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Review Article :

The Cytochrome P450s

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Abstract. Cytochrome P450 monooxygenases are versatile biocatalysts that incorporate oxygen into an enormous range of molecules. The cytochrome P450 proteins are involved in the catalysis of different reactions and these properties have been used for drug improvement. The protein family also includes compounds producing properties such as resistance to insecticides, and the synthesis of valuable chemicals. In this review, we will discuss various aspects of the structure and function of the cytochrome P450s.

Keywords: Cytochrome P450, Localization and function, P450 Clans.

INTRODUCTION

The cytochromes P450 enzymes (P450s) are the oldest and the largest super family of haem proteins involved in the metabolism of both exogenous compounds and endogenous compounds (Feyereisen, 1999; Werck-Reichhart and Feyereisen, 2000). They are found in the genomes of nearly all organisms, from bacteria to humans, and the multiplicity of these proteins has led to the classification of CYP genes into more than 270 different gene families. At least 12 cytochrome P450 gene families, 20 subfamilies and more than 57 active genes have been identified in humans, 29 alone contained in the Human cytochrome P450 Allele (CYP-allele) (Nebert and Russell, 2002; Sigel *et al.*, 2007; Sim and Ingelman-Sundberg, 2013), and 58 pseudogenes (Rodriguez-Antona and Ingelman-Sundberg, 2006). Bacteria contain around 11 different P450 genes (Sigel *et al.,* 2007) while *Saccharomyces cerevisiaehas* has three P450 genes. In insects, the fruit fly *Drosophila melanogaster* has 83 P450 genes and seven pseudo genes whilst 111 P450 genes exist in Anopheles mosquitoes and 46 P450 genes have been identified in the termite (Bignell *et al.*, 2011). Additionally, the nematode *Caenorhabditis elegans* has 80 P450 genes, and the mouse has 102 functional CYP genes and 88 pseudo genes (Preissner *et al.*, 2010; Sigel *et al.*, 2007). Higher plants have more P450 genes than animal species, with nearly 280 being found in *Arabidopsis thaliana,* and up to 323 different CYP proteins found in rice. Key early discoveries in the area of cytochrome P450s go back to the 20th Century when Cytochrome P450s were first discovered in 1958 by Martin Klingenberg and David Garfinkel (Garfinkel, 1958; Goeptar *et al.*, 1995; Klingenberg, 1958). Early research on the cytochrome P450s was largely based on *in vitro* biochemical studies. The cytochrome P450 enzymes were first discovered in rat liver microsomes, characterized by an intense absorption at 450 nm in the presence of carbon monoxide (Omura and Sato, 1962).

The term P450 enzymes is used to describe the enzymes encoded by cytochrome P450 genes, which derive their name from the discovery of a liver microsomal pigment (P) 51 years ago (Omura and Sato, 1962), and a maximum absorption at 450 nm in the UV spectrum in the presence of carbon monoxide. Cytochrome P450s are designated various names, including polysubstrate monooxygenases (PSMOs), cytochrome P450 monooxygenases, microsomal oxidizes, heme thiolate proteins and mixed function oxidases (MFOs) (Omura and Sato, 1962). The standard nomenclature of P450s was introduced by David Nelson; nomenclature of the P450s and sequence date can be found at http://drnelson.utmem.edu/cytochromeP450.html. This is a systematic naming of P450s which has been in place since 1989 and has been periodically updated since then based on the recommendations of a nomenclature

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committee on the basis of amino-acid identity, phylogenetic criteria and gene organization. The nomenclature system for all members of the P450 super family of genes starts with CYP (CYtochrome P450) followed by a numeral for the family, a letter for the subfamily and a numeral for an individual gene. All members of the same family contain more than 40% similarity in amino acid sequence whereas members of the same subfamily consist of more than 55% similarity in amino acid sequence. For instance, a P450 gene in family 27, subfamily A, gene 1 is named *CYP27A1* (Figure 1) (Nebert *et al.*, 1996).

More than 660 alleles have been designated into the P450 Allele Nomenclature (CYP-allele) since 2008, found at http://www.cypalleles.ki.se, following the guidelines of the CYP-allele Nomenclature Committee (Sim and Ingelman-Sundberg, 2013).

LOCALISATION AND FUNCTION

Cytochrome P450s are categorized into two types. The first type are present in the mitochondrial matrix and called Mitochondrial P450s, and the second types are present in the endoplasmic reticulum, the microsomal *CYPs*. These types of P450 differ in the amino-terminal sequence, that determines the sub-cellular localization and influences the electron donor system (Omura, 2010; Werck-Reichhart and Feyereisen, 2000).

