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Sterol 14 α -Demethylase Cytochrome P450 (CYP51), a P450 in all Biological Kingdoms

Galina I. Lepesheva and Michael R. Waterman[†]

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, 37232-0146, USA

Summary

The CYP51 family is an intriguing subject for fundamental P450 structure/function studies and is also an important clinical drug target. This review updates information on the variety of the CYP51 family members, including their physiological roles, natural substrates and substrate preferences, and catalytic properties *in vitro*. We present experimental support for the notion that specific conserved regions in the P450 sequences represent a CYP51 signature. Two possible roles of CYP51 in P450 evolution are discussed and the major approaches for CYP51 inhibition are summarized.

Keywords

sterol 14 α -demethylase (CYP51); sterol biosynthesis; substrate preferences; catalytic activity; inhibition

Family overview

Sterol 14 α -demethylation as a general part of sterol biosynthetic pathways in eukaryotes [1] has been known and studied for more than 30 years [2-7]. The enzyme catalyzing this reaction was first purified from yeast in 1984 (*Sacharomyces cerevisiae* [8]), and following determination of its primary structure [9] the cytochrome P450 sterol 14 α -demethylases were placed into the CYP51 family, a number reserved for fungal sequences [10]. In 1986 the orthologous mammalian P450 was purified from rat liver microsomes [11], in 1996 the first sterol 14 α -demethylase was found in plants (*Sorghum bicolor* [12]), and in 2000 the orthologous nature of a CYP51-like gene [13] from *Mycobacterium tuberculosis* to eukaryotic CYP51s was confirmed [14].

Currently the CYP51 family joins proteins found in 82 organisms from all biological kingdoms. In addition, several plants and fungi contain multiple *CYP51* genes (e.g. rice (10), black oats (2), tobacco (2), *Arabidopsis thaliana* (2), *Fuzarium graminearum* (3) or *Aspergillus* species: *A. fumigatus* (2), *A. nidulans* (2), *A. orizae* (3)). As a result, the number of known CYP51 sequences exceeds 100. The reasons for the existence of homologous *CYP51* genes in the same species or their precise functions remain unknown, though it was reported that only one of the two *CYP51* genes from *A. thaliana* (CYP51A2) is functional, while the other is an expressed pseudogene [15]. Mammalian genomes contain only one *CYP51* gene yet sometimes

[†]Corresponding author: Michael R. Waterman, Tel.: 615-343-1373; Fax: 615-322-4349; E-mail: michael.waterman@vanderbilt.edu

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nonfunctional processed *CYP51* pseudogenes (e.g. 3 in human and 1 in rat [16, <http://drnelson.utmem.edu/biblioC.html>]) have been found.

The average amino acid sequence identity in the CYP51 family is about 30%, varying from relatively high between the proteins from evolutionary closely related species (e.g. 95% in mammals) to lower values within the highly diverse kingdoms of lower eukaryote (41%) and decreasing to 23-34% across the biological kingdoms (Figure 1). Interestingly, sequence identity between multiple genes in the same organism can also be rather moderate. Thus, the average identity for the ten putative CYP51s from rice is only 45% ranging from 78 to 38%; the sequences from three *Aspergillus* species are about 60% identical to each other, and the identity between the two proteins from *Arabidopsis thaliana* is 72%.

CYP51 function and physiological role *in vivo*

Though the cut off for the level of identity within a P450 family is accepted to be > 40%, sterol 14 α -demethylases from all phyla are assigned to the same family because they appear to retain strict catalytic function. All forms studied catalyze one regio- and stereospecific reaction of oxidative removal of the 14 α -methyl group from sterol precursors formed in the sterol biosynthetic pathway downstream from cyclization of squalene 2,3-epoxide (Figure 2).

The CYP51 reaction occurs in three steps, each requiring one molecule of oxygen and two NADPH-derived reducing equivalents [17]. During the first two cycles, which are typical P450-monooxygenations, the 14 α -methyl group is converted successively into the 14 α -carboxyalcohol and then into the 14 α -carboxyaldehyde. In the final step, the 14 α -aldehyde group is released as formic acid with concomitant introduction of the $\Delta^{14,15}$ double bond into the sterol core. The catalysis of the C-C bond cleavage is more complicated and two possible mechanisms (radical reaction or ionic (Beayer-Villiger) process) have been proposed [18,19].

