Endoplasmic Reticulum

The *endoplasmic reticulum* (ER) is a continuous system of cell membranes, which are about 6nm thick. Dependent on cell specialization and activity, the membranes occur in different forms, such as stacks or tubules. The ER double membranes may be smooth or have granules attached to their outer surfaces. These granules are about 25nm in diameter and have been identified as membrane-bound ***ribosomes***. Therefore, two types of ER exist: the granular or **rough form (rER, rough ER)** and the agranular or **smooth form (sER, smooth ER).** Elaborate systems of rER membranes are found predominantly in cells that **biosynthesize protein**. Proteins, which are synthesized on membranes of the rER, are mostly exported from the cell. They may be secreted from the cell (including hormones and digestive enzymes, etc.) or become part of intracellular vesicles (*membrane proteins*).

Dioxygenases Incorporate Both Atoms of Molecular Oxygen into the Substrate

The basic reaction is shown below.

$$A + O_2 \rightarrow AO_2$$

Monooxygenases (Mixed-Function Oxidases, Hydroxylases) Incorporate Only One Atom of Molecular Oxygen into the Substrate

The other oxygen atom is reduced to water, an additional electron donor or co substrate (Z) being necessary for this purpose.

$$A—H + O_2 + ZH2 \rightarrow A—OH + H_2O + Z$$

Cytochromes P450 Are Monooxygenases Important for the Detoxification of Many Drugs & for the Hydroxylation of Steroids

Cytochromes P450 are an important superfamily of heme-containing monooxgenases, and more than 1000 such enzymes are known. Both NADH and NADPH donate reducing equivalents for the reduction of these cytochromes, which in turn are oxidized by substrates in a series of enzymatic reactions collectively known as the **hydroxylase cycle**. In liver microsomes, cytochromes P450 are found together with **cytochrome *b*5** and have an important role in detoxification. Benzpyrene, aminopyrine, aniline, morphine, and benzphetamine are hydroxylated, increasing their solubility and aiding their excretion. Many drugs such as Phenobarbital have the ability to induce the formation of microsomal enzymes and of cytochromes P450. Mitochondrial cytochrome P450 systems are found in steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta and are concerned with the biosynthesis of steroid hormones from cholesterol (Murray *et al.,* 2003).

Isoforms of cytochrome P450 hydroxylate many of xenobiotics in phase 1 of their metabolism

Hydroxylation is the chief reaction involved in phase 1. The responsible enzymes are called **monooxygenases** or **cytochrome P450s;** the human genome encodes at least 14 families of these enzymes. Estimates of the number of distinct cytochrome P450s in human tissues range from approximately 35 to 60. The reaction catalyzed by a monooxygenase (cytochrome P450) is as follows:

$$RH + O_2 + NADPH + H^+ \rightarrow R— OH + H_2O + NADP$$

RH above can represent a very wide variety of xenobiotics, including drugs, carcinogens, pesticides, petroleum products, and pollutants (such as a mixture of PCBs). In addition, **endogenous compounds,** such as certain steroids, eicosanoids, fatty acids, and retinoids, are also substrates. The substrates are generally **lipophilic** and are rendered more **hydrophilic** by hydroxylation. Cytochrome P450 is considered the **most versatile biocatalyst** known. It has been shown by the use of $^{18}O_2$ that one atom of oxygen enters R— OH and one atom enters water. This dual fate of the oxygen accounts for the former naming of monooxygenases as **"mixedfunction oxidases."** The reaction catalyzed by cytochrome P450 can also be represented as follows:

$$\text{Reduced cytochrome P450} \rightarrow \text{Oxidized cytochrome P450}$$

$$RH + O_2 \rightarrow R—OH + H_2O$$

The major monooxygenases in the endoplasmic reticulum are **cytochrome P450s**—so named because the enzyme was discovered when it was noted that preparations of microsomes that had been chemically reduced and then exposed to carbon monoxide exhibited a distinct peak at 450 nm. Among reasons that this enzyme is important is the fact that approximately 50% of the drugs humans ingest are metabolized by isoforms of cytochrome P450; these enzymes also act on various carcinogens and pollutants.

The following are important points concerning cytochrome P450s.

