

## **Mammalian cytochromes P450 – importance of tissue specificity**

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## **Abstract**

Mammals express multiple cytochromes P450 simultaneously in a variety of tissues, including the liver, kidney, lung, adrenal, gonads, brain, and most others. For cytochromes P450 that are expressed in many tissues or cell types, the tissue/cell type-specific expression might be associated with their special physiological roles. Several cytochrome P450 enzymes are found not only in different cell types and tissues, but also in different subcellular compartments. Generally, all mammalian cytochrome P450 enzymes are membrane bound. The two major groups are represented by microsomal cytochromes P450 that reside in the endoplasmic reticulum, and mitochondrial cytochromes P450, that reside in the inner mitochondrial membrane. However, the outer nuclear membrane, different Golgi compartments, peroxisomes and the plasma membrane are also sites where cytochromes P450 were observed. For example, CYP51 is an ER enzyme in majority of tissues but in male germ cells it trafficks through the Golgi to acrosome, where it is stabilized for several weeks. Surprisingly, in brains of heme synthesis deficient mice, a soluble form of CYP1A1 was detected whose activity has been restored by the addition of heme. In the majority of cases each cytochrome P450 enzyme resides in a single subcellular compartment in a certain cell, however, examples of simultaneous localization in different subcellular compartments have also been described, such as endoplasmic reticulum, Golgi and plasma membrane for CYP2E1. This review will focus on the physiological importance of mammalian cytochrome P450 expression and localization in different tissues or cell types and subcellular compartments.

Keywords: Cytochrome P450, endoplasmic reticulum, signal sequence, mammals, subcellular localization, tissue distribution

## 1. Introduction

With sequencing of genomes of many different organisms, the number of different cytochrome P450 sequences is raising fast. Currently, the cytochrome P450 superfamily consists of over 5,500 named sequences, from which approximately 1300 sequences were found in animals (<http://drnelson.utmem.edu/CytochromeP450.html>). The number of cytochromes P450 is quite different from one species to another, from 3 in *S. cerevisiae* (baker's yeast), 38 in *Neurospora crassa* (fungus), 74 in *C. elegans*, to 57 in human, 102 in the mouse and several hundreds in many plant species. A vast majority of cytochromes P450 have unknown functions, especially in the microbial world. Therefore, one of the challenges in the future is to find a faster way for assigning function to unknown cytochromes P450. From 57 putatively functional cytochrome P450 genes in the human [1], the function for fifteen is still unknown. Others can be divided according to their major substrate class. Fifteen human cytochromes P450 metabolize xenobiotics, all of them being from CYP1, CYP2 and CYP3 families. The majority of them are concentrated in the endoplasmic reticulum of the liver. Other cytochromes P450 are devoted mainly to the metabolism of endogenous substrates as presented in Table 1 [2]. Due to involvement of the cytochromes P450 in the production and metabolism of many molecules with important physiological functions, many of them have been studied in detail in different tissues and cell types. Tissue and cell type specific expression and regulation is very important for cytochromes P450 involved in steroid and eicosanoids biosynthesis and catabolism of vitamins. See Figure 1 for the schematic presentation of cytochromes P450 involved in conversion of endogenous substrates. Some results show how important the localization of certain cytochromes P450 inside the cell is and also the importance of proper distribution in different cell types or tissues. For cytochromes P450 with yet unknown function data on tissue or cell specific expression may be valuable for the prediction of function. We will start this text with reviewing the subcellular localization and targeting of cytochromes P450.

