ROLES OF CYTOCHROME P450 IN DEVELOPMENT

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CONTENTS

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SUMMARY

Cytochrome P450 (CYP) forms are ubiquitous in nature, appearing in almost all phyla, with many forms appearing in any organism. About 50 different forms have been identified in man, and some of these are found in the embryo, some showing temporal dependence. Many of the forms of cytochrome P450 present in one species have homologues in other species. For example, CYP1A2 is present in many species, including man, rabbits, rodents, fish and fowl. The amino acid sequence identity of these homologues is often in excess of 70%. CYP26, too, has more than 61% identity in amino acid sequence between fish, fowl and mammals. In view of the high degree of conservation of sequence as well as of enzymatic activities, it is only reasonable to assume that such strong conservation of sequence also reflects a conservation of function. Since the 'xenobiotic metabolizing' enzymes predate the production of the many xenobiotics they are known to metabolize, perhaps it is reasonable to consider endobiotics as natural substrates for their metabolism. Of the identified forms of cytochrome P450 that are present in embryonic tissue, we consider the possibility that they serve the organism in support of morphogenesis of the embryonic tissue. These forms may either function to generate morphogenic molecules or to keep regions free of them, thereby creating temporal and spatial regions of morphogen action and supporting region-specific changes in cells. One known morphogen, retinoic acid, has the enzymes retinal dehydrogenase (RALDH) and CYP26 maintaining its actions, the former responsible for its generation and the latter for its elimination. Another form of cytochrome P450, CYP1B1 appears also to be involved in differentiation of tissue, with its absence resulting in primary congenital glaucoma. However, the nature of the morphogen it may maintain still remains to be elucidated.

KEY WORDS

cytochrome P450, embryogenesis, developmental regulation, morphogen metabolism

1. INTRODUCTION

The recognition of the existence of cytochrome P450 hemoproteins dates back to the late 1950s, when a carbon-monoxide-binding pigment was reported to be present in the endoplasmic reticulum of liver /1,2/, and to the later identification of the pigment as a b-type cytochrome /3-5/. Shortly thereafter, the ability of cytochrome P450 to serve as a terminal oxidase in the metabolism of steroids /6/ and xenobiotics /7/ was demonstrated. These observations were quickly followed by the recognition that multiple forms of cytochrome P450 exist in the fragments of endoplasmic reticulum, the microsomes /8-12/.

Studies demonstrating the importance of the different cytochromes P450 in metabolism of drugs and chemicals, and in the activation of various toxicants, teratogens and carcinogens quickly followed /13-19/. Attention subsequently turned to human polymorphisms in cytochrome P450 forms, and their effects on xenobiotic metabolism /20-28/. The importance of the cytochrome P450 enzymes with respect to development of new therapeutic agents was immediately recognized and considerable resources are currently devoted to the interactions of these agents with the different forms of cytochrome P450. Newly developed chemicals being considered for use as drugs are routinely examined for metabolism by different forms of human cytochrome P450, since these represent the major routes of elimination from the body, and metabolites are routinely screened for pharmacological activities. The focus of studies on drug metabolism and xenobiotic activation has resulted in inertia in inquiries as to whether endogenous substrates of cytochrome P450 exist and how such substrates or their metabolites might influence physiological functions. Our objective in the present paper is the review of the literature with respect to our hypothesis that specific members of the cytochrome P450 superfamily may exist which have a role in normal development. By "development" we mean the basic biological phenomena occurring during the generation of a multicellular organism from a single fertilized egg: cell division, pattern formation, morphogenesis, cell differentiation and growth. Such cytochrome P450s might also be capable of *in vitro* metabolism of xenobiotics, but their appearance in the developing embryo at a specific stage of development would suggest a specific role in the development of the organism or of a specific tissue.

Our argument is based on three main elements:

1. Genetic studies have established linkage between P450 mutations and developmental defects.
2. In the developing embryo, a number of cytochrome P450 forms are expressed in extrahepatic tissues undergoing morphogenic transformations.
3. Some cytochrome P450 forms have been shown to be involved in the metabolism of signaling molecules essential for normal development.