(1) Because of the large number of isoforms (about 150) that have been discovered, it became important to have a **systematic nomenclature** for isoforms of P450 and for their genes. This is now available and in wide use and is based on structural homology. The abbreviated root symbol CYP denotes a cytochrome P450. This is followed by an Arabic number designating the **family;** cytochrome P450s are included in the same family if they exhibit 40% or more sequence identity. The Arabic number is followed by a capital letter indicating the **subfamily,** if two or more members exist; P450s are in the same subfamily if they exhibit greater than 55% sequence identity. The **individual** P450s are then arbitrarily assigned Arabic numerals. Thus, CYP1A1 denotes a cytochrome P450 that is a member
of family 1 and subfamily A and is the first individual member of that subfamily. The nomenclature for the **genes** encoding cytochrome P450s is identical to that described above except that *italics* are used; thus, the gene encoding CYP1A1 is *CYP1A1*.

(2) Like **hemoglobin,** they are hemoproteins.

(3) They are widely distributed across species. Bacteria possess cytochrome P450s, and P450cam (involved in the metabolism of camphor) of *Pseudomonas putida* is the only P450 isoform whose crystal structure has been established.

(4) They are present in highest amount in **liver** and **small intestine** but are probably present in all tissues. In liver and most other tissues, they are present mainly in the **membranes of the smooth endoplasmic reticulum,** which constitute part of the **microsomal fraction** when tissue is subjected to subcellular fractionation. In hepatic microsomes, cytochrome P450s can comprise as much as 20% of the total protein. P450s are found in most tissues, though often in low amounts compared with liver. In the **adrenal,** they are found in **mitochondria** as well as in the endoplasmic reticulum; the various hydroxylases present in that organ play an important role in cholesterol and steroid biosynthesis. The mitochondrial cytochrome P450 system differs from the microsomal system in that it uses an NADPH linked
flavoprotein, **adrenodoxin reductase,** and a nonheme iron-sulfur protein, **adrenodoxin.** In addition, the specific P450 isoforms involved in steroid biosynthesis are generally much more restricted in their substrate specificity.

(5) At least six isoforms of cytochrome P450 are present in the endoplasmic reticulum of human liver, each with wide and somewhat overlapping **substrate specificities** and acting on both xenobiotics and endogenous compounds. The genes for many isoforms of P450 (from both humans and animals such as the rat) have been isolated and studied in detail in recent years.

(6) NADPH, not NADH, is involved in the reaction mechanism of cytochrome P450. The enzyme that uses NADPH to yield the reduced cytochrome P450, is called **NADPH-cytochrome P450 reductase.** Electrons are transferred from NADPH to NADPH cytochrome P450 reductase and then to cytochrome P450. This leads to the **reductive**

activation of molecular oxygen, and one atom of oxygen is subsequently inserted into the substrate. **Cytochrome *b*5,** another hemoprotein found in the membranes of the smooth endoplasmic reticulum, may be involved as an electron donor in some cases.

(7) Lipids are also components of the cytochrome P450 system. The preferred lipid is **phosphatidylcholine,** which is the major lipid found in membranes of the endoplasmic reticulum.

(8) Most isoforms of cytochrome P450 are **inducible.** For instance, the administration of Phenobarbital or of many other drugs causes hypertrophy of the smooth endoplasmic reticulum and a three- to four fold increase in the amount of cytochrome P450 within 4–5 days. The mechanism of induction has been studied extensively and in most cases involves increased transcription of mRNA for cytochrome P450. However, certain cases of induction involve stabilization of mRNA, enzyme stabilization, or other mechanisms (e.g., an effect on translation). Induction of cytochrome P450 has important clinical implications, since it is a biochemical mechanism of **drug interaction.** A drug interaction has occurred when the effects of one drug are altered by prior, concurrent, or later administration of another. As an illustration, consider the situation when a patient is taking the anticoagulant **warfarin** to prevent blood clotting. This drug is metabolized by CYP2C9. Concomitantly, the patient is started on **phenobarbital** (an inducer of this P450) to treat a certain type of epilepsy, but the dose of warfarin is not changed. After 5 days or so, the level of CYP2C9 in the patient's liver will be elevated three- to four fold. This in turn means that warfarin will be metabolized much more quickly than before, and its dosage will have become inadequate. Therefore, the dose must be increased if warfarin is to be