The 14 α -demethylated products are intermediates in the pathways leading to formation of cholesterol in animals, ergosterol in fungi and a variety of 24-alkylated and olefinated sterols in plants, algae and protozoa [1,20]. *De novo* sterol biosynthesis takes place in the majority of eukaryotic cells (except for insects and nematodes, which consume sterols from the diet). Generally, the pathway occurs in the endoplasmic reticulum, but there is evidence that *Kinetoplastidae* might also synthesize sterols in mitochondria [21]. Bulk sterols, such as cholesterol, ergosterol and sitosterol (plants) are ubiquitous components of the plasma membranes, where they play an important structural role to regulate membrane fluidity and permeability and indirectly modulate the activity and distribution of integral membrane proteins, including enzymes, ion channels and components of signal transduction pathways [1,22]. The sterols serve as precursors for bioactive molecules such as mammalian steroid hormones, plant brassinosteroid hormones and insect ecdysteroid to control developmental processes. Amongst bacteria sterol biosynthesis is known in *Methylococcus capsulatus* [23], but the function of the CYP51 genes in *Mycobacteria* and *Nocardia* remains unclear.

In addition to being an invariant part of an essential biosynthetic pathway, the CYP51 reaction has other role(s) (Figure 2). Thus, in mammals a reaction intermediate (14 α -carboxyaldehyde derivative of lanosterol demethylation) is known to downregulate cholesterol production acting as suppressor of HMG-CoA reductase translation. [24-26]. Such a regulatory role of CYP51 might be of special importance in *Trypanosomatidae*, where the CYP51 reaction aldehyde intermediate of lanosterol can be used by the parasite to switch the pathway from endogenous sterol production to the energetically more favorable consumption of exogenous sterol precursors available from mammalian (host) blood [27].

Finally, in mammals, being expressed ubiquitously in all tissues, CYP51 is overexpressed in germ cells. Interestingly, in sperm in addition to endoplasmic reticulum active sterol 14 α -

demethylase and its electron donor cytochrome P450 reductase have been shown to be localized in Golgi apparatus and in the outer membrane of Golgi-derived vehicles called acrosomes [28]. In testis and follicular fluid the products of lanosterol 14 α -demethylation have been established to act as meiosis inducing substances ((meiosis activating sterols, MAS, see Figure 2) [29,30]. Detailed analysis of the role of mammalian CYP51 in spermatogenesis can be found in a recent review [31].

CYP51 *in vitro*

To date (May, 2006), 12 CYP51 have been purified, either from natural sources or by cloning and overexpression in heterologous systems, and characterized. Amongst them 9 are microsomal, eukaryotic enzymes (including 3 mammalian: rat [7], pig [32], and human [33]; 1 plant: *S. bicolor* [12], 3 fungal: 2 from yeasts (*S. cerevisiae* [8], *Candida albicans* [34]), and one from filamentous fungus *Ustilago maydis* [35] and 2 trypanosomal (*Trypanosoma brucei* [27] and *T. cruzi* [36]) while the other 3 are water soluble bacterial orthologs (from *M. tuberculosis* [14], *M. smegmatis* [37], and a fusion CYP51-ferredoxin protein from *Methylococcus capsulatus* [38]).

All the P450s are purified in the low-spin form with Soret maximum at 417 nm (Figure 3). In the reduced CO-complexes bacterial enzymes show maximum at 450 nm, while in the spectra of the eukaryotic orthologs the maximum is shifted to 446-447 nm, which might be connected with the formation of aggregates. Similar to other microsomal P450s, the eukaryotic forms of CYP51 require cytochrome P450 reductase as an electron donor partner; bacterial CYP51 from *M. tuberculosis*, *M. smegmatis* and *M. capsulatus* can be enzymatically reduced by flavodoxin/ flavodoxin reductase and ferredoxin/ferredoxin reductase systems [14,37].

Contrary to drug metabolizing P450s, which can accommodate a vast variety of structures, CYP51 has very narrow substrate specificity. The set of substrates is limited to the five naturally occurring 14 α -methylsterols (lanosterol, 24, 25-dihydrolanosterol, 24-methylenedihydrolanosterol, obtusifoliol and 4 β -desmethyllanosterol (norlanosterol [36]) (see Figure 2)). The reconstituted activity strongly depends on the reaction conditions, the highest reported turnover numbers being summarized in Table 1. Mammalian (human) and yeast (*C. albicans*) orthologs, which act *in vivo* directly downstream of formation of the first cyclized compound (lanosterol (Figure 2), can 14 α -demethylate all five substrates *in vitro* with similar rates and under the reconstitution conditions are the fastest sterol 14 α -demethylases. The activity of the single characterized CYP51 from filamentous fungi (*U. maydis*) was reconstituted with its physiological substrate (24, 25-methylenedihydrolanosterol) only [35]. CYP51 from the flowering plant *S. bicolor* is highly specific toward the C4-monomethylated sterols, the turnover number with the physiological substrate obtusifoliol being the highest (36), which is in good agreement with the results of the earlier work on characterization of this CYP51, yet in this earlier work norlanosterol was not tested [12]. Strict preference toward obtusifoliol was also reported for membrane fractions containing CYP51 orthologs from maize [39] and wheat [40] and thus it was considered a feature general for all plant orthologs until recently, when the ability of CYP51 from *S. chacoense* (chaco potato) to metabolize lanosterol (though at a rate considerably lower than that with obtusifoliol) was reported [41]. Strong specificity toward the C4-monomethylated substrates of CYP51 from the protozoan parasite *T. brucei* is similar to the CYP51 from *S. bicolor* while the ortholog from *T. cruzi* clearly prefers C4-dimethylated 24, 25-methylenedihydrolanosterol [36]. It is not excluded that there is a correlation between the strict substrate specificity of a CYP51 and the number of steps separating the first stylized sterol of the pathway from the CYP51 site in the biosynthetic scheme (Figure 2), the possible reason being to prevent the pathway from multiple bifurcations. *M. tuberculosis* CYP51 is able to demethylate all five sterols (turnover of obtusifoliol being the highest); the substrate preferences of CYP51 from *M. smegmatis* and *M. capsulatus* have

