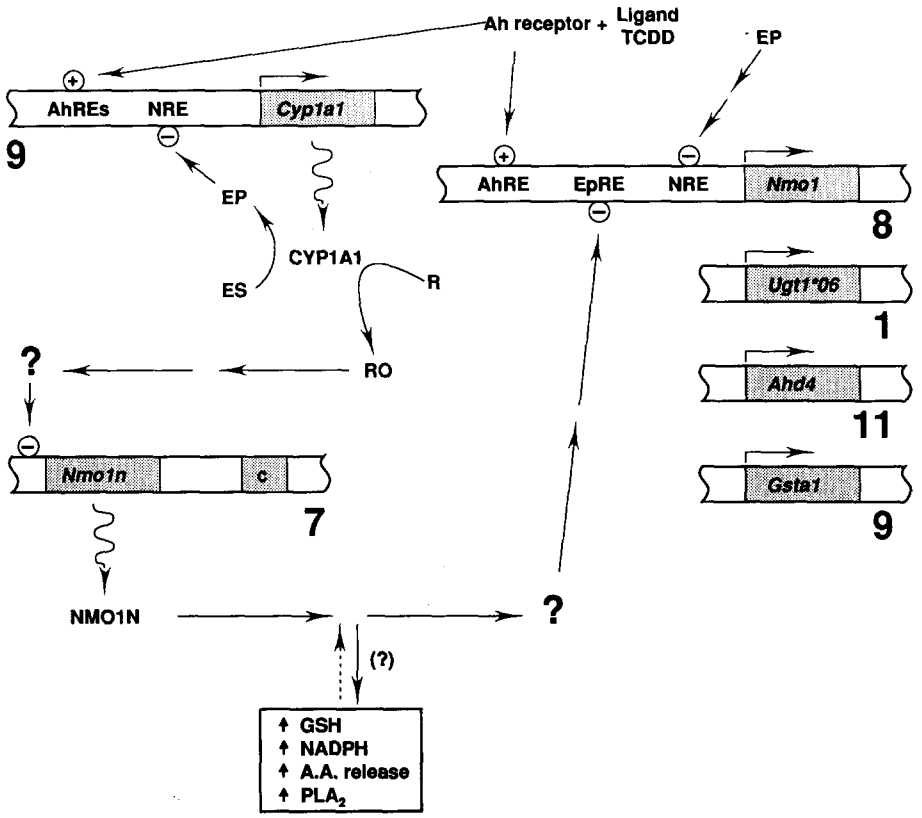

Fig. 7. Inter-relationship in the mouse, among the AhREs and the EpRE and NRE regulatory sequences, the TCDD-binding Ah receptor, the *Nmo1n* gene on chromosome 7 encoding the NMO1N negative regulatory protein, and the *Cyp1a1* and Phase II [*Ah*] genes. Regulation of the *Cyp1a2* gene is known to be similar to the *Cyp1a1* gene (reviewed in Refs. 46, 47) but has not been as thoroughly studied in cell cultures. The upstream regions of the *Ugt1*06*, *Ahd4* and *Gsta1* genes are believed to contain the same AhRE, EpRE and NRE regulatory regions as found in the *Nmo1* gene. Question marks denote an unknown number of steps between the NMO1N negative repressor protein and the EpRE, and an unknown number of steps between RO (endogenous or exogenous metabolites leading to oxidative stress) and the *Nmo1n* gene. The extent of involvement of the Ah receptor with the EpRE and NRE is not known. Because these genes exhibit varying tissue- and developmental-specific expression, as well as their own individual responses to other endogenous signals, there are undoubtedly additional transcription factors and response elements that govern the expression of each of these genes. R, endogenous or exogenous substrate for CYP1A1. RO, electrophilic metabolite. ES, unknown endogenous substrate of CYP1A1. EP, endogenous product formed by CYP1A1 and believed to be necessary for the negative regulation of not only the *Cyp1a1* gene but all four Phase II [*Ah*] genes. *c*, albino locus on chromosome 7. GSH, reduced glutathione. AA, arachidonic acid. PLA_2, phospholipase A_2. The large bold numbers identify the mouse chromosomes on which each gene resides. (Modified and reproduced, with permission from Nebert *et al.*, 1993; Ref. 45.)