2. CURRENT STATUS AND NOMENCLATURE

At present the superfamily of cytochrome P450 consists of about 1200 individual genes, including some 310 mammalian forms, according to the Russian cytochrome P450 database (http://cpd.ibmh.msk.su/online/main/htm). This includes forms in species of all phyla examined, from bacteria to yeast and other primitive eukaryotes to simple plants and trees. There are 17 mammalian families of cytochrome P450. The greatest variability in number of members of the subfamilies lies in families 2, 3 and 4, which contain the endoplasmic reticulum enzymes of xenobiotic metabolism. For example, family 2 has 10 subfamilies (14 if non-mammalian species are included). At present 52 different forms of cytochrome P450 have been identified for the human genome (Fig. 1). Fifteen of these forms are in family 2, four are in family 3 and 11 are in family 4. Thus, more than half of cytochrome P450 families contain single members, each presumably effecting a specific task related to homeostasis in the organism. A number of the forms of cytochrome P450 are present in many species as orthologous proteins. That is, they have at least 45% identity of amino acids in alignments between species (and many have greater than 90% identity) and catalyze the same reaction *in vivo*. These forms are given the same designation, e.g. CYP1B1, or CYP51, the latter an enzyme used in synthesis of cholesterol (or ergosterol in fungi), in the different species. In view of the very large number of forms of cytochrome P450 that exist and the high degree of sequence identity between orthologous forms in different species, it is difficult to consider that these enzymes have developed just to oxidize the drugs and chemicals developed by man in the last couple of centuries. It is possible that very similar orthologous cytochrome P450 enzymes have

retained their functions during evolution over the eons in maintenance of homeostasis and in aid of development of the organism. Below we discuss the functions of cytochrome P450 and its putative roles in the biology of development.

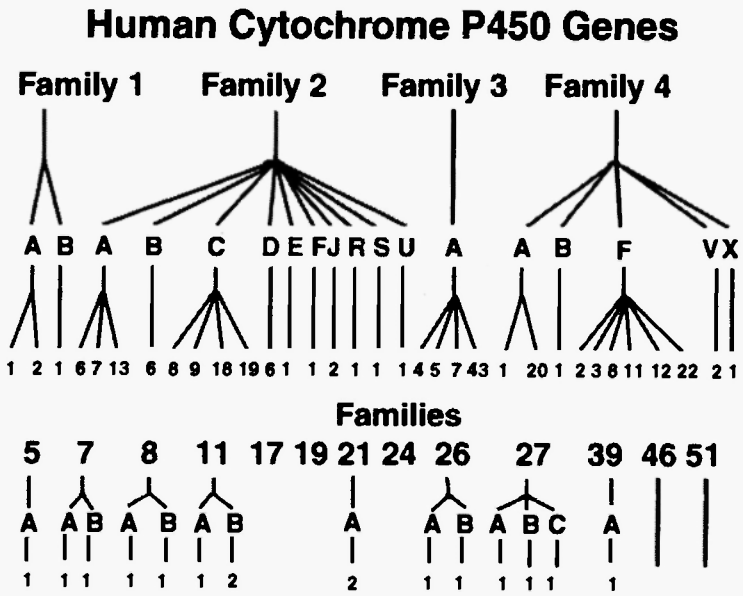

Fig. 1: Human cytochrome P450 genes. The families of cytochrome P450 (CYP) are designated by an Arabic numeral, e.g. CYP1. At present about 157 families are known. Subfamilies are designated by a letter, e.g. CYP1A, and members in the subfamilies by an Arabic numeral, e.g. CYP1A2. Thus, family 1 consists of two subfamilies, CYP1A and CYP1B. CYP1A contains two members, CYP1A1 and CYP1A2, while subfamily 1B only contains one member, CYP1B1. Most of the families of cytochrome P450 contain one or two members, and these often have orthologous forms in other species, e.g. CYP51 is present in fungi, mammals, plants, etc., indicating a high degree of specificity in its function in biological processes.