not been reported. Overall, the bacterial orthologs (at least with heterologous electron donor partners) operate one to two orders of magnitude slower than the characterized eukaryotic CYP51s but a larger number of CYP51 orthologs needs to be studied at comparable experimental conditions and preferably with their endogenous reductase systems to evaluate their relative turnover numbers more precisely.

CYP51 signature, structural basis of conservation in the CYP51 family

The complex mechanism of the CYP51 reaction suggests that critical geometry of the substrate is necessary to ensure its proper orientation during the three steps of catalysis in the enzyme active center. Therefore, CYP51s should have a common configuration of their substrate binding pockets, which in turn, regardless of low sequence identity in the family, requires certain amino acid conservation.

Yet, with the increasing number of known CYP51 sequences, the number of the invariant amino acid residues in the family within the last 3 years has dropped from 40 [42] to 29. Seven of them are glycines aligned with those in the *M. tuberculosis* CYP51 structure, which separate secondary structural elements. Based on the results of site-directed mutagenesis of these glycines in *M. tuberculosis* CYP51, we have hypothesized that they must be essential in the CYP51 structure to ensure functionally important conformational flexibility of the enzyme [43].

Most of the other conserved CYP51 residues are clustered into six regions, representing substrate recognition sites (SRS) 1-5 and that surrounding the heme-coordinating Cys. While conservation in the heme binding region is general amongst P450 families, high conservation within the SRSs (the sequences of highest variability in the drug metabolizing CYP2 family [44]) is a specific feature of CYP51 [45]. We believe that two of these regions, the most conserved and therefore the most thoroughly studied (SRS1 and SRS4) can be considered as a CYP51 signature (Figures 4, 5).

SRS1 (B' helix/B'C loop) forms the upper surface of a P450 substrate binding cavity (Figure 5). Substitution of the conserved Y, F and G in the B' helix results in complete loss of sterol 14 α -demethylase activity of *M. tuberculosis* and human CYP51 [42] and was shown to strongly affect activity of the rat ortholog (the corresponding Y131 and F139 were studied [46]). Another example provides mutation of conserved P81 in *M. tuberculosis* CYP51 (there is always P in this position with the exception of one sequence from filamentous fungi *Cunninghamella elegans* (Y)). In this case, the effect strongly depends on the substitution (Figure 6): thus alanine in this position decreases P450 expression but hardly influences the activity, but short polar G which does not affect P450 expression yet causes a 10-fold decrease in the activity and 5-fold decrease in the amplitude of type I spectral response. On the contrary, the long hydrophobic side chain of L increases two-times the activity and substrate binding but lowers P450 expression 5-fold, most likely as a result of strong destabilization. An example of the important role of phyla specific residues in the CYP51 B' helix is seen from our studies of CYP51 from *T. cruzi*. Expressing substrate preferences toward the C4-dimethylated 24, 25-methylenedihydrolanosterol, the enzyme has animal/fungi like I in the position where like all plant orthologs, CYP51 from *T. brucei* and CYP51s found in five other *Trypanosomatidae* contain F. Substitution of this I to F (I105F) in *T. cruzi* alters substrate preferences of the mutant more than two orders of magnitude increasing its ability to bind and metabolize C4-monomethyl sterols [36].

