Molecular Basis of Disease

Cytochrome P450s in humans Feb. 4, 2009

David Nelson (last modified Jan. 4, 2009)

Reading (optional)

Nelson D.R. Cytochrome P450 and the individuality of species. (1999)
Arch. Biochem. Biophys. **369,** 1-10.

Nelson et al. 2004 Comparison of cytochrome P450 (*CYP*) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes, and alternative-splice variants Pharmacogenetics 14, 1-18

Objectives:

This lecture provides a survey of the importance of cytochrome P450s in humans. Please **do not memorize** the pathways or structures given in the notes or in the lecture. Do be aware of the major categories of P450 function in human metabolism, like synthesis and elimination of cholesterol, regulation of blood hemostasis, steroid and arachidonic acid metabolism, drug metabolism. Be particularly aware of drug interactions and the important role of CYP2D6 and CYP3A4 in this process.

You will **not** be asked historical questions about P450 discovery.

You will **not** be asked what enzyme causes what disease.

Understand that P450s are found in two different compartments and that they have two different electron transfer chains in these compartments.

Understand that P450s are often phase I drug metabolism enzymes and what this means.

Be aware that rodents and humans are quite different in their P450 content. The same P450 families are present but the number of genes is much higher in the mouse. What is the relevance to drug studies?

Understand that P450s can be regulated or induced by certain hormones or chemicals.

Know that the levels of individual P450s can be monitored by non-invasive procedures.

Be aware of the significance of polymorphisms in human P450s and their effects on drug metabolism and drug interactions.

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Cytochrome P450s in humans

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Cytochrome P450 proteins in humans are drug metabolizing enzymes and enzymes that are used to make cholesterol, steroids and other important lipids such as prostacyclins and thromboxane A2. These last two are metabolites of arachidonic acid. Mutations in cytochrome P450 genes or deficiencies of the enzymes are responsible for several human diseases. Induction of some P450s (i.e. by cigarette smoking) is a risk factor in several cancers since these enzymes can convert procarcinogens to carcinogens.

The name cytochrome P450 derives from the fact that these proteins have a heme group, and an unusual spectrum. Mammalian cytochrome P450s are membrane bound. They were originally discovered in rat liver microsomes. Microsomes are turbid suspensions made by grinding up cells and isolating the membrane fraction that is still in suspension after the cell debris and mitochondria have been pelleted. These mixtures are very opaque to standard spectroscopy, because they scatter light so badly. The only way to measure a spectrum on turbid samples like these was to make a special instrument with the light detector very close to the cuvette, and to use dual beams and do difference spectroscopy. In this way all the interfering substances and the light scattering could be subtracted out. With this setup, microsomes treated with dithionite (reduced microsomes) and with carbon monoxide gas added to one cuvette only give a very strong absorption band at 450 nm, thus P450 (P is for pigment). This is called a reduced CO difference spectrum. The CO binds tightly to the ferrous heme, giving a difference between the absorbance of the two cuvettes. This spectrum was first observed in 1958.

Other heme containing proteins don't absorb at 450 nm. The reason why cytochrome P450 absorbs in this range is the unusual ligand to the heme iron. Four ligands are provided by nitrogens on the heme ring. Above and below the plane of the heme, there is room for two more ligands, the 5th and 6th ligands. In cytochrome P450s, the 5th ligand is a thiolate anion, a sulfur with a negative charge, S(-). The sulfur comes from a conserved cysteine at the heme binding region of the active site.

Over 150 X-ray crystal structures are now available for P450s in the Entrez Structure database. Stuctures are known for at least 21 different soluble P450s mostly from bacteria (CYP51B1 [Mycobacterium], 55A1 [fungal], 101A1, 102A1, 107A1 [eryF], 107L1, 111A1, 119A1, 119A2, 121A1 [P450Mt2], 130A, 152A1, 154A1, 154C1, 158A2, 165B3, 165C4, 167A1, 175A1, 203A1, 231A). The eukaryotic P450s except fungal CYP55s are membrane bound. There are 12 different mammalian P450 structures known. [1A2, 2A6, 2B4, 2C5, 2C8, 2C9, 2D6, 2R1, 3A4, 7A1, 8A1, 46A1]. These structures are similar to the soluble P450s except for the membrane anchoring parts. Here is a picture of the first P450 crystallized. This is P450 cam from Pseudomonas putida, a bacterium that can use camphor as its sole carbon source. 45 of the P450 structures in Entrez Structure are of P450 cam. This bug is found growing in soil under camphor trees. The protein is shaped like a triangle with the heme buried deep inside. In this structure, there is no access channel for substrate or products, even water, to enter or leave the active site. Therefore, we must assume that the structure breathes when it functions, so a channel will be open at some point in the catalytic cycle. The mammalian CYP2B4 P450 has been caught in an open configuration in a crystal structure. The mammalian P450s are similar to the bacterial fold, but with an N-terminal membrane anchor. A cartoon of one possible view is given here. This model shows a single transmembrane segment, but membrane attachment is more complex than that. When the N-terminal sequence is removed the protein