3. CYTOCHROME P450-PRODUCTION OF CHEMICAL MEDIATORS

3.1 Molecular structure

Cytochrome P450 proteins have an average mass of approximately 50 kDa, and all have an iron protoporphyrin IX (heme) prosthetic group liganded to a cysteine thiolate. All appear to be membrane proteins, with the exception of several bacterial forms. Structurally the proteins are anchored to the endoplasmic reticular membrane or the inner mitochondrial membrane by a transmembrane amino terminus. The very hydrophobic amino-terminal region of the protein contains a membrane insertion sequence as well as a stop-transfer sequence that functions as an anchor in the membrane determining the topological orientation of the cytochrome P450 /29-31/. This hydrophobic region is followed by a proline-rich 'hinge' region, which imparts flexibility between the transmembrane region and the globular catalytic part of the protein that resides in the cytosolic region of the cell or oriented toward the mitochondrial matrix. This flexibility may be necessary to orient the cytosolic portion of the molecule with respect to the membrane for substrate access and for interaction with the appropriate electron transfer partner. At least one of the bacterial forms (CYP102) and one of the mammalian forms of cytochrome P450 (NOS-I) exist as a fusion protein with an electron transfer partner, NADPH-cytochrome P450 reductase /32-36/. The carboxyl-terminal portion of the cytochrome P450 consists of a conserved core structure shared by all members of the cytochrome P450 superfamily /37/. These structures include a number of α-helices and β-sheets and a 'meander' region, all necessary for the proper structural orientation of the heme prosthetic group /37-39/ that makes this family of enzymes mono-oxygenases. Interestingly, as noted below, mutations affecting these structures in CYP1B1 result in abnormal eye development.

3.2 Biochemistry of cytochrome P450

The cytochromes P450 are monooxygenases. They accept two reducing equivalents sequentially and use these to reduce molecular oxygen to an oxidizing species that forms one molecule of water and one oxidized substrate molecule. The monooxygenase reaction can be described by the equation:

$$O_2 + 2e^- + SH + 2H^+ \longrightarrow H_2O + SOH$$

where SH is the substrate to be oxidized and SOH is the oxidized substrate. The types of reactions catalyzed by the different forms of cytochrome P450 are, perhaps, more varied than any other enzyme /40/. Different cytochromes P450 can hydroxylate aliphatic and aromatic carbons and form epoxides across double bonds. They can also remove alkyl groups from nitrogen, oxygen or sulfur atoms by inserting oxygen onto the alkyl moiety. Some are also capable of removing and replacing nitrogen and sulfur in molecules with oxygen, and of the formation of double bonds (dual hydrogen atom abstraction).

3.3. Substrates of cytochrome P450

In considering roles for cytochrome P450 forms in development and in maintenance of homeostasis in organisms it is helpful to recognize that while they may metabolize a wide variety of compounds foreign to the body (xenobiotics), *in vivo* these enzymes may utilize a specific endogenous substrate. Perhaps they generate a stereospecific metabolite targeting a specific receptor. However, members of cytochrome P450 families 1, 2, 3 and 4 are called xenobiotic metabolizing enzymes, and oxidize a large number of lipophilic drugs and chemicals of varying shapes and sizes (Fig. 2) as well as a number

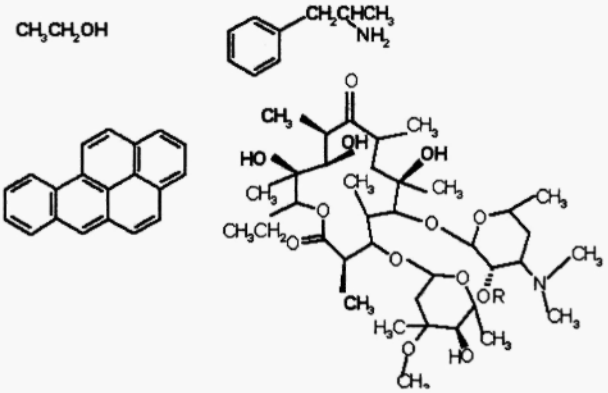

Fig. 2: Some xenobiotic substrates of cytochrome P450. Structures of ethanol, amphetamine, benzo[*a*]pyrene and erythromycin are shown. These are oxidized to the aldehyde, deaminated, oxidized to hydroxy or epoxide metabolite, and *N*-dealkylated, respectively.

of compounds of intermediary metabolism (endobiotics). It was suggested that these enzymes function to decrease the lipid/water partition coefficient of the xenobiotics and endogenous compounds and thereby make them more readily excreted by the kidneys /41/. Substrates range in size from a mass of 36 Da (ethanol), to planar, aromatic polycyclic hydrocarbons such as benzo[*a*]pyrene (252 Da), to large macrolide compounds such as erythromycin (734 Da). The substrate specificities of the different forms of cytochrome P450 are broad and overlapping. However, most will metabolize a number of physiologically relevant compounds of intermediary metabolism, producing different products. For example, the duration of action of a series of barbiturates was shown to be inversely related to their partition coefficients and to their rates of metabolism /40/. As with xenobiotics, elimination of lipophilic waste compounds of endogenous metabolism is also enhanced through oxidation by cytochrome P450 forms. For example, steroids are eliminated as multiple hydroxylated metabolites and their conjugates /42/.