In the B'C loop, mutation of V (87/143 (*M. tuberculosis*/human)) to A prevents expression of *M. tuberculosis* CYP51 in the P450 form and strongly (more than 10 fold) reduces P450 expression of the human enzyme [42]. The effect of the substitution of two other residues F89 [47] and D90 [42] in this loop is probably phylum-specific. F corresponding to F89 in *M.*

tuberculosis CYP51 is present in this position in all bacterial and fungal CYP51 but in the other species this position is occupied by Y. Mutation of F89 inactivates *M. tuberculosis* CYP51 but substitution of the corresponding Y in human or *C. albicans* orthologs has no effect [47] on their activities and interaction with the substrate. Similarly, mutation D90A inactivates *M. tuberculosis* CYP51 but the D132A mutant of the human ortholog partially retains its sterol 14 α -demethylase activity. Later it was found that this D, which is conserved across the rest of the CYP51 family, is replaced with A in all trypanosomal sequences, suggesting that the mutation studied in *M. tuberculosis* and human CYP51 has occurred in nature. It is possible that in eukaryotic and prokaryotic CYP51, involvement of the BC loop in catalysis is different, especially taking into account that mutagenesis of a number of other, nonconserved residues in the B'C loop of *M. tuberculosis* CYP51 also strongly affect its catalytic activity (Lepesheva, G.I., Seliskar, M., Waterman, M.R., unpublished data). *M. tuberculosis* CYP51 is the only known P450 structure where the B'C loop is in an open conformation. Probably, this open conformation represents the channel [48] through which the sterol enter the active site. Thus in the traditional view of P450 structure, *M. tuberculosis* CYP51 is the only example to date where the substrate enters from the top rather than from the front. It is possible that in the eukaryotic CYP51 the B'C loop is positioned differently so that the role of the specific set of amino acid residues discussed here becomes functionally less crucial because the substrate enters the active site from the front rather than the top. This is only speculation until the structure of a eukaryotic form of CYP51 is determined.

SRS4 is located in the C-terminal part of the P450 I-helix (Figure 5) forming the right wall of the distal surface of the substrate binding cavity. This region contains the unique triplet of the CYP51 family –HT/sS-. The role of each of these three residues has been thoroughly studied on rat CYP51 [46,49]. The first and the last residues in the triplet (Figure 4) are conserved throughout the CYP51 family, while the middle T, corresponding to the “conserved threonine” [50] in most P450s, is replaced with S in several sequences from filamentous fungi. Mutagenesis in rat CYP51 shows that substitution of T315 to S has little effect on the enzyme, but the other substitutions (V, K or N) cause loss of detectable activity. On the contrary, S316 which is conserved throughout the family can be easily substituted with nonpolar A in rat CYP51 but longer, especially hydrophobic side chains placed here have a strongly negative influence on the activity. The position H in the triplet, invariant amongst the CYP51 family, in most other P450s is occupied by an acidic amino acid. In CYP101 (P450 cam) the corresponding D (D251) has been found to play a key role in proton transfer [51]. Mutation H314D in rat CYP51, however, causes more than a 6-fold decrease in the activity. In our studies of mutagenesis of *M. tuberculosis* CYP51 the corresponding H259D mutant retained only 5% of the wild type protein activity and the H259N mutant was inactive. Mutation of the conserved G257 (preceding the triplet) which disrupts the I-helix into two helical segments also inactivated *M. tuberculosis* CYP51 as a sterol 14 α -demethylase (Lepesheva, G.I., Waterman, M.R., unpublished data).

Thus, the two motifs, -YxxF/L(i)xxPxFGxxVxF/YD/a- in SRS1 and –GQ/hHT/sS- in SRS4, can be used to identify a P450 sequence as a CYP51 family member (CYP51 signature) and the phyla-specific residue F/L(i), in the B' helix (F in plant and L in animal/fungal CYP51) allows one to predict substrate preferences (mono or dimethyl at C4) of newly identified sterol 14 α -demethylases.

CYP51 and P450 evolution

Sterol biosynthesis is known to be a very ancient metabolic pathway, arising during the latest stages of microbial evolution after the introduction of molecular oxygen into the atmosphere [1]. Once aerobic conditions developed, formation of squalene 2, 3-epoxide became possible. Contrary to the anaerobic cyclization of squalene in the bacterial hopanoid pathway, squalene

2, 3-epoxide is cyclized into the steroid precursors (lanosterol or cycloartenol (Figure 2)). Further conversions of the precursors, including oxidative removal of three methyl groups from the core, produce sterols known today. Insertion of sterols into cellular membranes may have paved the way toward the development of eukaryotic organisms [52]. The prokaryotic origin of the sterol biosynthetic pathway implies a common prokaryotic ancestor for all currently existing sterol 14 α -demethylases, the only invariant P450 in every known sterol biosynthetic pathway, and in turn provides a very logical explanation for the presence of CYP51 orthologs in all evolutionarily younger biological kingdoms.

