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Steroidogenic Cytochrome P450 17A1 Structure and Function

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Abstract

Cytochrome P450 17A1 (CYP17A1) is a critical steroidogenic enzyme, essential for producing glucocorticoids and sex hormones. This review discusses the complex activity of CYP17A1, looking at its role in both the classical and backdoor steroidogenic pathways and the complex chemistry it carries out to perform both a hydroxylation reaction and a carbon-carbon cleavage, or lyase reaction. Functional and structural investigations have informed our knowledge of these two reactions. This review focuses on a few specific aspects of this discussion: the identities of reaction intermediates, the coordination of hydroxylation and lyase reactions, the effects of cytochrome b_5, and conformational selection. These discussions improve understanding of CYP17A1 in a physiological setting, where CYP17A1 is implicated in a variety of steroidogenic diseases. This information can be used to improve ways in which CYP17A1 can be effectively modulated to treat diseases such as prostate and breast cancer, Cushing's syndrome, and glioblastoma.

Keywords

Cytochrome P450 17A1; Mechanism; Crystallography; Prostate; Cancer; Cushing's syndrome

1. INTRODUCTION

Cytochrome P450 enzymes are a superfamily of monooxygenases involved in numerous critical physiological functions. While some are best known for their roles in drug metabolism, human steroidogenesis involves six P450 enzymes. Of these, human cytochrome P450 17A1 (CYP17A1) plays a critical role in the production of glucocorticoids and sex hormones and is implicated in a variety of disease states. Numerous studies have investigated the structure and function of CYP17A1 as required to effectively modulate its activity through drug design.

This review discusses the major catalytic activities of CYP17A1 and how those reactions play into human steroidogenesis pathways. Various hypotheses surrounding the mechanism through which this enzyme accomplishes two distinct reactions will be examined from the

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DECLARATION OF COMPETING INTERESTS

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viewpoints of both functional and structural studies. Finally, the importance of CYP17A1 activity is highlighted by examination of various disease states associated with this enzyme, as well as CYP17A1 inhibitors that have been studied to treat disease.

2. FUNCTION

2.1 Classical Steroidogenic Pathway

CYP17A1 functions at an early stage in the classic steroidogenesis pathway. After cleavage of the cholesterol side chain, the resulting pregnenolone can be converted into mineralocorticoids, glucocorticoids, or sex hormones (Figure 1) (Miller and Auchus, 2011). In the absence of CYP17A1 activity, pregnenolone is converted to the mineralocorticoid aldosterone. Alternatively, CYP17A1 can hydroxylate pregnenolone at carbon 17 or its 3-keto, Δ4 version progesterone at either carbon 16 or 17. While the physiological significance of the minor 16α-hydroxyprogesterone product is poorly elucidated, hydroxylation at C17 leads to the generation of glucocorticoids such as cortisol (Swart et al., 2010; Turcu et al., 2015; Yoshimoto and Auchus, 2015; Yoshimoto et al., 2016; Yoshimoto et al., 2012). Finally, CYP17A1 can act on its initial 17-hydroxylated products to subsequently cleave the bond between C17 and C20. This lyase reaction leaves a ketone at C17, forming the androgen dehydroepiandrosterone or androstenedione. As the catalytic efficiency of 17OH-progesterone conversion to androstenedione is relatively low, androstenedione is primarily formed from dehydroepiandrosterone (Lee-Robichaud et al., 1995; Swart et al., 1993). These intermediates can subsequently be converted to testosterone and then into dihydrotestosterone or into estrogens or into 11OH-androstenedione. Notably, reduction of the C4 and C5-double bond by the enzyme 5α-reductase occurs late in the classical pathway, upon conversion of testosterone to dihydrotestosterone, which differs in the backdoor pathway discussed below (section 2.2).

2.2 Backdoor Pathway

While studying tammar wallabies, researchers discovered a second steroidogenic pathway now referred to as the “Backdoor Pathway” (Figure 1) (Auchus, 2004; Miller and Auchus, 2019; Renfree et al., 1996; Renfree et al., 1992; Shaw et al., 1988). In this pathway dihydrotestosterone is still the ultimate product but intermediates such as dehydroepiandrosterone, androstendione, and testosterone are bypassed. Instead reduction of the Δ4-double bond of progesterone occurs earlier in the pathway and subsequent intermediates include dihydroprogesterone, pregnan-17-ol-3,20-dione, allopregnanolone, and 17OH-allopregnanolone. These intermediates lead to the production of androsterone which can be converted to androstanediol and then dihydrotestosterone. Later studies of midgestation male human fetuses suggest the presence of backdoor pathway enzymes and intermediates in the placenta, liver, and adrenal rather than in the testis. Additionally, androsterone rather than testosterone was primarily observed in blood circulation (O’Shaughnessy et al., 2019). This suggests that the backdoor pathway also occurs in humans (O’Shaughnessy et al., 2019). This backdoor pathway is implicated in polycystic ovarian syndrome, congenital adrenal hyperplasia, minipuberty of infancy in males, and cytochrome P450 reductase deficiency in virilized females (Dhayat et al., 2017; Homma et

al., 2006; Kamrath et al., 2012; Krone et al., 2012; Marti et al., 2017; Shackleton et al., 2004).