still sticks to membranes. The mammalian CYP2C5 protein was the first mammalian P450 crystallized after removal of the N-terminal anchor peptide and replacement of an internal hydrophobic sequence with a more water soluble sequence from a related enzyme. The X-ray structure has been solved (Williams PA, Cosme J, Sridhar V, Johnson EF, McRee DE. J Inorg Biochem 2000 Aug 31;81(3):183-90 Microsomal cytochrome P450 2C5: comparison to microbial P450s and unique features.) A brief description of the main features had appeared earlier (Arch. Biochem. Biophys. 369 Sept. 1, 24-29 1999). The structure is similar to the soluble bacterial enzymes, but there are significant differences.

The phamaceutical industry is very interested in P450 crystal structures with drugs bound, so they can do improvement of drug design. Here we see a press release from May 3, 2001 about a research agreement between Astex and AstraZeneca to determine crystal structures of human P450s with AstraZeneca drugs bound in the active site. Look at the outlined sections. "Cytochrome P450 enzymes are the most prominent group of drug-metabolising enzymes in humans, and consequently are of great importance to the pharmaceutical industry. Application of Astex's technology to determine the three dimensional structures of human cytochrome P450 enxymes complexed with AstraZeneca's compounds will facilitate rapid design of drug candidates with greater potential for clinical success. AstraZeneca is one of the top five pharmaceutical companies in the world with 2000 healthcare sales of $15.8 billion." Astex has now solved the crystal structures of CYP2C9 and CYP3A4, two of the most significant drug metabolising enzymes in humans. These structures can now be used to modify existing drugs to make them poorer substrates for 3A4 (or better substrates). Poorer P450 substrates would last longer in the body before elimination, which is desirable for the pharmaceutical industry.

P450s catalyze many types of reactions, but the one that is most important for us is hydroxylation. These enzymes are called mixed function oxidases or monooxygenases, because they incorporate one atom of molecular oxygen into the substrate and one atom into water. They differ from dioxygenases that incorporate both atoms of molecular oxygen into the substrate.

Foreign chemicals or drugs are also called xenobiotics. Cytochrome P450s play an important role in xenobiotic metabolism, especially for lipophilic drugs. The metabolism of these compounds takes place in two phases. Phase I is chemical modification to add a functional group that can be used to attach a conjugate. The conjugate makes the modified compound more water soluble so it can be excreted in the urine. Many P450s add a hydroxyl group in a Phase I step of drug metabolism. The hydroxyl then serves as the site for further modifications in Phase 2 drug metabolism.

For cytochrome P450s to function, they also need a source of electrons. The addition of two electrons (reduction) to the heme iron makes the difficult chemistry of breaking the oxygen-oxygen bond possible. The electrons are donated by another protein that binds briefly to the P450 and passes an electron from a prosthetic group. This handoff of electrons between proteins is called an electron transfer chain, and it is similar to the electron transfers that go on in complexes I to IV of the electron transfer chain in mitochondria. (However, this is not the same electron transfer chain.)

There are two different kinds of electron transfer chains for cytochrome P450s. These depend on the location of the enzyme in the cell. Some P450s are found in the mitochondrial inner membrane and some are found in the endoplasmic reticulum (ER). Both types of P450s are membrane bound proteins. The protein that donates electrons to P450s in the ER is called NADPH cytochrome P450 reductase. It is also membrane bound by an N-terminal tail that crosses the ER membrane once. The bulk of this protein is on the cytosolic

side of the ER membrane. This protein has two domains that each contain one flavin. Two electrons are acquired from NADPH and migrate from FAD to FMN, then to the P450 heme iron.