## **2. Intracellular localization and trafficking of cytochromes P450**

Cytochromes P450 are membrane bound, with the exception of bacterial enzymes. Microsomal enzymes are tethered to the membrane through a hydrophobic transmembrane helix at N-terminus of the protein, which also serves as a targeting sequence for the signal recognition particle dependent cotranslational incorporation of a nascent cytochrome P450 into the ER membrane [3, 4]. About 90% of hepatic cytochrome P450 enzymes are present in microsomal membranes, with the remaining in mitochondria [1]. Mitochondrial cytochromes P450 reside in the inner mitochondrial membrane with their globular domain orientated into the matrix. In humans, mitochondrial cytochromes P450 are involved in steroid and vitamin D3 biosynthesis: CYP11A - cholesterol side-chain cleavage enzyme, CYP11B1 – mainly steroid 11-beta-hydroxylase, CYP11B2 - steroid 18-hydroxylase, CYP24 - 24-hydroxylation of 25-hydroxyvitamin D(3), CYP27A1- sterol 27-hydroxylase and CYP27B1 - calcidiol 1-monooxygenase.

### **2.1. Retention of cytochromes P450 in the endoplasmic reticulum**

After the proper folding, the ER resident proteins are retained in the ER while the majority of newly-made proteins are selectively incorporated into transport vesicles budding off from the ER for transport to the Golgi. Several studies have shown that transport from the ER to Golgi in mammalian cells is mediated by a pre-Golgi intermediate compartment. Experimental results support the idea that this compartment represents specialized regions of the ER devoted to vesicle budding, referred as ER exit sites [5]. Vesicular-tubular clusters represent post-ER and pre-Golgi membrane structures involved in anterograde transport to the Golgi and retrograde recycling of proteins back to the ER [6]. At first two models have been proposed regarding the selectivity of

ER export – one suggesting selective export [7] and another non-selective events [8]. Experimental results that followed are very much in favor of selective ER-Golgi transport and since then several integral membrane proteins have been implicated in the sorting of certain cargo molecules during the budding of vesicles from the ER.

It is not exactly known how cytochromes P450 are organized in the membrane of endoplasmic reticulum, but it is predicted that at least some cytochromes P450 form oligomeric complexes [9, 10], which may be important for their increased substrate affinity and interaction with P450 reductase based on *in vitro* results [11].

At first it was believed that all cytochromes P450 are ER resident proteins that are retained directly in the ER and do not undergo recycling through the pre-Golgi compartment [12, 13]. However, later studies on some cytochromes P450 showed this is not always the case. Lanosterol 14 $\alpha$ -demethylase (CYP51) is one example of recycling between ER and cis-Golgi in somatic cells [14, 15] and transport through Golgi to the acrosome in male germ cells [15]. The exact reason for the static retention or recycling of cytochromes P450 is not fully understood. ER resident proteins must possess specific motifs, which enable them to be excluded from transport through Golgi to other cell compartments or from exocytosis. In many luminal ER proteins sequence KDEL or a related signal at their C-terminus has this function, while some ER membrane proteins have a dilysine motif KKXX [16] at the C-terminal end of the cytoplasmic domain. Cytochromes P450 in most cases do not possess KKXX-like signals. Previously mentioned CYP51 has this motif at the C-terminus, although it has not been investigated whether it contributes to the ER retention or retrieval. Several studies proved that the hydrophobic N-terminal transmembrane region of different cytochromes P450 serves as ER retention signal [12, 13] and is sufficient to restrict localization to the ER membrane. In addition to that some other regions of P450 proteins were shown to have function in ER retention and most probably have a synergistic effect together

with the transmembrane region. CYP2C1 and CYP2C2 contain an additional ER retention signal in the juxtamembrane region immediately after the transmembrane sequence [17, 18]. Cytoplasmic domain of these cytochromes P450 is able to redirect the localization of a plasma membrane protein to the ER independently of the N-terminal region [19]. Two cytoplasmic regions of CYP2C2 were found to be responsible for the retention, one being the amino acid sequence 160-200 and other the C-terminal half of the protein. Possible reason for the retention is high hydrophobicity of those two segments and interactions with the membrane or formation of networks that are excluded from the transport vesicles [20], especially since cytochromes P450 are known to interact with other ER membrane proteins, such as cytochrome P450 reductase, and form oligomeric complexes [21]. CYP2E1 which was also detected in the plasma membrane and Golgi apparatus is retained in the endoplasmic reticulum membrane by different mechanisms than CYP2C1 and the cytoplasmic domain alone of CYP2E1 is not enough for efficient retention in the ER.