In contrast to the many forms of cytochrome P450 with broad overlapping substrate specificities, many cytochrome P450 forms in other families participate in fairly specific biosynthetic reactions, generating products involved in the homeostasis of the organism. Such forms generally exist in families with only one or two members. Examples include CYP51, involved in formation of cholesterol, phytosterol or ergosterol, CYP27A, which produces bile acids, CYP11A1, which forms progestanes, CYP11B and CYP21, which generate corticosteroids, CYP17, for production of androgens, CYP19 for production of estrogens, CYP2D25 for 25-hydroxyvitamin D_3 activation, and CYP26 which catalyzes catabolism of all-*trans* retinoic acid.

4. STUDIES IMPLICATING CYTOCHROME P450 IN TISSUE DEVELOPMENT

As indicated earlier, evidence has begun to appear that provides an indication that a number of different forms of cytochrome P450 may be involved in development of the organism. Such evidence includes genetic linkage between cytochrome P450 mutations that result in developmental defects, the discrete temporal and spatial localization of different forms of cytochrome P450 in extrahepatic embryonic

tissues, and the identification of the involvement of cytochrome P450 in the metabolism of signaling molecules essential for normal embryonic development.

4.1 Genetic studies link CYP1B1 to developmental eye disorder - primary congenital glaucoma

Genetic linkage analysis of families with primary congenital glaucoma (PCG) identified two chromosomal loci linked to the disease phenotyope - GLC3A on chromosome 2p21 and GLC3B on chromosome 1p36 /43,44/. Efforts to clone the PCG gene residing in locus GLC3A indicated that the gene mutated in individuals with PCG was a cytochrome P450, CYP1B1 /45/, and was demonstrated by quantitative PCR and Northern blot analysis. The genetic linkage of CYP1B1 defects and PCG has been confirmed by similar findings in other laboratories /46,47/. A total of 23 different mutations were shown to segregate with the PCG disease phenotype in affected families and not to be polymorphisms found in the general population /48/. A number of these mutations reported in patients with PCG were mapped against a 3D model of the CYP1B1 molecule constructed by homology modeling /49/. The missense mutations were found to affect highly conserved amino acid residues located predominantly either in the hinge region or the Conserved Core Structures (CCS) /50/ of the CYP1B1 molecule. These mutations therefore are expected to interfere with fundamental properties of the cytochrome P450 molecule, such as proper folding, heme binding, substrate accommodation and interaction with the redox partner. Another group of mutations was predicted to introduce premature stop codons by frameshifts in the CYP1B1 open reading frame. These mutations would eliminate at least the heme-binding region of CYP1B1, which is essential for the normal function of every P450 molecule. Therefore, it is expected that these mutations would result in functional null alleles.

How might these defects translate to PCG? Cytochrome P450 has been shown to be present in bovine eye /51/, and cytochrome P450 metabolic activities were shown to differ in different regions of the eye /52/. As shown in Figure 3, during normal eye development the trabecular meshwork cells are shifted from below the iris junction to a position above that junction to a point where it connects to the anterior chamber of the eye. From that position it can serve the function of filtering the anterior chamber fluid for drainage of that chamber. In