laboratory has used mutant inbred mouse strains as well as mutant cell culture lines to demonstrate that four Phase II [*Ah*] genes include: NAD(P)H: menadione oxidoreductase (*Nmo1*); a cytosolic "class 3" aldehyde dehydrogenase (*Ahd4*); a UDP glucuronosyltransferase having 4-methylumbelliferone as substrate (*Ugt1*06*); and a glutathione transferase having 2,4-dinitro-1-chlorobenzene as substrate (*Gsta1*, Ya). The Ah receptor-mediated coordinate induction is controlled positively and involves all six [*Ah*] battery genes. On the other hand, a gene on chromosome 7 is required for increases in the four Phase II [*Ah*] genes, and this up-regulation appears to occur via a negatively-controlled mechanism independent of Ah receptor-mediated *Cyp1a1* and *Cyp1a2* induction [44, 45].

A complex pattern of gene "cross-talk" is emerging from these studies (Fig. 7). The upstream regulatory regions of the *Cyp1a1* and *Cyp1a2* genes contain multiple AhREs (also termed dioxin-responsive elements and xenobiotic-responsive elements DREs, XREs), whereas all four murine Phase II [*Ah*] genes contain at least one AhRE and one EpRE (also termed antioxidant response element, ARE) [48, reviewed in Ref. 45, unpublished data of V. Vasiliou, S. F. Reuter and D. W. Nebert]. Heterodimers of the Ah receptor (AHR) and the Ah receptor nuclear translocator (ARNT) are known to bind to the

AhREs upstream of the *Cyp1a1* gene [21, 45], and there can be cooperativity amongst more than one AhRE [49]. The Ah receptor complex upstream of the other [*Ah*] genes has not been characterized nearly as completely as that of the *Cyp1a1* gene. The EpRE is independent of a functional Ah receptor and is an AP-1-like DNA motif [50] that responds to "oxidative stress", i.e. endogenous or exogenous lipid peroxidation, quinones and other electrophilic metabolites [51].

The *Nmo1n* gene on mouse chromosome 7 is involved in the negative regulation of *Nmo1* and the other three Phase II [*Ah*] genes, but not of the *Cyp1a1* or *Cyp1a2* gene [44]. Absence of the NMO1N gene product is associated with elevations in intracellular levels of glutathione [52] and NADPH [53], increases in arachidonic acid release and PLA_2 induction [54], and the presence of a putative transcription factor that binds to the EpRE [55]. In the absence of a functional *Nmo1n* gene (as well as in the presence of endogenous or exogenous electrophilic chemicals), therefore, the cell appears to "sense" danger in the form of oxidative stress; the response is to derepress the Phase II [*Ah*] genes, as well as other genes in the appropriate signal transduction pathways—including c-*fos* [56]—to rid itself of the oxidative stress.

A murine negative response element (NRE) has been proposed on the basis of several lines of indirect evidence (reviewed in Ref. 45). Recent DNase I footprinting analysis of the human CYP1A1 gene suggests that a 21-bp palindrome (inverted repeat) between 750 and 800 bases from the transcription start site is involved in negative regulation, and similar homologous regions have been identified upstream of the rat and mouse *CYP1A1* genes [57]. In the absence of endogenous CYP1A1 activity in a mutant cell line, CYP1A1 mRNA as well as the mRNAs and enzyme activities of several Phase II [*Ah*] genes are strikingly elevated. An exogenously expressed murine CYP1A1 or human CYP1A2 protein (i.e. functional enzyme) in this mutant cell line represses the expression of not only the endogenous *Cyp1a1* gene but also the expression of the Phase II [*Ah*] genes whose activities are elevated in these mutant cells [45, 58–60]; now that basal expression is repressed to low levels, inducibility by TCDD is also restored. The *level* of CYP1A1 or CYP1A2 protein expression is noteworthy in these experiments: levels of expression in which there is no detectable exogenous CYP1A1 or CYP1A2 mRNA by routine northern hybridization analysis, are sufficient for full repression of the endogenous genes [58, 59]. The exact role of the AhREs and Ah receptor in the interaction with the putative NRE and in the CYP1A1/CYP1A2-mediated repression of the Phase II [*Ah*] genes is not yet understood.

In summary, why are the six genes of the [*Ah*] battery acting like a glorified bacterial operon? The CYP1A1 (or CYP1A2) gene product acts as quarterback of the team; the functional enzyme is able to repress not only the *Cyp1a1* gene but also the Phase II [*Ah*] genes. There is also an Ah receptor-mediated positive regulation of all six genes. In addition, there is the chromosome 7-mediated repression of the *Nmo1n* putative repressor "master switch" gene; oxidative signals turn off the *Nmo1n* gene, leading to derepression of a large number of genes and responses designed to combat oxidative stress.