In the mitochondria, the electron transfer chain is a little longer. Ferredoxin (called adrenodoxin in the adrenals, but exactly the same gene codes for both proteins) is the immediate donor of electrons to the P450s in mitochondria (CYP11A1, CYP11B1, CYP11B2, CYP24, CYP27A1, CYP27B1, CYP27C1). Ferredoxin has an iron sulfur cluster instead of a flavin, however, ferredoxin is reduced by ferredoxin reductase (or adrenodoxin reductase in the adrenals) that does contain a flavin. NADPH is the source of electrons that flow from ferredoxin reductase to ferredoxin and then to P450. A few P450s also can accept electrons from cytochrome b5. This is a small membrane bound heme containing protein that gets its reducing equivalents (electrons) from NADH.

The families of human P450s

The P450 proteins are categorized into families and subfamilies by their sequence similarities. Sequences that are greater than 40% identical at the amino acid level belong to the same family. Sequences that are greater than 55% identical are in the same subfamily. There are now more than 9000 named cytochrome P450 sequences.

Humans have 18 families of cytochrome P450 genes and 44 subfamilies

CYP1 drug metabolism (3 subfamilies, 3 genes, 1 pseudogene)
CYP2 drug and steroid metabolism (13 subfamilies, 16 genes, 16 pseudogenes)
CYP3 drug metabolism (1 subfamily, 4 genes, 2 pseudogenes)
CYP4 arachidonic acid or fatty acid metabolism (6 subfamilies, 12 genes, 10 pseudogenes)
CYP5 Thromboxane A2 synthase (1 subfamily, 1 gene)
CYP7A bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus (1 subfamily member)
CYP7B brain specific form of 7-alpha hydroxylase (1 subfamily member)
CYP8A prostacyclin synthase (1 subfamily member)
CYP8B bile acid biosynthesis (1 subfamily member)
CYP11 steroid biosynthesis (2 subfamilies, 3 genes)
CYP17 steroid biosynthesis (1 subfamily, 1 gene) 17-alpha hydroxylase
CYP19 steroid biosynthesis (1 subfamily, 1 gene) aromatase forms estrogen
CYP20 Unknown function (1 subfamily, 1 gene)
CYP21 steroid biosynthesis (1 subfamily, 1 gene, 1 pseudogene)
CYP24 vitamin D degradation (1 subfamily, 1 gene)
CYP26A retinoic acid hydroxylase important in development (1 subfamily member)
CYP26B retinoic acid hydroxylase (1 subfamily member)
CYP26C retinoic acid hydroxylase important in development (1 subfamily member)
CYP27A bile acid biosynthesis (1 subfamily member)
CYP27B Vitamin D3 1-alpha hydroxylase activates vitamin D3 (1 subfamily member)
CYP27C Unknown function (1 subfamily member)
CYP39 7 alpha hydroxylation of 24 hydroxy cholesterol (1 subfamily member)
CYP46 cholesterol 24-hydroxylase (1 subfamily member)
CYP51 cholesterol biosynthesis (1 subfamily, 1 gene, 3 pseudogenes) lanosterol 14-alpha demethylase

Humans have 57 sequenced CYP genes and 58 pseudogenes.

only full length functional genes are named below. All these names include the prefix CYP.

1A1, 1A2, 1B1, 2A6, 2A7, 2A13, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2F1,
2J2, 2R1, 2S1, 2U1, 2W1, 3A4, 3A5, 3A7, 3A43, 4A11, 4A22, 4B1, 4F2, 4F3,
4F8, 4F11, 4F12, 4F22, 4V2, 4X1, 4Z1 5A1, 7A1, 7B1, 8A1, 8B1, 11A1, 11B1,
11B2, 17, 19, 20, 21A2, 24, 26A1, 26B1, 26C1, 27A1, 27B1, 27C1, 39, 46, 51,

Detailed information on mouse, human, dog, cattle, rat and other species P450s
can be found on my website http://drnelson.utmem.edu/CytochromeP450.html

A P450 name followed by P stands for a pseudogene. A pseudogene is a defective gene that does not produce a functional protein. There are several reasons why this happens, but in the end, the protein product is not made. Pseudogenes are relics of gene duplications where one of the copies has degenerated and lost its function.

Induction of P450 enzymes.

P450 enzymes have a variety of gene regulatory mechanisms. Many of these genes can be turned on or induced by a chemical signal. The steroid hormones are under strict endocrine control. Their levels are tightly regulated. One example is the induction of steroid biosynthetic P450s by ACTH adrenocorticotropic hormone. ACTH stimulates production of cAMP that presumably activates a protein kinase that phosphorylates some unidentified protein, leading to an increase in gene transcription.