In mammals, these proteins are primarily found in liver cells, with the highest concentrations found in the liver (hepatocytes) and the small intestine. However, the cytochrome P450 proteins are also found in cells throughout the body (Werck-Reichhart and Feyereisen, 2000). Inside the cells, cytochrome P450 enzymes are located in a structure involved in protein processing and transport, the endoplasmic reticulum, as well as in mitochondria (Omura and Sato, 1962). Insect P450 genes are expressed in many tissues, including in the digestive tract and a rich source of P450s is the fat body. Some P450s are larval stage specific, whereas others are expressed only in adults (Feyereisen, 1999).

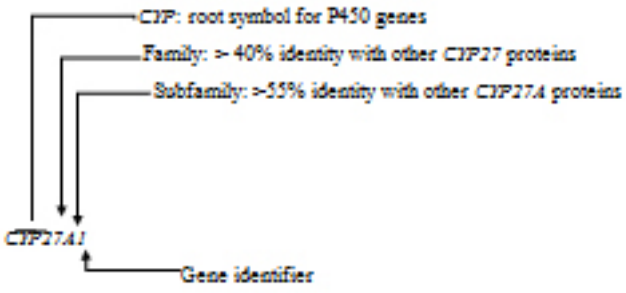

Figure 1. Scheme of the P450 nomenclature.

Currently, a total of > 18,000 *CYP* sequences are listed on the "Cytochrome P450" homepage curated by Nelson at the University of Tennessee, USA and there are approximately 6,000 more that are known but yet to be named (Nelson, 2011). The role of these enzymes in the detoxication and activation of drugs and carcinogens has created enormous research interest into the cellular and molecular mechanisms of the regulation of cytochrome P450s in the areas of biochemistry, genetics, medicine and toxicology (Guengerich, 2004; Preissner *et al.*, 2010). Cytochrome P450s comprise a varied function monooxygenase system, participating in both catabolic and synthetic reactions (Simpson, 1997) (Figure 1). Cytochrome P450s metabolize endogenous substances including steroids, vitamins, fatty acids and prostaglandins and also play an important role in metabolizing (exogenous) xenobiotics into detoxified forms. These include drugs and chemicals including environmental pollutants and carcinogens (Simpson, 1997; Scott, 1999; Preissner *et al.*, 2010)

In insects, the cytochrome P450s perform a variety of functions that parallel those performed in humans. Insect cytochrome P450s participate in the biosynthesis of juvenile hormone, ecdysteroids, cuticular hydrocarbons and pheromones (Feyereisen, 1999). Some of these enzymes are involved in the detoxication or in the activation of insecticides (Berge *et al.*, 1998; Scott, 1999; Le Goff *et al.*, 2003). Insect cytochrome P450s also have an important role in the metabolism of plant allelo-chemicals in herbivorous insects as they adapt to their plant hosts (Danielson *et al.*, 1998; Petersen *et al.,* 2001; Petersen *et al.,* 2003; Wen *et al.,* 2003; David *et al.,* 2006). For instance, *CYP6A1* is involved in the metabolism of the insecticides aldrin and heptachlor in Musca domestica, along with *CYP6D1* which is responsible for the metabolism of deltamethrin. It has been suggested that the *CYP4* family is primarily involved in the metabolism of toxic compounds (Danielson *et al.*, 1998). Additionally, previous studies have measured the cytochrome P450-dependent monooxygenase activity by the effect of atrazine in the midge *Chironomus tentans,* and have found increased activity in larvae as a result of atrazine exposure. Another study has reported the cloning and expression of the *C. tentans CYP4* gene that is responsive to atrazine induction, with the results indicating a high degree of similarity to other insect *CYP4* genes(Londono *et al.,* 2007).

P450 enzymes vary in their selectivity for substrates and inhibitors, and two enzymes may metabolize the same substrate. They are capable of exhibiting extremely variable turnover numbers. In spite of their broad range of substrates,

a lot of common structural features are conserved among all P450s as evidenced by the structures of ten published mammalian P450 enzymes.

In humans, the most abundant cytochrome proteins are members of the *CYP3A* subfamily, accounting for 30% of the cytochrome enzymes in the liver and 70% of those in the gastrointestinal tract. This enzyme is the most important form of cytochrome P450 in the adult liver and it metabolizes the greatest proportion of drugs of all cytochromes. Members of the *CYP3A4* group are 97% identical and cannot be distinguished from each other based on the substrates that they metabolize; these are the major enzymes expressed in the small intestine, while in the stomach the most important enzymes expressed are *CYP3A5* genes(Kosuge *et al.*, 2007).