Why does such a complex pattern of "cross-talk" exist among the genes in this battery? The hypothesis set forth in this paper appears to be a likely explanation. When presented with TCDD as a signal, those enzymes (subsets of DMEs, as shown in Table 4) that normally degrade what the cell "perceives" in TCDD as the endogenous ligand are "turned on" in order to restore "normal, physiologic" steady-state levels of the endogenous ligand.

So, what does enzyme induction really signify to the cell?

In pharmacology or toxicology experiments, therefore, a large concentration of foreign chemical, which mimics the structure of an endogenous ligand, is presented to the cell; the cell's answer is to respond to this foreign chemical as if it were an inordinately large amount of "effector molecule", by increasing the appropriate subsets of DMEs so as to degrade and otherwise return this "effector level" to safe, physiologic concentrations. "Drug metabolism induction/deinduction" studies [5, 17, 19, 61] might thus be viewed as our attempts to elucidate the sensor mechanisms by which genes are turned on or off in response to subcellular concentrations of effector ligands.

DMEs in the arachidonic acid cascade

Involvement of DMEs in such second-messenger pathways as the arachidonic acid cascade [1] is further evidence that DMEs provide important functions other than hepatic drug detoxification. Although generally regarded as manifesting pronounced effects in the kidney and lung [23], arachidonic acid metabolites undoubtedly exist in all mammalian cells. Virtually all enzymes in the arachidonic acid cascade may be regarded as DMEs, including at least six different P450 enzymes [3, 62]. P450 metabolism of arachidonic acid leads to formation of epoxyeicosatrienoic acids (EETs), hydroxyeicosatetraenoic acids (HETEs), and ω and ω-1 alcohols [23]. As summarized in Table 5, these metabolites are known to possess a broad spectrum of physiologic effects on growth, differentiation and homeostasis.

The PPAR-α has recently been shown to heterodimerize with the retinoid X receptor RXR-β in order to activate the acyl-CoA oxidase gene responsible for peroxisomal β-oxidation of fatty acids and retinoids [63]. Since the synthetic arachidonic acid analog 5,8,11,14-eicosatetraynoic acid was found to be an extremely potent ligand for PPAR-α [63], these data suggest that arachidonic acid metabolites might be the true endogenous ligands for the PPAR and certain other endogenous receptors involved in growth, homeostasis and differentiation. Interestingly, the *Nmo1n*-mediated putative repression of the arachidonic acid release and PLA_2 induction, illustrated in Fig. 7, suggest the possible involvement of arachidonic acid metabolites in the oxidative stress response by the [*Ah*] battery genes.

Table 5. Physiologic and subcellular effects of P450-mediated metabolites of arachidonic acid*

Effects of EETs
Bronchodilation, renal vasoconstriction, intestinal vasodilation, inhibition of cyclooxygenase, mitogenesis, inhibition of platelet aggregation, modulation of ion transport, enhanced peptide hormone secretion, mobilization of intracellular Ca^{2+}
Effects of HETEs
Inhibition of Na,K ATPase, vasodilation, chemotaxis of neutrophils
Effects of ω- and ω-1 alcohols
Stimulation of Na,K ATPase, vasoconstriction, bronchoconstriction

* EETs, epoxyeicosatrienoic acids; HETEs, hydroxyeicosatetraenoic acids.

MTD, MTD_{50} of test compounds might act as agonists and antagonists for endogenous receptors

The fact that MTD and MTD_{50} doses of many test compounds cause cancer, has been a recent, lively topic in the scientific literature and lay press. There is a number of noteworthy examples in which foreign test compounds, presumably acting as agonists or antagonists, might bind to receptors and effect growth and proliferation. For example, certain P450-mediated arachidonic acid metabolites are capable of causing mitogenesis (Table 5). Chemicals that induce cancer at maximally tolerated, often toxic, doses frequently increase the rate of cell division [64–66]. Clonal expansion of initiated cells (in which an oncogene has been activated) is the commonly accepted mechanism of tumor promotion. Formation of preneoplastic nodules and primary hepatocarcinomas in rats fed 2-acetylaminofluorene, or other toxic chemical [67], is a classical example of proliferation caused by foreign compounds. In fact, *CYP1A1* and *CYP1A2* are down-regulated, while the Phase II [*Ah*] genes are up-regulated during preneoplastic nodule formation (reviewed in Refs. 19, 67). Another example is a recently characterized dominant repressor gene, linked to the *c* locus on mouse chromosome 7, which has been shown to suppress *N*-methyl-*N*-nitrosourea-induced thymic lymphomas [68]; the *Nmo1n* gene represents an excellent candidate for being this dominant repressor gene.