## **2.2. Cytochromes P450 in mitochondria and other cellular compartments**

Initially it was believed that cytochromes P450 reside either in ER or in mitochondria. However, a dual (mitochondrial and microsomal) association of some cytochrome P450 isoforms has been reported lately, including CYP1A1/2, CYP2B1/2, CYP2E1, CYP3A1/2, CYP2D6 and CYP2C12 [22]. Many of the proteins destined for import into the mitochondrial matrix, contain a signal sequence that directs these proteins to the mitochondria. In most cases these signal sequences are at the N-terminus of cytochrome P450 proteins [23], but can also be found at the C-terminus of some cytochromes P450 [24, 25]. A general consensus sequence for mitochondrial matrix targeting of proteins does not exist. Many of mitochondrial signal sequences are rich in positively charged and hydrophobic amino acid residues, which enables them to form an amphiphilic secondary structure [26]. Import receptor in the mitochondrial outer membrane is

rich in negatively charged amino acid residues. It is believed that hydrophobic interactions and interaction between positively charged residues in mitochondrial targeting sequence and negatively charged residues of import receptor play an important role in signal-receptor recognition [27]. Translocase of the inner mitochondrial membrane complex can further transfer proteins into the matrix, where the N-terminal signal sequence of the protein is proteolytically removed by proteases [28].

Avadhani and his associates have identified mechanisms by which proteolytic cleavage in the N-terminus of CYP1A1 can mark this protein for import into mitochondria [29, 30]. CYP1A1 is an extrahepatic xenobiotic metabolizing enzyme involved in the activation of polycyclic aromatic hydrocarbons, for which it was shown that N-terminal truncation of 33 amino acids changes the substrate specificity when compared to the intact protein [30]. Transient transfection of COS cells and site-specific mutagenesis studies showed that the N-terminus of CYP1A1 contains a chimeric signal for targeting the protein to both the ER and mitochondrial compartments. The fraction of the pool of this enzyme that can move to the mitochondria is considerable, 20-25% [30]. Influence of CYP1A1 N-terminal signal sequence (residues 1-44) on targeting of two heterologous proteins was studied. Dihydrofolate reductase and the mature portion of rat CYP27 with the entire signal sequence were targeted to both the ER and mitochondria, whereas the 33-44 sequence strictly functions as a mitochondrial targeting signal. Interestingly, cholesterol 27-hydroxylase activity of the ER-associated CYP27 fusion protein with CYP1A1 signal sequence can be reconstituted with cytochrome P450 reductase, but the mitochondrial associated fusion protein is functional with adrenodoxin and adrenodoxin reductase. The two fusion proteins exhibited distinctly different membrane topologies in the two organelle compartments, which is consistent with the necessity for different redox partner [31].

The rat CYP2B1 also contains a chimeric mitochondrial and endoplasmic reticulum targeting signal at its N-terminus. It has been shown that targeting of this cytochrome P450 to mitochondria or endoplasmic reticulum is modulated through phosphorylation of Ser 128. Phosphorylation activates cryptic mitochondrial targeting signal at amino acids 20-36 and modulates ER or mitochondrial destination of the CYP2B1 protein. The role of Ser 128 phosphorylation was confirmed with recombinant dihydrofolate reductase containing the sequence 1-160 of CYP2B1 on N-terminus. This fusion protein was transported to mitochondria upon Ser 128 phosphorylation and activation of a cryptic mitochondrial targeting signal [32].

A mitochondrial targeting signal in the rat CYP2E1 has been studied in detail on several constructs with different deletions on the N-terminus of protein [33]. Contrary to CYP1A1 and CYP2B1, where the transmembrane domain is almost immediately followed by the mitochondrial targeting signal, these two sequences are further apart in CYP2E1.