Another type of P450 gene regulation is that shown by peroxisome proliferators like clofibrate. These drugs act through a binding protein called the PPAR or peroxisome proliferator activated receptor. When drug is bound to this protein it migrates to the nucleus, heterodimerizes with retinoid X receptor (RXR) and binds to specific DNA sequences in the regulatory region of genes that are needed for peroxisome generation. The CYP4A1 gene is turned on by this mechanism. Peroxisomes oxidize fatty acids and the CYP4A1 is a known fatty acid hydroxylase.

The members of the CYP1 family are induced by aromatic hydrocarbons. The activation involves a specialized receptor called the Ah receptor. Ah stands for aryl hydrocarbon. This receptor protein binds the aromatic hydrocarbon, but it cannot reach the nucleus to activate gene transcription without another protein called arnt for Ah receptor nuclear translocator. These two proteins bind and together, they enter the nucleus, then bind DNA and activate transcription.

Other chemicals also induce P450s. Ethanol induces the CYP2E enzymes. Phenobarbital induces the rat CYP2B enzymes 40-50 fold, through a phenobarbital receptor called CAR. This receptor also dimerizes with RXR as seen above with the PPAR receptor. The heterodimer binds to a phenobarbital response element in the DNA to activate the gene. For details on these receptor mediated induction mechanisms see the review by Waxman (Archives Biochem. Biophys. 369, 11-23, 1999). The general feature that many

P450 enzymes are inducible is probably related to P450's role in detoxification of foreign chemicals found in plants.

Noninvasive markers for measuring levels of P450 enzymes in humans

P450 enzymes catalyze specific reactions that can be monitored by sampling the urine, blood or breath of patients given a noninvasive marker. Caffeine is a marker for CYP1A2. It is demethylated, and the rate at which it is demethylated is related to the amount of CYP1A2 in a person's liver. By administering caffeine and measuring the rate of demethylation, it is possible to estimate the level of CYP1A2 in a human. This can show if a person has been induced by exposure to polycyclic aromatic hydrocarbons (PAHs). There are a variety of non-invasive markers for different P450s. Assays of CYP1A enzymes from fish livers can also be used to monitor water pollution levels, since certain types of pollutants will induce the enzyme. This is also being done in soil using nematodes like C. elegans.

Functions of human P450s and diseases caused by defects in P450s

The **CYP1** family of P450s can hydroxylate estrogen (CYP1A2 and 1B1) and oxidize uroporphyrinogen to uroporphyrin (CYP1A2) in heme metabolism, but they may have additional undiscovered endogenous substrates. These enzymes are inducible by some polycyclic hydrocarbons, some of which are found in cigarette smoke and charred food. These enzymes are of interest, because in assays, they can activate compounds to carcinogens. High levels of CYP1A2 have been linked to an increased risk of colon cancer. Since the 1A2 enzyme can be induced by cigarette smoking, this links smoking with colon cancer. The CYP1A2 crystal structure is now known.

The **CYP1B1** gene has been linked to primary congenital glaucoma (See Human Molecular Genetics 6, 641-7, 1997; Am J Hum Genet. 62, 325-33 1998; Am J Hum Genet. 62, 573-84 1998; J Med Genet. 36, 290-4 1999). The normal substrate in mammals is not known, but it is speculated that this P450 may be required to eliminate a signaling molecule. Defects in the gene could lead to chronic high concentrations of the signaling molecule that lead to glaucoma. The molecule affected may be a steroid.

As you can see from the table of human P450s, the 2 family is the largest family in humans. 28% of human P450s are in this family. Many of these proteins can hydroxylate steroids, and some of them are expressed in a sex specific manner. This would be expected for enzymes that only act on sex specific steroids. Some of these may also be drug metabolism enzymes that are defensive, to protect us from toxins in our food. Plants especially make many toxic components that are probably defensive for the plants. Since we eat almost anything, it is necessary to have a detoxification system coded in our genes. This idea has been called plant animal warfare on the chemical level.

CYP2B is inducible by barbiturates in rodents. It was one of the first P450s to be purified from mammals, but its role in humans is not understood.

CYP2C8 is known to catalyze the 6-alpha hydroxylation of taxol. This is a drug used in treating breast cancer. The crystal structure of human 2C8 is now known.