Supported by multiple sequence alignment analysis, the idea of the prokaryotic origin of sterol 14 α -demethylase might indicate that it is evolutionarily the oldest P450 recognizable today [45,53-55]. Even though an option of horizontal CYP51 gene transfer from plants to currently existing bacteria [31,56] (convolutionary process with loss of function) is not excluded, the CYP51 family remains an intriguing subject to study from two key evolutionary aspects: 1) evolution into different phyla with preserved metabolic role yet sometimes slightly different substrate preferences and 2) possible diversification into other P450 species with altered catalytic function.

Upon evolution within the family, alterations in the CYP51 sequences had to occur generally by mutagenesis in the regions which do not play crucial functional roles. As a result of such processes amino acid sequences of CYP51 from phylogenetically distant species became very diverse (e.g. CYP51 orthologs from *B. fuckeliana* (filamentous fungi) and *M. truncatula* (plant) or *T. cruzi* (protozoa) and onion (plant) have only 21 % identity and 42% similarity), yet the set of functionally essential amino acids (part of which are described above) must remain conserved.

Interesting from the evolutionarily viewpoint is identification of phyla-specific residues, switching CYP51 preferences from C4-double to C4-monomethylated substrates [36. Thus, in ancient *Euglena* CYP51 preferences toward C4-monomethylated obtusifolliol (F as the phyla specific residue in the B' helix (Figure 4)) could have developed with cycloartenol formation. However, the origin for the same preferences in *Trypanosomatidae* is likely different. *Trypanosomatidae* evolved independently from *Euglena* [57. Besides, the first cyclized product of their sterol biosynthesis is lanosterol and not cycloartenol. It suggests that either CYP51 from early photosynthetic organisms and *Kinetoplastida* share a common, more ancient (most likely prokaryotic), ancestor or that mutation in this key position (L to F) occurred repetitively. Of course the latter option is possible (e.g. in CYP51 from *T. cruzi* F in this position is substituted to I, as in CYP51 from *M. capsulatus*, both enzymes favoring C4-dimethylated sterols as substrates). On the other hand, it is not excluded that at first sterol 14 α -demethylase could have contained F in this position but (similar to *M. tuberculosis* CYP51 or I105F mutant of CYP51 from *T. cruzi* or CYP51 from *S. chacoense*) was able to metabolize both C4 mono- and C4 dimethylated substrates. If so, then F in this position might be evolutionarily older than L (or I).

Diversification of CYP51 into other P450 species could result from gene duplications followed by mutations in the substrate binding cavity and other areas which influence substrate binding. Potentially, three possibilities exist: if a mutation did not prevent binding of the sterol core, the mutant P450s could have developed the ability to metabolize other sterol molecules and to be included into evolutionarily newer metabolic pathways downstream of the CYP51 reaction. Such divergence could have occurred after the separation of the ancestors of animals from the fungal/plant branches [58] (sterol 22 desaturase (CYP61 in fungi [58] and CYP710 in plants [59]. Mammalian steroidogenic CYPs (e.g. CYPs 7, 8, 11, 17, 19, 21, 27, 39, 46) might be another example of this type of diversification. Sterol metabolizing P450s often preserve narrow substrate specificity and among them there are other examples which catalyze multiple

step reactions, e.g. CYP11A1, CYP17, CYP19. The last two were found to have essentially the same reaction mechanism as CYP51 [19], sequence identity/similarity to human CYP51 being 12/29, 16/37 and 14/33 %, respectively. If mutations caused loss of ability to bind sterols, the resultant protein either was not required, which could lead to formation of pseudogenes with future elimination or it could have acquired the ability to metabolize completely different structures [60] e.g. exogenous) and thus remain important for the organism

In general, detailed investigation of CYP51 evolution could be of great help for better understanding evolutionary processes in the P450 superfamily, which would possibly provide insight into creation of artificial P450 enzymes with desired properties and to uncover general directions of physiology accommodating the changing environment.

Inhibition of CYP51

Being an interesting subject for fundamental P450 studies, CYP51 is also of great practical importance as a drug target. Inhibition of sterol 14 α -demethylase activity blocks sterol biosynthesis, which is lethal in unicellular organisms, influences growth and developmental processes in plants, and lowers endogenous cholesterol production in animals. Though CYP51 inhibitors are being thoroughly studied as potential herbicides and cholesterol lowering drugs, they are most widely used as fungicides to treat human mycoses (clinical antifungals) and prevent food infections (agricultural antifungals). The demands for CYP51 inhibitors are increasing continuously because of drug resistance, worldwide increase in the incidence of opportunistic fungal infections as a consequence of the rising number of immunocompromised hosts (HIV-infections, cancer chemotherapy, organ and bone marrow transplantation) and patients with primary infections such as tuberculosis, etc. Quite a new aspect for CYP51 inhibitors is opened by the findings that they might be helpful in treatment of deadly diseases caused by trypanosomal parasites.