Last year an article was published in Cancer Research where authors describe a unique subcellular localization of enzymatically active CYP1A1 splice variant (CYP1A1v) formed by excision of an 84-bp cryptic intron in exon 2 [34]. They found that CYP1A1v is overexpressed in ovarian cancer cell lines and exhibits subcellular distribution restricted to the nucleus and mitochondria, contrary to the mostly endoplasmic reticulum localization of the wild-type enzyme. In concordance, the total CYP1A1 activity, as measured by the ethoxyresorufin O-deethylase assay, was detected in mitochondrial, nuclear, and microsomal fractions of ovarian cancer cells but was notably absent in all subcellular fractions of normal human ovarian surface epithelium cells. Immunocytochemistry studies in 30 clinical specimens revealed overexpression of CYP1A1 in various types of ovarian cancers compared with benign epithelia and frequent localization of the enzyme to cancer cell nuclei. Forced expression of CYP1A1wt or CYP1A1v in human ovarian surface epithelium cells resulted in nuclear localization of the enzyme and acquisition of



anchorage-independent growth, which was further exacerbated following exposure to benzo(a)pyrene or 17beta-estradiol [34]. This reminds us that subcellular distribution of cytochromes P450 may change with the level of expression. It is important to have this in mind when we try to monitor intracellular localization or trafficking of overexpressed cytochrome P450 enzymes. In another study on transgenic mouse with chronic impairment of heme synthesis, the effect of heme deficiency on CYP1A1 subcellular localization in brain was observed. CYP1A1 protein was detected not only on the endoplasmic reticulum, but also in the cytosol, where activity could be restored by the addition of heme [35]. The cytosolic appearance of CYP1A1 most likely did not originate from mutant alleles. It seems that heme deficiency in the brain leads to incomplete heme saturation of CYP1A1, which causes its improper incorporation into the ER membranes and persistence in the cytosol.

Small amounts of some cytochromes P450 can be translocated to the outer layer of the plasma membrane. The specific content of cytochromes P450 in plasma membrane was determined to be 9% of that in the microsomes as concluded from studies on plasma membrane preparations. [36]. Pulse chase labeling experiments performed later gave very similar results. Specific content of cytochromes P450 in plasma membrane was found to be about 10% with much faster turnover in this location compared to endoplasmic reticulum [37]. When molecular basis for the transport of CYP2E1 to the plasma membrane was studied, conclusion was that the N-terminal transmembrane domain of CYP2E1 has a critical role in directing the protein to the cell surface. Topological inversion of a small fraction of CYP2E1 in endoplasmic reticulum from cytosolic to luminal orientation directs the protein to the plasma membrane [38]. Pulse chase experiments with CYP1A2 showed that only the newly synthesized P450 molecules are transferred to the plasma membrane of hepatocyte, whereas microsomal CYP1A2 was stably radiolabeled for several hours [39]. Several studies also confirm location of NADPH cytochrome P450 reductase on the hepatocyte surface [36, 39]. Cell surface expression of cytochromes P450 has generated

interest because it has a role in autoimmune hepatitis, chronic necroinflammatory disease of the liver. Autoimmune hepatitis type II is characterized by the presence of specific autoantibodies against liver and kidney microsomal antigens (anti-LKM-1 and anti-LKM-2). Identity of these antigens was unknown at first. Eventually CYP2D6 has been documented as the major target-autoantigen of anti-LKM-1 autoantibodies [40] and CYP2C9 for anti-LKM-2 autoantibodies. Some other cytochromes P450 were also found to be target autoantigens in hepatitis, including CYP2E1, CYP3A4, CYP1A2, CYP17 and CYP21. Reactive metabolites of cytochromes P450 are capable of altering the shape of the molecule to a degree not tolerated by the immune system, when such enzyme is exposed to the cell surface.