CYP2C9 is one of 12 human P450s that has a known crystal structure (see page 1 for a list). The CYP2C9 structure was published in Nature (Williams et al. Crystal structure of human cytochrome P450

2C9 with bound warfarin. Nature. Jul 24; 424, 464-468 2003.) CYP3A4 in Yano et al. The structure of human microsomal cytochrome P450 3A4 determined by X-ray crystallography to 2.05-A resolution. J Biol Chem. Sep 10, 279, 38091-4. 2004 Williams et al. Crystal structures of human cytochrome P450 3A4 bound to metyrapone and progesterone. Science. Jul 30 305, 683-6 2004. CYP2A6 structure: Nat Struct Mol Biol. 12, 822-823 (2005).

CYP2C19 metabolizes omeprazole, a common ulcer medication. Polymorphisms in this gene cause a higher incidence of poor metabolizer phenotypes in Asians (23%) vs caucasians (3-5%).

Drug metabolism differences caused by polymorphisms in P450s.

A polymorphism is a difference in DNA sequence found at 1% or higher in a population. These differences in DNA sequence can lead to differences in drug metabolism, so they are important features of P450 genes in humans. CYP2C19 has a polymorphism that changes the enzyme's ability to metabolize mephenytoin (a marker drug). In Caucasians, the polymorphism for the poor metabolizer phenotype is only seen in 3% of the population. However, it is seen in 20% of the asian population. Because of this difference, it is important to be aware of a person's race when drugs are given that are metabolized differently by different populations. Some drugs that have a narrow range of effective dose before they become toxic might be overdosed in a poor metabolizer. Very recently, Roche has marketed a CYP450 DNA chip to detect major known polymorphisms in human CYP2D6 and CYP2C19. For about $400 you can test a person to see if they are a poor metabolizer, normal metabolizer or ultra metabolizer, for a large number of drugs. Since 1A2, 2C9, 2C19, 2D6 and 3A4 are responsible for oxidizing more than 90% of currently used drugs (2C9 paper above), this is a significant beginning to characterizing risk of adverse drug reactions in people.A cytochrome P450 allele website is available from Sweden at http://www.imm.ki.se/CYPalleles/

CYP2D6 is perhaps the best studied P450 with a drug metabolism polymorphism. This enzyme is responsible for more than 70 different drug oxidations. Since there may be no other way to clear these drugs from the system, poor metabolizers may be at severe risk for adverse drug reactions. I heard a statistic at a meeting that adverse drug reactions are the number 4 cause of hospitalization in the US. There are at least 72 named alleles identified in CYP2D6. The crystal structure is known: J Biol Chem. 281, 7614-7622 (2006).

CYP2D6 Substrates

Antiarrhythmics: Flecainide, Mexiletine, Propafenone
Antidepressants: Amitriptyline, Paroxetine, Venlafaxine, Fluoxetine (Prozac), Trazadone
Antipsychotics: Clorpromazine, Haloperidol, Thoridazine
Beta-Blockers: Labetalol, Timolol, Propanolol, Pindolol, Metoprolol
Analgesics: Codeine, Fentanyl, Meperidine, Oxycodone, Propoxyphene

CYP2E1 is induced in alcoholics. There is a polymorphism associated with this gene that is more common in Chinese people. The mutation correlates with a 2-fold increased risk of nasopharyngeal cancer linked to smoking. This is the second P450 enzyme that may be related to smoking induced cancer (see 1A2 above).

The **CYP3A** subfamily is one of the most important drug metabolizing families in humans. CYP3A4 is "the most abundantly expressed P450 in human liver". (Arch. Biochem. Biophys. 369, 11-23 1999) The

color of perfused liver is due to this protein. CYP3A4 is known to metabolize more than 120 different drugs. Some of these are well known and I give a list here of some of the recognizable ones.

CYP3A4 Substrates
Acetominophen (Tylenol)
Codeine (narcotic)
Cyclosporin A (an immunosuppresant),
Diazepam (Valium)
Erythromycin (antibiotic)
Lidocaine (anaesthetic),
Lovastatin (HMGCoA reductase inhibitor, a cholesterol lowering drug),
Taxol (cancer drug),
Warfarin (anticoagulant).