Azoles (imidazoles and triazoles) are the most broadly known CYP51 inhibitors. They coordinate to the heme iron through a basic nitrogen and inhibit activity preventing substrate binding and metabolism. Azoles play a pivotal role in the treatment of systemic and dermal mycoses [61] and have been found effective to cure murine models of leishmaniasis and Chagas disease [62-64]. Azoles are less toxic than other antifungal and anti-trypanosomal drugs, inexpensive and broadly available, yet have several disadvantages. Their long term usage can inhibit other P450 enzymes [65] and leads to resistance allowing the drugs tolerance in the pathogen. Amongst possible reasons for resistance are 1) mutations in fungal CYP51 (dozens of mutations which might be connected with azole resistance have been reported [e.g. 65-69]) 2) increase in the CYP51 gene expression and 3) faster azole efflux or decreased permeability [70-72]. To overcome this problem (or at least to make it less severe), *in vitro* testing of each particular inhibitor with the target fungal enzyme might be useful, because it is well known that affinities of different CYP51 orthologs to the same azole drug may vary significantly (up to 3-4 orders of magnitude [61,73]). Also, it is well known that the lower drug susceptibility a fungal strain has, the more frequently and rapidly resistance develops [70,74]. Besides, selecting the most powerful CYP51 inhibitors would help to shorten treatment time and lower the necessary doses.

An alternative approach to avoid azole resistance might be inhibition of CYP51 with substrate analogs. Contrary to azole derivatives sterols would have longer life-time in water solution and better membrane permeability. Attempts to use CYP51 substrate analogs to block cholesterol biosynthesis in humans have described that 7-oxo, 15-keto, 15-oxime, 15-hydroxy, 26-oxo derivatives of lanosterol are effective [24,75-77]; derivatives of the 14 α -carboaldehyde intermediate of lanosterol are known as hypocholesterolemic agents having dual effect *in vivo* (competitive CYP51 inhibitors and suppressors of 3-hydroxy-3-methylglutaryl coenzyme

A (HMG-CoA) reductase) [76-78]. Finally, 14 α -aminoderivatives of lanosterol were found effective to inhibit *C. albicans* and *T. cruzi* CYP51 orthologs [36,79]. Another option might be random search for other types of highly selective CYP51 inhibitors through high throughput screening of chemical libraries and selecting the compounds of interest based on their binding to the P450 and subsequently inhibition of its reaction.

CONCLUSION

Sterol 14 α -demethylase is a very important enzyme throughout biology because of its essential role in sterol biosynthesis. It is a primary drug target for a number of microbial infections in both animals and plants. CYP51 targeted drugs may also prove useful in treatment of other human conditions such as elevated cholesterol levels and as a herbicide in raising crops around the world. Since CYP51 is the most widely distributed member of the cytochrome P450 superfamily it also plays significant roles in our understanding of these monooxygenases. We have presented here a summary of the biochemical features of CYP51. We expect that this information will continue to expand in the coming years, particularly more detailed analysis of CYP51 structure/function relationships, a better understanding of why some plants and microbes contain what appear to be multiple copies of functional CYP51 genes and development of species specific inhibitors. We encourage readers to keep their eyes on developments in understanding CYP51 because they will continue to provide important insights into the P450 superfamily.

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Figure 1.

Amino acid sequence identity in the CYP51 family. Alignment of 71 sequences was performed using ClustalW 1.82, percentage of amino acid identity was calculated in GeneDoc (2.6). *(number of sequences in the alignment/ percentage of identity). Complete alignment of CYP51 family can be found at the web site <https://medschool.mc.vanderbilt.edu/watermanlab/index.php>

Figure 2.

CYP51 reaction in sterol biosynthesis. D, 24, 25-dihydrolanosterol; L, lanosterol; M, 24-methylenedihydrolanosterol; N, norlanosterol; O, obtusifoliol. *We have found recently that N is likely to be the preferred CYP51 substrate in *Trypanosomatidae* with the exception of *T. cruzi* [36]. R₁, alkyl or alkylene group; R₂, C6 or C7 double bond. The dashed line shows a major site of regulation of sterol biosynthesis.

Figure 3. Typical spectra of eukaryotic CYP51 (sterol 14 α -demethylase from *T. cruzi*). A, absolute oxidized; B, reduced CO difference; C, type I spectral response to substrate 24-methylenedihydrolanosterol; D, type II spectral response to azole inhibitor ketoconazole.