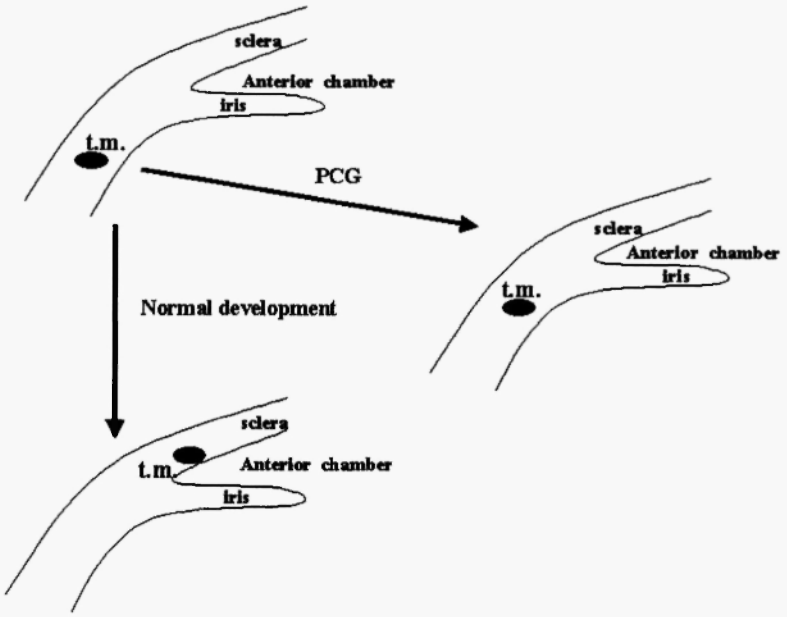

Fig. 3: Scheme of the movement of the trabecular meshwork (t.m.) in the formation of the eye during embryonic development.

PCG the development of that region of the eye is arrested at about that of the 7 month fetus at the time of birth, and pressure in the anterior chamber begins to rise even prior to birth /53/. Two possible scenarios on how CYP1B1 mutations may trigger pathogenic responses resulting in abnormal eye development are: 1) The spatial and temporal expression of genes controlling the anterior chamber angle development may be altered by the absence of a regulatory molecule (such as steroid or lipid metabolite) produced by CYP1B1. 2) Alternatively, the signs of developmental arrest may reflect the toxic effect of a metabolite that is normally eliminated by CYP1B1.

A CYP1B1-null mouse strain has been constructed in which the homozygous animals were reported not to show any evidence of glaucoma /54/. Unfortunately, the methods used to evaluate the mouse (gross examination and standard behavioral comparisons) may not be sensitive enough to detect glaucomatous changes in the mouse eye /55/. In addition, the mouse phenotype may differ from the human, since the anterior chamber angle has undergone some very recent evolutionary changes. For example, only humans and higher apes have

the typical trabecular-type meshwork, while reticular-type meshwork is present in lower organisms.

4.2. Expression of cytochrome P450s in embryonic tissues undergoing morphogenic transformation

Genetic studies mentioned implicate cytochrome P450 in control of normal development. The next step in identifying its role in morphogenic conversion would be to demonstrate that cytochrome P450 is present in embryonic tissue undergoing morphogenic transformation. Numerous studies have reported the presence of individual forms of cytochrome P450 in the developing embryo. Early studies on the involvement of cytochrome P450 in embryogenesis and tissue development demonstrated the presence of mRNA of NADPH-cytochrome P450 reductase and CYP51 in the 4-day (preimplantation) mouse blastocyst. Specific tests for other forms of cytochrome P450 involved in steroid metabolism, CYP17, CYP11A1, CYP19 and CYP27, were negative /56/. In another study, CYP1A1 and CYP1A2 were not found in the rabbit fetus, while CYP3A6 appeared on day 30, the last day prior to birth /57/. In mouse fetus, mRNA encoding CYP2B19 appears in developing keratinocytes in the upper skin layer on day 15 /58/. The distribution of this mRNA was specific to the fetal mouse epidermis. The temporal appearance of this enzyme during initiation of the epidermal stratification suggests a possible involvement in this function. The recombinant protein expressed in *Escherichia coli* was capable of arachidonate metabolism, forming two metabolites, 11,12-epoxyeicosatrienoic acid and 14,15-epoxyeicosatrienoic acid, also found in murine skin. Other metabolites were also found in the *in vitro* assays. An orthologous form of cytochrome P450, CYP2B15, found in rat, has 86% sequence identity to CYP2B19, and like it is specific to keratinocytes /58/.