Poisoning by acetominophen overdose is caused by CYP2E1 in the liver and kidney that convert acetominophen into a very toxic intermediate that can react with cellular macromolecules to damage cells and eventually kill them. This intermediate normally reacts with glutathione, a natural antioxidant in cells. It is only when the glutathione is depleted that cell death can occur. That's why acetominophen overdoses don't have any serious symptoms until 3-4 days later. This problem is worse in alcoholics, since they have induced CYP2E1 that makes more of the toxic intermediate. The antidote to acetaminophen overdose is N-acetylcysteine (NAC) to restore glutathione levels. It is most effective when given within 8 hours of ingesting acetaminophen.

There are common drugs given for special purposes that inhibit P450 enzymes. These include erythromycin (an antibiotic), ketoconazole, and itraconazole (both antifungals that inhibit the fungal CYP51 and unintentionally they also inhibit CYP3A4). If these drugs are given with other drugs that are normally metabolized by P450 enzymes, the lifetime of these other drugs will be prolonged, and plasma levels will be increased, since they won't be cleared as fast. If these drugs affect heart rhythms or other critical systems, the result can be fatal. For example, inhibition of CYP3A4 in a patient taking warfarin can cause bleeding.

This is called a **drug interaction**. Drug interactions are one of the major causes of death in hospitalized patients. The risk of an adverse drug interaction increases with the number of drugs taken, with a probability of 40% when 10 drugs or more are taken. The most serious cases are due to drug metabolism by P450 enzymes. A case report of a 63 year old man receiving medication for major depression showed he boarded a plane in Toronto to fly to London. On arrival he was unrousable. In his Carry-on bag he had Mefadazone (for depression), Ketoconazole (for fungal infection) and Triazolam (an antipsychotic also used for insomnia). All three of these drugs bind to CYP3A4. Ketoconazole inhibits CYP3A4 and probably caused the other two drugs to become overdosed.

Another example is terfenadine (a non-sedating anti-histamine) with ketoconazole. Studies in 1993 (Honig) showed a 15-72 fold increase in terfenadine AUC (Area under the curve) due to inhibition of CYP3A4 by ketoconazole. Torsades de pointes (TDP) is a potentially fatal ventricular tachycardia. TDP is a side-effect that has led to withdrawal of several drugs from the market including terfenadine. This is a case where a 72 fold increase in drug dose might harm or even kill a patient.

Another factor in drug dosage is interfering substances from food. Grapefruit juice contains a CYP3A4 inhibitor (6',7'- dihydroxybergamottin) that causes about a 12 fold increase in some drug concentrations. And the effect lasts for several days. It is advisable to discourage your patients from drinking grapefruit juice while on medication metabolized by CYP3A4. I have read that some drugs of abuse are being taken with grapefruit juice to enhance their effect.

Now we will leave the drug metabolizing enzymes behind and talk about P450s that are very specific in their reactions, just the opposite of CYP3A4. These enzymes tend to be in families with one or two members and they have only one substrate. Most of these enzymes use steroids or steroid precursors as their substrates.

CYP5 is the thromboxane A2 synthase. Thromboxane A2 is a fatty acid in the arachidonic acid cascade. Arachidonic acid can be metabolized in two pathways, the linear pathway that leads to leukotrienes, and the cyclic pathway that leads to prostaglandins and thromboxanes. The first enzymes leading to cyclic products of arachidonic acid are cyclooxygenases 1 and 2. These enzymes are inhibited by aspirin and non-steroidal antiinflammatory drugs (NSAIDS). Aspirin acetylates a serine in the enzyme that blocks the binding of arachidonic acid. Current research shows that COX2 is inducible and is found to be induced in inflammation. COX1 is constitutive. This difference suggests that COX2 specific inhibitors would block inflammation while not interfering with the beneficial effects of COX1, such as maintaining the stomach lining. One of these drugs, VIOXX, was recently taken off the market. After this step the pathway branches. Two of the branches include cytochrome P450 reactions. One leads to thromboxane A2 (CYP5) and the other to prostacyclin (CYP8A1). Thromboxane A2 causes platelet aggregation and that is why aspirin prevents platelet aggregation. Prostacyclin acts in opposition to thromboxane A2. It is a vasodilator and an inhibitor of platelet aggregation. The acetylation of COX1 and COX2 in platelets is critical since the platelets have no nucleus and cannot resynthesize the inhibited enzymes.

CYP7A is the first and rate limiting step of bile acid synthesis. This pathway is the only means the body has of eliminating cholesterol in liver. As we will see later, CYP51 is a key enzyme in cholesterol biosynthesis, so P450s are active at both ends of cholesterol metabolism. In the summer of 2003, patients were found with defects in this gene. They had elevated levels of cholesterol, decreased levels of bile acids and increased triglycerides as a compensation for the reduced bile acids. John Kane et al. Journal of Clin. Invest. July 2002.