### **3. Organ, tissue and cell type specific expression of CYP enzymes**

#### **3.1. Specific roles of CYP26B1 and CYP51 in gonads**

Koopman with co-workers recently discovered an interesting role of CYP26B1 in the development of germ cells in mouse embryo to either oocytes or spermatogonia [41]. Their results demonstrated that retinoic acid, produced by mesonephroi of both sexes, causes germ cells in the ovary to enter meiosis and initiate oogenesis. However, in the fetal testis, meiosis is retarded by the action of the retinoid-degrading enzyme CYP26B1, which is ultimately leading to spermatogenesis. This was confirmed in *Cyp26b1*-knockout mouse embryos – germ cells in testes enter meiosis prematurely, as if in a normal ovary. Gene expression analysis showed initial expression of *Cyp26b1* in gonads of both sexes, but became very male specific by 12.5 days post coitum. Before this study, the existence of a meiosis-inhibiting factor was postulated [42]. Interestingly, this factor is not a secreted signaling molecule as predicted, but instead a

cytochrome P450 enzyme of retinoid metabolism, whose activity is ascribed to peritubular myoid cells.

Another cytochrome P450 for which localization in the cell and differences in the expression and regulation in various tissues/organs has important role, is CYP51. The CYP51 gene family is evolutionary very conserved and has orthologues in animals, fungi, plants and few prokaryotic groups [43]. It has been well characterized in five mammalian species, the human [44, 45], mouse [46], rat [47, 48], pig [49] and bull [15, 50, 51]. CYP51 is one of the enzyme in cholesterol biosynthesis, which is a tightly regulated housekeeping pathway composed of over 20 enzymatic steps. It catalyzes the first step in the post-squalene part of cholesterol biosynthesis, conversion of lanosterol to FF-MAS (4,4-dimethyl-5 $\alpha$ -cholesta-8,14,24-triene-3 $\beta$ -ol). CYP51 is ubiquitously expressed with highest levels in the testis, ovary, adrenal gland, prostate, liver, kidney and lung. The highest level of CYP51 mRNA expression is observed in testis (germ cells) of different mammals (human, pig, rat). The CYP51 catalytic product, sterol FF-MAS, is further converted to testis meiosis-activating sterol (T-MAS). These are the first sterols in this pathway whose function was proposed to be dedicated to the biological processes that are distinct from the biosynthesis of cholesterol. FF-MAS and T-MAS show the ability to trigger resumption of oocyte meiosis *in vitro*. They are short-lived products of the CYP51 demethylation reaction in somatic cells, but accumulate in gonadal tissues. The different roles of cholesterol and proposed roles of meiosis activating sterol intermediates suggest that regulation of enzyme activities of cholesterologenic genes differ between tissues. Generally CYP51 resides in the ER, but during male germ cell development, the enzyme is detected also on the Golgi and on the acrosome during all phases of acrosome development (Fig. 2). NADPH cytochrome P450 reductase is co-expressed with CYP51 on acrosomal membranes and supports its enzymatic activity. This is indicating an intracellular transport of CYP51 from ER through the Golgi to the acrosome [15].

### **3.2. Localization of cytochromes P450 in some other tissues**

Study conducted on normal pancreases from donors and early autopsy cases as well as on cultured human islet cells showed considerable differences in distribution of xenobiotic-metabolizing cytochromes P450 [52]. Nine cytochromes P450 and the NADPH cytochrome P450 oxidoreductase were analyzed by immunohistochemistry, western blot analysis, and/or reverse-transcription polymerase chain reaction. Remarkable differences in the cellular distribution of these enzymes were found between the individuals and between different pancreatic cells within the same individual. A majority of the enzymes were localized in islet cells and were either distributed in all islet cells or were restricted to, or expressed in a higher concentration in, glucagon and/or pancreatic polypeptide cells. A different cellular localization of the enzymes was found in some individuals, that is cytoplasmic vs. Golgi pattern of staining and a frequent nuclear localization of CYP2E1 in females. Authors of the study conclude that the expression of individual enzymes and their distribution in acinar, ductal, and islet cells may determine individual susceptibility to pancreatic diseases.