MT	AYP-FMTPTFGCGVVDA	: 91	MFACHHTSSG	: 263
M.bovis	ATP-FMTPTFGCGVVDA	: 91	MFACHHTSSG	: 263
M.smegmatis	AYP-FMTPTFGCGVVDA	: 92	MFACHHTSSG	: 264
M.avium	AYP-FMTPTFGCGVVDA	: 90	MFACHHTSSG	: 262
N.farcinica	AYP-FMKPTFGCGVVDA	: 91	MFACHHTSSG	: 263
M.capsulatus	AYR-IMTPTFGCGVVDA	: 94	MFACHHTSSG	: 264
S.chacoense	VYQ-FNVPTFGCGVVDA	: 122	LFACQHTSSI	: 292
potato	VYQ-FNVPTFGCGVVDA	: 122	LFACQHTSSI	: 292
tomato	VYQ-FNVPTFGCGVVDA	: 122	LFACQHTSSI	: 292
tobacco	VYQ-FNVPTFGCGVVDA	: 122	LFACQHTSSI	: 292
cotton	VYQ-FNVPTFGCGVVDA	: 121	LFACQHTSSI	: 290
poplar	VYQ-FNVPTFGCGVVDA	: 114	LFACQHTSSI	: 284
clover	VYQ-FNVPTFGCGVVDA	: 98	LFACQHTSSI	: 268
M.truncatula	VYQ-FNVPTFGCGVVDA	: 161	LFACQHTSSI	: 331
lotus	VYQ-FNVPTFGCGVVDA	: 109	LFACQHTSSI	: 279
soybean	VYQ-FNVPTFGCGVVDA	: 121	LFACQHTSSI	: 291
A.thaliana	VYQ-FNVPTFGCGVVDA	: 125	LFACQHTSSI	: 295
onion	VYQ-FNVPTFGCGVVDA	: 123	LFACQHTSSI	: 293
sorghum	VYR-FNVPTFGCGVVDA	: 126	LFACQHTSSI	: 296
cane	VYK-FNVPTFGCGVVDA	: 89	LFACQHTSSI	: 259
rice	VYK-FNVPTFGCGVVDA	: 122	LFACQHTSSI	: 292
wheat	VYR-FNVPTFGCGVVDA	: 126	LFACQHTSSI	: 296
barley	VYR-FNVPTFGCGVVDA	: 93	LFACQHTSSI	: 263
corn	VYR-FTVPTFGCGVVDA	: 85	LFACQHTSSI	: 255
D.discoideum	AYD-FVTPTFGCGVVDA	: 109	LFACQHTSSI	: 293
T.brucei	VYS-FMVPTFGCGVAYA	: 118	MFACQHTSSI	: 298
T.gambiense	VYS-FMVPTFGCGVAYA	: 118	MFACQHTSSI	: 298
T.congolense	VYS-FMVPTFGCGVAYA	: 118	MFACQHTSSI	: 298
T.vivax	VYA-FMVPTFGCGVAYA	: 118	MFACQHTSTI	: 298
T.cruci	VYT-IMTPTFGCGVAYA	: 118	MFACQHTSTI	: 298
L.major	VYS-FMVPTFGCGVAYA	: 117	MFACQHTSTI	: 297
L.infantum	VYS-FMVPTFGCGVAYA	: 117	MFACQHTSTI	: 297
pig	VYSRLTTPWFGKGVANDV	: 147	LLACQHTSST	: 318
dog	VYSRLTTPWFGKGVANDV	: 147	LLACQHTSST	: 318
human	VYSRLTTPWFGKGVANDV	: 147	LLACQHTSST	: 318
bull	VYSRLTTPWFGKGVANDV	: 148	LLACQHTSST	: 319
rat	VYGRLTTPWFGKGVANDV	: 147	LLACQHTSST	: 318
mouse	VYGRLTTPWFGKGVANDV	: 130	LLACQHTSST	: 301
chicken	VYSRLTTPWFGKGVANDV	: 139	LLACQHTSST	: 310
frog	VYSRLTTPWFGKGVANDV	: 145	LLACQHTSST	: 316
killifish	VYSRLTTPWFGKGVANDV	: 143	LLACQHTSST	: 314
sergeant.major	VYSRLTTPWFGKGVANDV	: 141	LLACQHTSST	: 312
O.latipes	VYSRLTTPWFGKGVANDV	: 87	LLACQHTSST	: 257
zebrafish	VYARLTTPWFGKGVANDV	: 139	LLACQHTSST	: 310
sea.urchi	VYSNLTTPWFGKGVANDV	: 138	LLACQHTSST	: 308
C.albicans	AYKHLTTPWFGKGVYDC	: 134	LMCQHTSAS	: 314
C.dubliniensis	AYKHLTTPWFGKGVYDC	: 134	LMCQHTSAS	: 314
C.tropicalis	AYTHLTTPWFGKGVYDC	: 134	LMCQHTSAS	: 314
C.krusei	AYTHLTTPWFGKGVYDC	: 80	LMCQHTSAA	: 254
C.glabrata	AYSHLTTPWFGKGVYDC	: 143	LMCQHTSAA	: 322
S.cerevisiae	AYAHLTTPWFGKGVYDC	: 142	LMCQHTSAA	: 321
E.gossypii	AYTKLTTPWFGCGVVYDC	: 140	LMCQHTSAA	: 319
M.acuformis	IYTVLTTPWFGKGVVYDC	: 138	LMACQHTSSS	: 314
M.yallundae	IYTVLTTPWFGKGVVYDC	: 138	LMACQHTSSS	: 314
B.fuckeliana	IYTVLTTPWFGKGVVYDC	: 138	LMACQHTSSS	: 315
M.fruticola	IYTVLTTPWFGKGVVYDC	: 138	LMACQHTSSS	: 315
B.graminis	IYTVLTTPWFGKGVVYDC	: 138	LMACQHTSSS	: 315
U.necator	IYTNLTTPWFGKGVVYDC	: 138	LMACQHTSSS	: 315
M.graminicola	IYSPLTTPWFGKGVVYDC	: 139	LMACQHTSSA	: 314
V.inaequalis	IYSPLTTPWFGKGVVYDC	: 135	LMACQHTSSS	: 311
V.nashicola	IYSPLTTPWFGKGVVYDC	: 135	LMACQHTSSS	: 311
P.digitatum	IYCKLTTPWFGKGVVYDC	: 128	LMACQHTSSA	: 312
P.italicum	IYCKLTTPWFGKGVVYDC	: 128	LMACQHTSSA	: 311
A.fumigatus	IYSPLTTPWFGKGVVYDC	: 123	LMACQHTSSS	: 300
A.oryzae	IYSPLTTPWFGKGVVYDC	: 121	LMACQHTSSS	: 296
A.nidulans	IYSPLTTPWFGKGVVYDC	: 121	LMACQHTSSS	: 296
S.pombe	AYSHLTTPWFGKGVVYDI	: 119	LMACQHTSAA	: 295
C.neoformans	AYTHLTTPWFGKGVVYDC	: 147	LMACQHTSSA	: 324
P.chrysosporium	IYCKLTTPWFGKGVVYDC	: 141	LMACQHTSSA	: 323
U.maydis	AYTHLTTPWFGKGVVYDV	: 146	LMACQHTSSA	: 332
C.elegans	AYNHMTKYWFGKGVVYDA	: 132	LFCEQHTSAT	: 304