CYP7B a novel brain cytochrome P450, catalyzes the synthesis of neurosteroids 7-alpha hydroxy dehydroepiandrosterone and 7-alpha hydroxy pregnenolone Proc. Natl. Acad. Sci. USA 94, 4925-4930 (1997)

CYP8A is prostacyclin synthase (prostaglandin I2). It is part of a regulatory component of hemostasis that opposes CYP5 that makes thromboxane A2. Crystal structure: J. Mol. Biol. 364, 266-274 (2006).

CYP8B is the 12-alpha hydroxylase needed in bile acid biosynthesis

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CYP11A1 is the side chain cleavage enzyme that converts cholesterol to pregnenolone. This is the first step in steroid biosynthesis. Defects in this enzyme lead to a lack of glucocorticoids, feminization and hypertension. [mitochondrial]

CYP11B1 is the 11-beta hydroxylase enzyme that can act on 11-deoxycortisol to make cortisol or it can hydroxylate 11-deoxycorticosterone to make corticosterone. [mitochondrial] Defects in this gene lead to congenital adrenal hyperplasia.

CYP11B2 is aldosterone synthase that hydroxylates coricosterone at the 18 position. [mitochondrial] Defects in this gene lead to congenital hypoaldosteronism.

CYP17 is the 17 alpha hydroxylase and 17-20 lyase (two enzymes in one). A mutation in this gene is described in Nature Genetics 17, 201-205 (1997) that causes the loss of the 17-20 lyase activity without affecting the 17 hydroxylase activity. This enzyme is required for production of testosterone and estrogen. Defects in this enzyme affect proper development at puberty.

CYP19 is aromatase that makes estrogen by aromatizing the A ring of the steroid nucleus. Lack of this enzyme causes a lack of estrogen and failure of women to develop at puberty. An interesting defect found in a male was an overactive CYP19 enzyme with about 50 times normal activity. This boy developed breasts at a young age. Aromatase inhibitors are new estrogen positive breast cancer drugs.

CYP20 is a new P450 that may be involved in development. It has orthologs in sea urchin, seq squirts and sponges so it is an old animal specific P450. Nothing is known yet. About it s function.

CYP21 is the C21 steroid hydroxylase. Defects in this gene cause congential adrenal hyperplasia due to lack of cortisol synthesis. Since cortisol is not made, the precursor 17 hydroxy progesterone builds up and this causes excessive androgen (testosterone) biosynthesis resulting in virilization.

CYP24 is a 25-hydroxyvitamin D(3) 24-hydroxylase used in the degradation or inactivation of vitamin D metabolites. [mitochondrial]

CYP26A1 is an all trans retinoic acid hydroxylase. It does not recognize 9-cis or 13-cis retinoic acid. CYP26A1 has been mutated in zebrafish and it causes a developmental defect. The human and mouse cDNAs have been cloned, but the effects of a mutation in mammals is not yet determined. Retinoic acid is known to be an important molecule in vertebrate development. It operates through several retinoic acid receptors. The hydroxylase degrades the retinoic acid signal and thus turns off a developmental switch. Cyp26a1 is expressed in the anterior hindbrain down to the rhabdomere r2/r3 boundary to keep the retinoic acid concentration low.

CYP26B1 is a human P450 that metabolizes retinoic acid and its expression is induced by retinoic acid during development in chickens (and probably all vertebrates). (See Nelson, D.R. A second CYP26 P450 in humans and zebrafish: CYP26B1. Archives of Biochem. Biophys. 371, 345-347 1999 and Reijntjes S, Gale E, Maden M. Expression of the retinoic acid catabolising enzyme CYP26B1 in the chick embryo and its regulation by retinoic acid. Gene Expr Patterns. Oct; vol. 5, 621-627 2003.

CYP26C1 CYP26C1 hydroxylates all trans and 9-cis retinoic acid and it is induced in rhabdomere r4 during development. It seems to be involved in shifting the hoxb1 expression boundary from r2/r3 to r4/r5. Development 132, 2611-2622 (2005). Retinoic acid is required for hoxb1 expression in r4 of the developing hindbrain. Therefore, the transient expression of of hoxb1 in the r4 segment from embyronic day E7.5 to E8.25 is stopped by the action of CYP26C1.