By what mechanism might such test compounds cause cell proliferation? The hypothesis set forth in this paper (and Refs. 2, 3) would offer a reasonable explanation. Consider, for example, an endogenous effector binding to its receptor with a theoretical dissociation constant in the picomolar range (Fig. 8). Competition with a relatively nonspecific test compound, given to the laboratory animal at concentrations in the micromolar or millimolar range, might "flood" the growth transduction pathway. A large quantity of test compound that binds to a growth transduction receptor, albeit with relatively low affinity, would therefore elicit the same effect (e.g. cell type-specific proliferation) as a small, physiologic concentration of endogenous effector that binds to the receptor with high affinity.

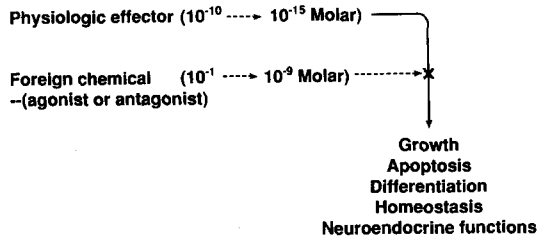

Fig. 8. Simplified illustration of the mechanism by which test compounds might compete with physiologic ligands for numerous endogenous receptors. The result might explain why the MTD or MTD_{50} of many test compounds can lead to tumor formation via such cellular processes as mitogenesis, apoptosis or dedifferentiation.

There are certain published observations, which have been difficult to explain, but which would also be consistent with the hypothesis set forth here. For example, millimolar concentrations of trichloroacetic acid have been shown to bind to the PPAR and effect the signal transduction pathway; this effect was more than three orders of magnitude higher than the concentrations of Wy 14,643, the most potent ligand [20] until the recently described arachidonic acid analog [63]. Furthermore, virtually any drug or chemical in the $M_r = 250 \pm 200$ range, when added to hepatoma cell cultures, will cause increases in CYP1A1 activity—although, in some cases, concentrations in the millimolar range, e.g. 5 mM phenobarbital, are necessary [69]. Hence, when a large dose of test compound is administered to the intact laboratory animal, one can test for levels that approach lethality. In cultured cells, if toxicity requires paracrine or humoral factors, one can often test chemicals at much higher concentrations than in the intact animal without seeing toxicity. Due to the possible requirements of humoral factors for many of these processes, the effect in the intact animal might differ markedly from that in cell culture. In either MTD studies in the intact animal or in cell culture studies of a test compound, however, an endogenous receptor in the tissue or cells might easily become overwhelmed by excessive doses of a foreign chemical bearing a structural resemblance to that of endogenous growth effectors. And the subcellular response might have little relationship to real-life situations.

Closing remarks

In conclusion, the so-called DMEs and endogenous receptors are intimately interrelated in signal

transduction pathways involving growth- and differentiation-related processes. The hypothesis described in this paper, and previously [2, 3], is that the steady-state levels of many important growth effector ligands—in prokaryotes as well as eukaryotes—might be controlled by the DMEs. Furthermore, I suggest that the DME control point is *upstream* in the regulatory cascade, before the interaction of endogenous ligands with their receptors (Fig. 4). This proposal would also suggest that, when an "inducer of drug metabolism" is administered to a cell or the intact animal, the cell or organism "senses" the foreign compound as an abnormally elevated concentration of an endogenous ligand (probably, but not always, having a chemical structure similar to the foreign ligand); the response includes the turning on of subsets of DME genes in order to degrade the excessive amounts of this signal and restore "safe" levels once again.

The concept that DMEs are needed only for hepatic drug detoxification is only a small part of the total picture. It is difficult to imagine that there would be any eukaryotic cell without multiple P450 genes and other DME genes expressed. A more appropriate name for enzymes of drug metabolism might therefore be "effector ligand-metabolizing enzymes".

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