Cytochromes P450 have important roles in the physiology and patho-physiology through the involvement in the metabolism of arachidonic acid to various active substances, including epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs). This enzymatic pathway was first described in the liver [53], however, it became clear that arachidonic acid can be metabolized by cytochromes P450 in many tissues, including the brain, kidney, lung, heart, and blood vessels [54]. Recent reports suggest that tissue specific arachidonic acid metabolizing cytochromes P450 localize in these tissues and contribute to the regulation of homeostasis [55, 56]. For example, some cytochromes P450 have prominent roles in vascular regulation, like epoxygenases of the CYP2 family (2B, 2C8, 2C9, 2C10, 2J2 in humans) which generate a series of regiospecific and stereospecific epoxides, and the arachidonic acid  $\omega$ -hydroxylases belonging

to the CYP4A family, which form  $\omega$  and  $\omega$ -1 hydroxyeicosatetraenoic acids. Imaoka with co-workers has investigated expression of arachidonic acid metabolizing cytochromes P450 in rat tissues by RT-PCR. They have found important differences for some of them. CYP2B1 was specifically expressed in the lung, CYP2C23 was detected in all tissues used in the study, that is liver, lung, kidney, spleen, heart, brain, and testis, while CYP4F1 was expressed in the kidney as well as in the liver [57]. Another study focused on the expression pattern of human CYP2C8, CYP2C9 and CYP2J2 and the soluble epoxide hydrolase, which hydrolyzes a wide variety of endogenous and exogenous epoxides. A large number of different tissues from various organs were evaluated using high-throughput tissue microarrays (see ref. 31 for detailed results). CYP2C9 and soluble epoxide hydrolase displayed very similar tissue-specific patterns of expression. They were both found in the liver, kidney, and many other organs, including adrenals, pancreatic islets, pituitary gland, lymphoid tissues, muscles, certain vascular smooth muscles, and epithelial cells in the skin, prostatic ducts, and the gastrointestinal tract. CYP2C8 and CYP2J2 were also widely distributed in human tissues but were not present in some tissues and the pattern of their expression was not so similar to that of soluble epoxide hydrolase. The results suggest potentially distinct pathways of endogenous fatty acid epoxide production and hydrolysis in a variety of human tissues.

Little is known about the expression and function of cytochrome P450 enzymes in the human skin. Some evidence indicates that xenobiotic metabolizing enzymes and transport proteins function as a second – biochemical – barrier of the skin [58, 59]. After penetration, the xenobiotics are first chemically activated or inactivated by oxidative reactions [60]. The most important family of enzymes involved in the reactions are the cytochrome P450 enzymes. In addition to their detoxifying functions they may be involved in allergic reactions to substances of low molecular weight, e.g., contact dermatitis. This has been shown for compounds such as eugenol, which is metabolized by 3-methylcholantrene inducible cytochrome P450 [61]. Eugenol

is a sensitizer only in those mice which have an inducible CYP1A1 phenotype, giving further evidence that this enzyme system is important with regard to the formation of the related antigens. In one study the expression pattern of cytochrome P450 enzymes and multidrug resistance-associated transport proteins has been analyzed in proliferating human epidermal keratinocytes under constitutive conditions and after induction with various inducers [62]. Reverse transcription-polymerase chain reaction revealed constitutive expression of CYP1A1, CYP1B1, CYP2B6, CYP2E1, and CYP3A5 in keratinocytes and showed expression of CYP3A4 after incubation with dexamethasone. Immunoblots as well as immunofluorescence in skin specimens showed expression of CYP1A1, CYP2B6, CYP2E1, and CYP3A and their activity was confirmed. It was concluded that cytochrome P450 enzymes play a complementary role in drug disposition by biotransformations and act synergistically with other enzymes as a drug bioavailability barrier.