While these studies have established that cytochrome P450 forms are present in the developing embryo, studies on CYP26 have provided a model for investigating the relevance of cytochrome P450 expression during development. *In situ* hybridization analysis of the spatio-temporal pattern of expression of this orthologous form of cytochrome P450 in the various species demonstrates a relevance of its expression to the pattern of development (see below). The high degree of sequence identity of the CYP26 protein between orthologous forms in different species suggests that the protein is carrying out a

function important for the development of the embryo. For example, the degree of sequence identity between the human and mouse forms of this enzyme is 93%, and the sequence identity between human and zebrafish is 68%. *Xenopus laevis* CYP26 was reported to have 68% amino acid identity to the mouse enzyme /59/. CYP1B1, as indicated above, has similarly been shown to be expressed in the developing eye (mouse) in a pattern consistent with its proposed function as regulator of the anterior chamber angle development. In the case of CYP1B1, the sequence identity between mouse and rat is 93%, and between rat and human 80%. In agreement with a role for the cytochrome P450 monooxygenases in embryogenesis and development is the observation that the electron transfer protein, NADPH-cytochrome P450 reductase, necessary for the proper function of endoplasmic reticular forms of cytochrome P450, also shows differential tissue distribution in developing embryos /60/.

4.3. Cytochrome P450 functions in morphogen metabolism

In the present review we consider the role of cytochrome P450 forms in the development of the organism. Our goal is to provide information supporting a role for this family of enzymes, suggesting forms functioning in morphogenesis and development make use of small molecules (morphogens), which they synthesize or destroy, in support of the gradient concept, as discussed by Crick /61/:

"...One postulates a source - a cell which produces the chemical (which I shall call a morphogen) and maintains it at a constant level. At the other end the extreme cell acts as a sink: that is, it destroys the molecule..." "I doubt if morphogens will turn out to be large proteins or common ions like K^+ or Na^+. The obvious choice would be an organic molecule of the size of, say, cyclic AMP or a steroid. That is, with the molecular weight in the range of 300 to 500."

That vitamin A (retinol) is a requirement for normal embryonic development has been known for almost 75 years. It is a small molecule (Fig. 4) of mass 294 and consists of a benzene ring coupled to a linear 9 carbon polyene sidechain. Its absence results in structural defects, tissue morbidities such as defective heart, nervous system, urogenital system, and eye, in the embryo and newborn animal, and embryonic lethality if the deficiency is severe enough. Similar effects are seen in retinoid receptor-null mutants /62/. Retinol is obtained in

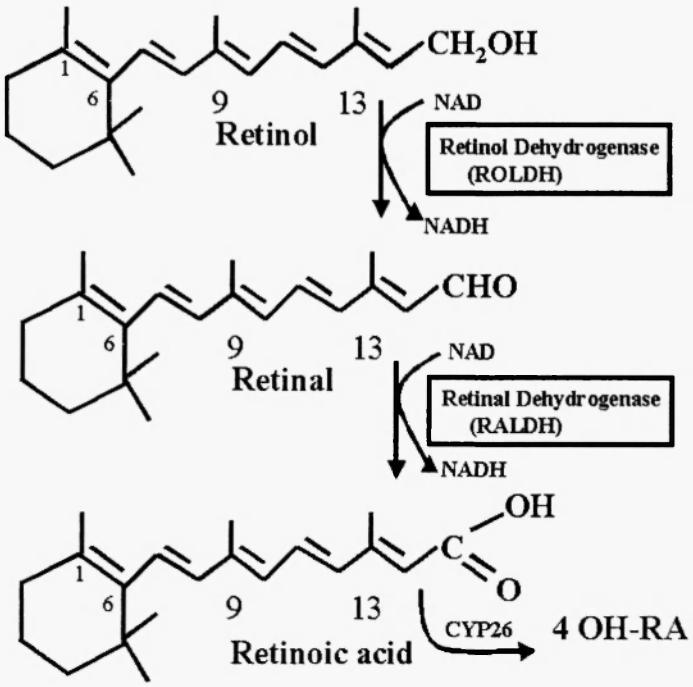

Fig. 4: Steps in the metabolism of vitamin A (retinol) to retinoic acid *in vivo* and the enzymes involved.

the diet from animal sources and β-carotene, a bis-retinal compound that yields retinal on oxidation *in vivo*, is obtained from plant sources. Retinol dehydrogenase, an enzyme distinct from alcohol dehydrogenase, converts the retinol to retinal whereupon another distinct enzyme, retinal dehydrogenase-2 (RALDH-2), converts it to retinoic acid, the active compound /63/. In studies with RALDH-2-null mice it could be shown that embryos negative for RALDH-2 generally die at mid-gestation /63/. At least two retinoid receptor families exist in the nucleus and serve as ligand-activated transcriptional regulators. These include the all-*trans* and 9-*cis* retinoic acid activated receptors (RAR) and the retinoid X-receptor (RXR), which is only activated by 9-*cis* retinoic acid, which bind to a retinoic acid response element in the promoter region of target genes /64/. Each of the two receptor family types consists of three isotypes, α, β, and γ /65/. Two cytoplasmic binding proteins (CRABP I and CRABP II) exist which have been