CYTOCHROME P450 PROTEIN STRUCTURE

There is an increasing number of P450s sequences being listed in GenBank with time, including many P450 sequences from insects (Feyereisen, 1999). P450 proteins are made up of about five hundred amino acids with molecular weights in the range of 45-55 kDa, with several conserved domains (Feyereisen, 1999). In addition, P450s have characteristics such as a Cysteine axial ligand to the heme iron, and also a Cysteine located near the *C terminus* of the protein. Upstream of the L-helix, another characteristic is the I- helix which contains a Thr (T) residue that is involved in a highly conserved oxygen binding region with the sequence FXXGXXXCXG (X representing any amino acid). Identification of a

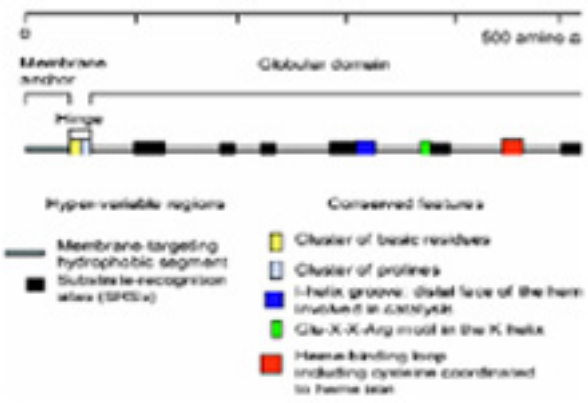

Figure 3. The primary structure of P450s. Typical features of an ER bound protein, the function of the diverse domain and regions indicated via colors are described in the text. Modified from (Werck-Reichhart and Feyereisen, 2000).

P450 sequence is straightforward from this "signature" (Figure 3) (Feyereisen, 1999; Phillips and Shephard, 2006; Werck-Reichhart and Feyereisen, 2000). Moreover, an ExxR motif in the K-helix is highly conserved and probably required to stabilize the core structure.

In addition, EVDTFMFEGHDTT is a 13-residue sequence in the I-helix region that is relatively conserved in proteins belonging to the *CYP4* family. The non-conserved regions are usually associated through substrate binding, although there is a remarkable general level of sequence diversity. The P450s from bacteria and P450s from mammals are increasingly being characterized in terms of their crystal structures, mostly in their soluble form.

P450 CLANS

The nomenclature for P450s used to be simple, with the enzymes numbering a few dozen families, and taxa such as vertebrates having less than 20 families; one could easily memorize the family names. Many more different groups have now been identified, with plants alone containing 62 families of P450. Fungi and bacteria are more varied, with every second or third sequence belonging to a new family. Clans are higher-order groups and they are basically similar to clades, although clades technically refer to species with a common ancestor and not to sequences.

Clans have been defined as groups of P450 families that consistently cluster together on phylogenetic tree. The 62 families of plant P450s arrange into just 10 clans. The animal clans are still being defined, however it is clear that the insect *CYP6* and *CYP9* families belong in a clan with vertebrate *CYP3* and *CYP5.* This has been named the *CYP3* clan for the lowest family number in the group. Insects have four clans comprising *CYP2, CYP3, CYP4,* and mitochondrial *CYP11, CYP24, CYP27* families. Vertebrate P450s belong to around 10 clans, whereas some, such as *CYP19,* have only one family. The clans that are in common between insects and vertebrates must have had a common ancestor sequence in the bilaterian ancestor of the species (Phillips and Shephard, 2006). The *CYP4* family includes a large number of sequences from vertebrates, *Caenorhabditis elegans* and insect species. The *CYP4* family consists of 72 subfamilies, mostly in invertebrate species, including *CYP4C, CYP4D, CYP4E, CYP4G, CYP4P,CYP4S,* CYP4AA, CYP4AE, CYP4AD and *CYP4AC* (Kirischian and Wilson, 2012). *CYP4A - CYP4M* consist of a group of 63 members encoding omega-hydroxylates (Simpson, 1997). In mammals, six subfamilies have been recognized (*CYP4A, CYP4B, CYP4F, CYP4V, CYP4X* and *CYP4Z)* (Hardwick, 2008).

Human gene polymorphisms, particularly in *CYP2* family members, are proven to result in tremendous differences in an individual's xenoboiotic metabolism abilities. The analyses of polymorphisms in the

Figure 2. Functions of mammalian cytochrome P450s (modified from (Wolf, 1986).

human *CYP2D6* gene in European people resulted in the identification of almost 50 mutations that could be grouped according to the metabolic abilities of every single allele (Sigel *et al.,* 2007).

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