In brain the total content of cytochromes P450 was found to be at least nine times higher in the mitochondrial fraction than in the microsomes in various regions studied [63]. Advances in a multitude of disciplines support an emerging role for cytochrome P450 enzymes and their metabolic substrates and end-products in the pathogenesis and treatment of central nervous system disorders. These include acute cerebrovascular injury, such as stroke, chronic neurodegenerative disease, such as Alzheimer's and Parkinson's diseases, as well as epilepsy, multiple sclerosis and psychiatric disorders, including anxiety and depression. The neural tissue contains its own unique set of cytochrome P450 genes that are regulated in a manner that is distinct from their molecular regulation in peripheral tissue. Furthermore, brain cytochromes P450 catalyze the formation of important brain signaling molecules, such as neurosteroids and eicosanoids, and metabolize substrates as diverse as vitamins A and D, cholesterol, bile acids, as well

as centrally acting drugs, anesthetics and environmental neurotoxins [64]. These unique characteristics allow this family of proteins and their metabolites to perform vital functions in the brain, such as neurotrophic support, neuroprotection, control of cerebral blood flow, temperature control, neuropeptide release, maintenance of brain cholesterol homeostasis, elimination of retinoids from CNS, regulation of neurotransmitter levels and other functions important in brain physiology, development and disease.

#### **4. Conclusion**

It is evident that the expression pattern of many cytochromes P450 in different tissues or cell types of an organ has important physiological roles and that sometimes the different cell-type distribution is combined with a different sub-cellular localization. Involvement of a cytochrome P450 enzyme in certain physiological processes can thus easily be missed when expression is studied in the entire organ. Currently the knowledge of detailed distribution of human cytochromes P450 in different organs, tissues and cell types is quite incomplete. Several studies of cytochrome P450 distribution and expression pattern have been performed in animal models. However, it is not always easy to translate these results to humans with certainty. In the mouse, for example, there are 102 putatively functional cytochrome P450 genes [1], while human possesses only 57 putatively functional cytochromes P450. In human and mouse the 34 cytochrome P450 orthologous pairs were identified with sequence alignment (see ref. 1 for the alignment), presumably carrying out similar or identical functions in both species. It is reasonable to predict the same or very similar expression pattern for these orthologous cytochromes P450. Consequently, we can conclude that for several human cytochrome P450 enzymes their tissue and cell-type specific physiological roles await determination.