suggested to have a modulating effect on retinoic acid levels reaching the nucleus /64/. While retinoic acid has been unequivocally demonstrated to have positive influences on the development of specific regions of the embryo, based upon teratogenic effects of pharmacological levels applied, abnormalities resulting from deficiency, and on RAR and RXR (null) constructs, in order to be considered a morphogen another criterion must be met, i.e. the existence of a sink. Such a sink was discovered in the form of CYP26 /66/, a form of cytochrome P450 that specifically catabolizes retinoic acid to the less effective 4-hydroxyretinoic acid metabolite (Fig. 4). CYP26 and RALDH-2 provide the necessary functions that make retinoic acid a morphogen. They are differentially and exclusively distributed in the embryo, with levels both temporally and spatially distinct (Fig. 5). Their distinctly regionalized and nonoverlapping boundaries of distribution create a morphogenic gradient of retinoic acid that explains the head-to-tail axis formation /67/. As shown in Figure 5, retinoic acid is generated by RALDH in the posterior region of the embryo and its diffusion is curtailed by CYP26 in the anterior portion of the embryo, resulting in a sharp boundary between areas impacted by retinoic acid and areas not impacted by the agent.

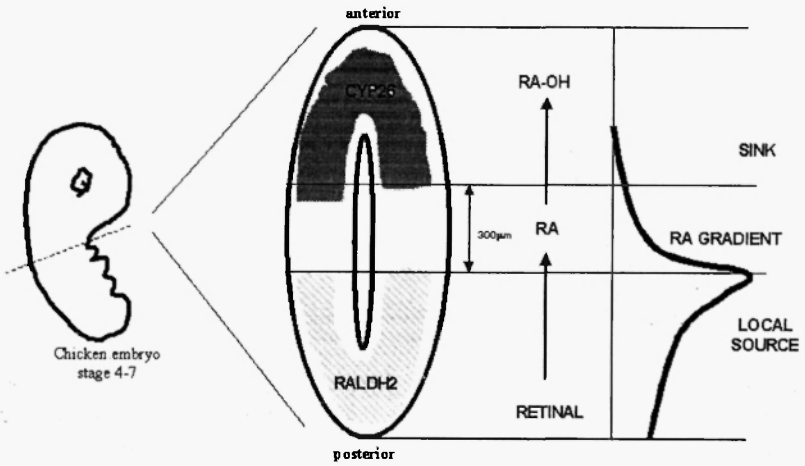

Fig. 5: Scheme depicting the retinoic acid gradient serving as a morphogen in the developing embryo.

Interestingly, a number of other forms of human cytochrome P450 have been identified that can also 4-hydroxylate retinoic acid /68/.

These include CYP2C8, CYP3A4, and CYP2C9. This is not unexpected, in view of the broad, overlapping spectrum of substrates of cytochrome P450 forms. However, these enzymes do not appear to be expressed in the developing embryo and thus do not appear to be involved in morphogenesis. Based upon inhibition of *trans* retinoic acid 4-hydroxylation in human fetal liver by the CYP3 inhibitor, troleandomycin, it was suggested that CYP3A7 plays a role in detoxifying this compound /69/. In contrast, based upon lack of effect of specific inhibitors, it was concluded /69/ that hepatic CYP1A1, CYP1A2, CYP1B1, CYP2C8 and CYP2E1 did not metabolize retinoic acids. At present, only CYP26, like CYP1B1, has been shown clearly to have a role in morphogenesis. However, discovery of CYP26 followed establishment of retinoic acid as a morphogen. In the case of CYP1B1, the opposite is true. The involvement of CYP1B1 in normal eye development was discovered by genetic linkage. The more difficult task that remains is to determine the nature of the morphogen involved in normal eye development and influenced by CYP1B1.