therapeutically effective. To pursue this example further, a problem could arise later on if the phenobarbital is discontinued but the increased dosage of warfarin stays the same. The patient will be at risk of bleeding, since the high dose of warfarin will be even more active than before, because the level of CYP2C9 will decline once phenobarbital has been stopped. Another example of enzyme induction involves **CYP2E1,** which is induced by consumption of **ethanol.** This is a matter for concern, because this P450 metabolizes certain widely used solvents and also components found in tobacco smoke, many of which are established **carcinogens.** Thus, if the activity of CYP2E1 is elevated by induction, this may increase the risk of carcinogenicity developing from exposure to such compounds.

(9) Certain isoforms of cytochrome P450 (e.g., CYP1A1) are particularly involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs) and related molecules; for this reason they were formerly called aromatic hydrocarbon hydroxylases (AHHs). This enzyme is important in the metabolism of PAHs and in carcinogenesis produced by these agents. For example, in the lung it may be involved in the conversion of inactive PAHs (procarcinogens), inhaled by smoking, to active carcinogens by hydroxylation reactions. Smokers have higher levels of this enzyme in some of their cells and tissues than do nonsmokers. Some reports have indicated that the activity of this enzyme may be elevated (induced) in the placenta of a woman who smokes, thus potentially altering the quantities of metabolites of PAHs (some of which could be harmful) to which the fetus is exposed.

(10) Certain cytochrome P450s exist in polymorphic forms (genetic isoforms), some of which exhibit low catalytic activity. These observations are one important explanation for the variations in drug responses noted among many patients. One P450 exhibiting polymorphism is CYP2D6, which is involved in the metabolism of debrisoquin (an antihypertensive drug) and sparteine (an antiarrhythmic and oxytocic drug). Certain polymorphisms of CYP2D6 cause poor metabolism of these and a variety of other drugs so that they can accumulate in the body, resulting in untoward consequences. Another interesting polymorphism is that of CYP2A6, which is involved in the metabolism of nicotine to conitine. Three *CYP2A6* alleles have been identified: a wild type and two null or inactive alleles. It has been reported that individuals with the null alleles, who have impaired metabolism of nicotine, are apparently protected against becoming tobacco-dependent smokers (Table 53–2). These individuals smoke less, presumably because their blood and brain concentrations of nicotine remain elevated longer than those of individuals with the wild-type allele. It has been speculated that inhibiting CYP2A6 may be a novel way to help prevent and to treat smoking. Table 53–1 summarizes some principal features of cytochrome P450s (Murray *et al.,* 2003).

Table 53–1. Some properties of human cytochrome P450s.

- Involved in phase I of the metabolism of innumerable xenobiotics, including perhaps 50% of the drugs administered to humans
- Involved in the metabolism of many endogenous compounds (eg, steroids)
- All are hemoproteins
- Often exhibit broad substrate specificity, thus acting on many compounds; consequently, different P450s may catalyze formation of the same product
- Extremely versatile catalysts, perhaps catalyzing about 60 types of reactions
- However, basically they catalyze reactions involving introduction of one atom of oxygen into the substrate and one into water
- Their hydroxylated products are more water-soluble than their generally lipophilic substrates, facilitating excretion
- Liver contains highest amounts, but found in most if not all tissues, including small intestine, brain, and lung
- Located in the smooth endoplasmic reticulum or in mitochondria (steroidogenic hormones)
- In some cases, their products are mutagenic or carcinogenic
- Many have a molecular mass of about 55 kDa
- Many are inducible, resulting in one cause of drug interactions
- Many are inhibited by various drugs or their metabolic products, providing another cause of drug interactions
- Some exhibit genetic polymorphisms, which can result in atypical drug metabolism
- Their activities may be altered in diseased tissues (eg, cirrhosis), affecting drug metabolism
- Genotyping the P450 profile of patients (eg, to detect polymorphisms) may in the future permit individualization of drug therapy

REFERENCES:

Murray R., Granner D., Mayes P. and Rodwell V. Harper's Illustrated Biochemistry, Lange Medical Books/McGraw-Hill 26th Ed, 2003