CYP27A1 is a sterol 27-hydroxylase that catalyzes the first step in side chain oxidation of sterol intermediates in bile acid biosynthesis. The sterol storage disorder cerebrotendinous xanthomatosis (CTX) is characterized by abnormal deposition of cholesterol and cholestanol in tissues like the Achilles tendon and nervous tissues. This disease is caused by mutations in the CYP27A1 gene. Remember that formation of bile acids is the only way the body can eliminate cholesterol, so if this pathway becomes blocked, then cholestrol can build up and become a problem. The end products of bile acid synthesis are cholic acid and chenodeoxycholic acid. These are the feedback inhibitors that shut down the biosynthesis of bile acids. This disease can be treated by giving cholic acid to shut down the bile acid pathway. CYP7A and CYP8B are two other enzymes in this bile acid biosynthesis pathway. CYP27A1 also 25 hydroxylates vitamin D3.

CYP27B1 is the 1-alpha hydroxylase of vitamin D3 that converts the D3 precursor to the active vitamin form. This gene was cloned after much effort, because the product acts to feedback inhibit mRNA systhesis. The paper appeared in Science (Sept. 19, 1997) . Because of this mechanism, it was very hard to get enough mRNA to clone this cDNA. The trick that was used was to make a knockout mouse that was missing the vitamin D3 receptor. This prevented the feedback inhibition and allowed a buildup of mRNA for the gene. [mitochondrial]

CYP27C1 is only known from genomic DNA sequencing. The function is not known.
This gene appears to be missing in rodents, so it cannot be essential for vertebrate develpoment.

CYP39 is the 7 hydroxylase of 24 hydroxy cholesterol. It is expressed in the liver and the eye and it may have a special role in the eye. (May be related to CYP1B1 function that is defective in glaucoma)

CYP46 is the brain cholesterol 24 hydroxylase and it is part of the cerebral cholesterol elimination pathway. Defects in this pathway are associated with Alzheimer's disease.

CYP51 is the lanosterol 14-alpha demethylase that is key in making cholestrol from lanosterol. This is the target of the triazole antifungal drugs like ketoconazole. This enzyme is evolutionarily conserved in plants, fungi, animals, and bacteria. It is found in Mycobacterium tuberculosis. This is the only P450 to be so highly conserved and it may have been the ancestor to all eukaryotic P450s.

Differences in humans, mice and rats.

Not all mammals have the same exact sets of P450 enzymes. They do tend to have the very specific ones we talked about for making steroids and bile acids, but they do not always have the same xenobiotic metabolizing P450s. The 2D subfamily is an interesting example. In humans there is only one active 2D P450, the 2D6 enzyme. The 2D6 enzyme in humans is also the enzyme responsible for the debrisoquine hydroxylase polymorphism we talked about earlier. In mice there are nine different functional Cyp2d P450 enzymes. Humans have one CYP2J2 while mice have eight Cyp2j P450s. Humans have 4 CYP2Cs

while mice have 15. This has implications for drug testing in animals. One has to be concerned that studying the effect of a drug in rats may not be relevant to humans, since the drug metabolizing systems are different. Beagle dogs are sometimes used in drug experiments, because their drug metabolism is supposed to be closer to humans than rodents. For a more detailed discussion see Nelson, D.R. Cytochrome P450 and the individuality of Species. Archives of Biochem. Biophys. 369, Sept. 1 issue 1-10, 1999. Nelson et al. 2004 Comparison of cytochrome P450 (*CYP*) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes, and alternative-splice variants Pharmacogenetics 14, 1-18

Use of a P450 for gene therapy in cancer

I mentioned earlier that CYP1A2 can activate procarcinogens to carcinogens. The induction of this enzyme may be a cancer risk. The activation of a prodrug to an active form by a P450 mediated reaction has been exploited to fight cancer. A vector with a P450 gene on it (and a P450 reductase gene) can be injected into cancer tumors. Some of these cells take up the vector and express The P450 and its reductase. Then a non-toxic prodrug is administered that is converted by the P450 into a toxic compound that kills the cells. Since the cancer cells have cellular connections, the toxin gets shared around and the tumor dies. For a review on this approach to cancer therapy see Waxman DJ, Chen L, Hecht JE, Jounaidi Y Cytochrome P450-based cancer gene therapy: recent advances and future prospects. Drug Metab Rev 31,503-22 1999.