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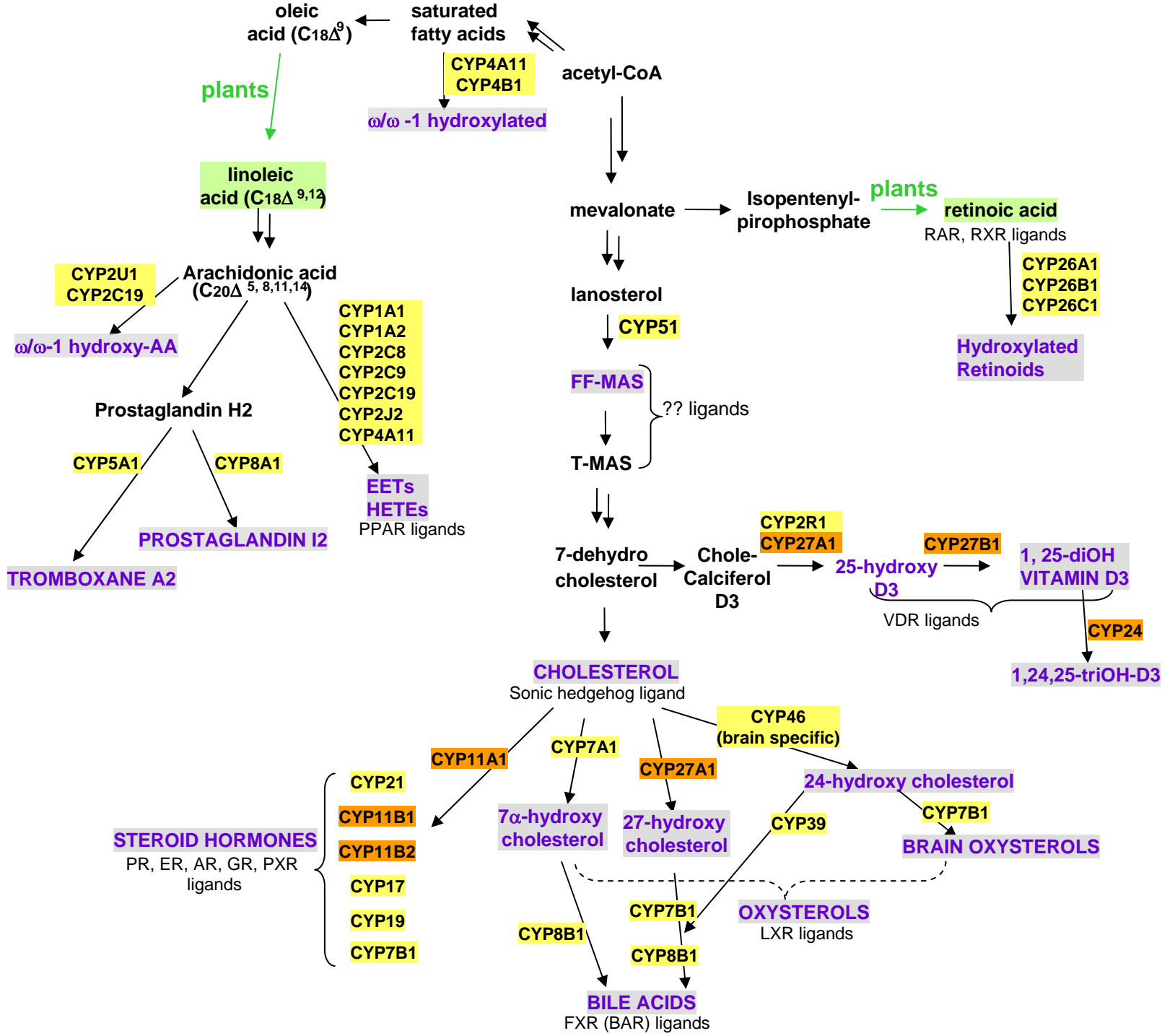
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Fig. 1. Schematic presentation of human cytochromes P450 involved in conversion of endogenous substrates. For some conversions not all possible cytochromes P450 are listed. An example is hydroxylation of retinoic acid – a number of cytochromes P450 from families 1-3 beside CYP26 family have also been shown to be capable of retinoic acid hydroxylation, in particular CYP3A7, CYP3A5, CYP2C8, CYP2C18 [65] and CYP2S1 [65]. Linoleic acid and retinoic acid are essential for humans and can only be produced in plants.

Fig. 2. Intracellular transport of CYP51 protein during spermatogenesis and transport in liver cells. (A) CYP51 intracellular transport in male germ cells during different phases of spermatogenesis (Golgi + Cap, Acrosomal, Maturation), with shown approximate duration of each phase (Days). After maturation phase spermatozoa can be stored in epididymis for several weeks. CYP51 protein seems to be stable for all that time, since enzymatic activity is still detected in acrosomal membranes of the ejaculate sperm. (B) The proposed scheme of the intracellular transport of CYP51 in liver cells [15].

N...nucleus, ER...endoplasmic reticulum, A...acrosome, GA...Golgi apparatus, RB...residual body.



A

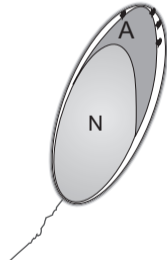
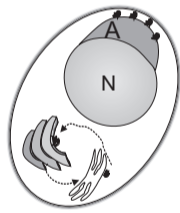
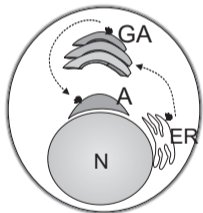
Days



Golgi+Cap

Acrosomal

Maturation



..... CYP51 protein

B