5. EXAMINATION OF PUTATIVE MORPHOGENIC AND PROMORPHOGENIC SUBSTRATES OF CYP1B1

If cytochrome P450 forms are responsible for morphogenic activity, we would expect the promorphogen or morphogen to be a small molecule of lipophilic nature, i.e. a molecule with characteristics similar to that of other known cytochrome P450 substrates. Examples of some known cytochrome P450 substrates and metabolites are shown in Figure 6. With the exception of nitric oxide synthetase (e.g. NOS-1), all of the forms of cytochrome P450 metabolize lipophilic compounds. Substrates include fatty acids, producing ω- and (ω-1)-hydroxylation products /58,70-73/, prostaglandins, yielding ω- to (ω-2)-hydroxylation metabolites /74-76/, and leukotriene ω- and (ω-1)-hydroxylation products /77/. Other metabolites of endogenous substrates include isomeric and epimeric-specific androgen, estrogen and progestane hydroxylation products /78-85/. The many potential endogenous substrates of the different cytochrome P450 forms and their potential metabolites, that may be involved in morphogen formation, make it difficult to predict what may or may not be a morphogen, as in the case of the CYP1B1-deficiency PCG phenotype. However, continued studies on the substrate specificity and metabolite profile of

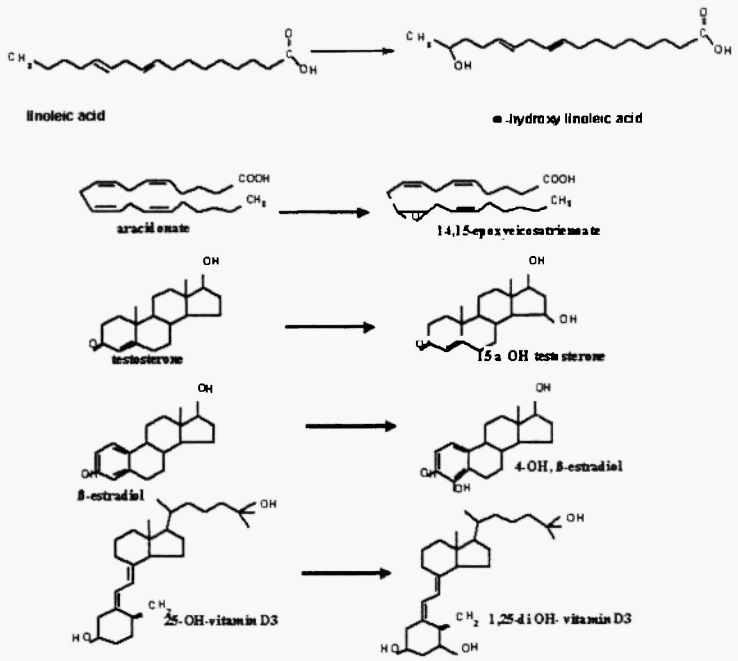

Fig. 6: Some of the endogenous (endobiotic) substrates of cytochrome P450. All of the substrates are converted to numerous metabolites by the different forms of cytochrome P450, but only a single metabolite of each is shown.

this enzyme may yield information, perhaps identifying the specific agent serving as morphogen.

From the data reviewed, it appears that different forms of cytochrome P450 are present in different regions of the adult eye, and in the developing eye tissue, based upon differences in location of xenobiotic metabolizing activities and NADPH-cytochrome P450 reductase. It was hypothesized that metabolites of cytochrome P450 forms might have physiological functions. Indeed, the ciliary body of the eye was seen to have the greatest xenobiotic metabolizing activity, followed by the retinal pigment epithelium /52/. An extension of this hypothesis was the proposal that the development of the different "drug metabolizing (P450) enzymes" from early evolutionary forms might relate to their ability to utilize endogenous substrates necessary for the regulation of processes of growth and differentiation /86/.

6. CONCLUSIONS

Cytochrome P450 forms are very strong suspects as potential players in the development of the organism. Because of their great diversity in both substrate recognition and stereochemical diversity in product production, they are strong candidates for morphogen production. Which form of cytochrome P450 appears in a tissue at a particular time will determine what will be produced from a particular endogenous substrate that presents itself at that time. Depending upon the form of cytochrome P450 that appears, the endogenous substrate may be converted to a metabolite for elimination or to a morphogen, or activated to a teratogen or carcinogen. At least one instance, the conversion of the metabolite retinoic acid to a less active 4-hydroxy-retinoic acid, enables developmental changes during embryonic development. In another instance, that of eye development, absence of CYP1B1 causes cessation of the trabecular meshwork development in the eye and results in PCG. Studies are currently underway to determine the nature of the morphogen in normal eye development.

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