YxxFLxxPxFGxxVxFYD/a

GQ/HT/s

Figure 4.
Conservation in the CYP51 SRS1 and 4. A CYP51 signature is written below the alignment.
 Phyla-specific residues are shadowed in gray.

Figure 5.
Location of the CYP51 signature in the P450 structure. A, upper view, B, distal view. The conserved regions within the B'helix/BC loop and in the I-helix are colored in red. Heme is shown in green.

Figure 6.
Mutagenesis of conserved P81 in the B' helix of *M. tuberculosis* CYP51.

Table 1

Reconstituted CYP51 activity

Species/ phylum	Turnover* (nmol substrate/nmol P450/min)				Reference
	24,25-Dihydrolanosterol	Lanosterol	24-Methylenedihydrolanosterol (eburecol)	Norlanosterol	Obtusifolol
<u>Eukaryote</u>					
Human/Mammal	30	29	27	23	29
Rat/Mammal	20	14	ns	ns	ns
<i>C. albicans</i> /Yeast	25	24	29	17	27
<i>S. cerevisiae</i> /Yeast	3.9	6.2	6.8	ns	ns
<i>U. maydis</i> / Filamentous	ns	ns	7.7	ns	ns
<u>Fungi</u>					
<i>T. cruzi</i> / Kinetoplastida	1.6	0.6	2.4	0.03	0.06
<i>T. brucei</i> / Kinetoplastida	<0.01	<0.01	<0.01	3.0	6.2
<i>S. bicolor</i> / Flowering plant	<0.01	<0.01	<0.01	0.9	5.1
<u>Prokaryote</u>					
<i>M. tuberculosis</i> / Bacteria	0.18	0.15	0.12	0.18	0.45
<i>M. smegmatis</i> / Bacteria	ns	0.7	ns	ns	ns
<i>M. capsulatus</i> / Bacteria	ns	0.26	ns	ns	ns

* The highest published values are presented. The reasons for the differences in the published calculated rates of the CYP51 turnovers are likely in part connected with differences in the final P450 concentrations in the reconstituted reaction mixture. ns-not studied.