2.3 Hydroxylase versus Lyase Activity

It is critical to understand that both the classical and backdoor pathways require CYP17A1 hydroxylase and lyase activity. There is no pathway to synthesize dihydrotestosterone that does not involve CYP17A1. P450 enzymes are monooxygenases that utilize molecular oxygen and electrons to carry out chemistry at their catalytic heme, but this usually results in substrate hydroxylation, which may be followed by product decomposition such as to a dealkylated product. CYP17A1 is unusual because in addition to the typical hydroxylation activity, it also performs lyase reactions. While the two disparate reactions were initially ascribed to two separate enzymes, in 1981 it was demonstrated that CYP17A1 purified from pig testes could perform both reactions (Auchus, 2001; Nakajin and Hall, 1981). Furthermore, raising the temperature and enzyme inhibition affected lyase and hydroxylase activities to the same extent, consistent with the idea that both activities were accomplished by the same enzyme (Nakajin and Hall, 1981). This idea was confirmed in 1986 when CYP17A1 transformed into non-steroidogenic COS-1 cells conferred both hydroxylase and lyase activities (Auchus, 2001; Zuber et al., 1986). Computational studies suggested that these two chemical reactions occurred via differential positioning in two different lobes of a CYP17A1 active site (Haider et al., 2010), but later experimental structures revealed a single active site. Functional studies revealed that the catalytic efficiencies of these reactions vary with substrate. While CYP17A1-mediated hydroxylation of pregnenolone and progesterone occur at comparable rates, the lyase reaction is much more efficient for 17OH-pregnenolone than 17OH-progesterone (Auchus et al., 1998; Khatri et al., 2014; Mak et al., 2018). These functional complexities with different substrates and different reactions have prompted significant mechanistic inquiries (*vide infra* section 3).

2.3 Pregnenolone 16-ene Synthase Activity

One study has additionally shown that human CYP17A1 also has the ability to generate a C16-C17 double bond in pregnenolone to form androstenol (5α−16-androstene-3α-ol) in transiently transformed HEK293 cells (Soucy et al., 2003). This reaction was highly dependent on cytochrome b_5 concentrations but remarkably insensitive to NADPH-cytochrome P450 levels beyond the endogenous level already in HEK293 cells. Androstenol is a precursor for androstanol, but the physiological significance in humans is poorly understood.

3. MECHANISM

In P450 enzymes, the orientation of a substrate relative to the heme prosthetic group typically dictates the identity of the product that is formed. For this reason, in promiscuous, drug-metabolizing P450 enzymes a single substrate can often each bind in more than one orientation, thereby producing multiple metabolites by interacting with a single P450 enzyme. P450 enzymes with more selective roles in the interconversion of endogenous compounds, like steroids, are more selective in the compounds that can bind in the active site and their orientations and thus tend to produce more strictly controlled metabolites. The

fact that CYP17A1 can produce both 16- and 17-hydroxylated progesterone products suggests some degree of flexible positioning in the active site. Studies suggest that Ala105 is crucial and orients the substrate for this 16-hydroxylation activity (Swart et al., 2010). However, the 16α-hydroxyprogesterone product is typically observed in only minor quantities compared to the major 17-hydroxylated products, consistent with one major and one minor orientation in the CYP17A1 active site.

Substrate binding in the active site is usually the first step of catalysis (Figure 2, top left, RH). This binding event typically displaces water from the ferric heme iron, resulting in an increased redox potential, and thereby favoring acceptance of an electron from cytochrome P450 reductase (Daff et al., 1997; Guengerich et al., 1975; Oprian and Coon, 1982; Sligar, 1976; Sligar et al., 1979). The now ferrous (Fe^{2+}) heme is able to bind molecular oxygen (O_2). Delivery of the second electron forms a peroxoanion intermediate. This sets the stage for a series of steps in which catalysis occurs, but the precise catalytic intermediate may vary for hydroxylation vs. lyase reactions as described below (sections 3.1 and 3.2).

3.1 Hydroxylation Chemistry

For a typical P450 hydroxylation reaction, employed for example in CYP17A1-mediated pregnenolone or progesterone hydroxylation, the next chemical steps after formation of the peroxoanion are relatively well understood (Figure 2, A-D). The ferric peroxoanion intermediate is protonated twice, with the second protonation resulting in cleavage of the O-O bond to release water. The remaining ferryl iron oxo species, called compound I, attacks the substrate, which has been colocalized in the active site during all the proceeding chemical steps, and abstracts hydrogen from what is usually the nearest substrate position. After this hydrogen abstraction, rebound then rapidly occurs to generate the hydroxylated product and the initial ferric heme. Products are thus formed and can be released from the active site allowing the catalytic cycle to repeat.

3.2 Lyase Chemistry

For CYP17A1's lyase activity, however, the mechanism after formation of the peroxoanion intermediate has been the subject of much debate. Two primary oxidants occurring in the catalytic cycle (Figure 2) have been suggested for the lyase reaction: the peroxoanion intermediate itself or the subsequent compound I intermediate. It is also possible that both intermediates are catalytically competent under subtly different conditions.

3.3 Lyase Reaction by Compound I

A combination of experimental and computational methods have suggested that the ferryl oxygen (Fe^{IV}=O) species compound I from the typical catalytic cycle can also serve as the primary oxidant for the lyase reaction. Compound I would abstract a hydrogen from the substrate to form compound II and a substrate radical, in this case potentially from the C17 alcohol (Figure 2, E). Compound II can then oxidize the substrate a second time and cleave the carbon-carbon bond. This type of reaction is feasible based on the reduction potential of Compound II (Mittra and Green, 2019).

Although artificial catalytic partners were employed, strong evidence for the participation of compound I in this reaction is the ability of the lyase reaction to be driven by iodosylbenzene (C_6H_5IO). Iodosylbenzene provides only a single oxygen and can thus generate compound I, but not the peroxo intermediate. In contrast, the hydroxylation reaction, but not the lyase reaction, occurred when supported artificially by hydrogen peroxide (H_2O_2) which can form the peroxo intermediate (Yoshimoto et al., 2016). Additionally, the same study revealed that both 17OH-steroids can also be hydroxylated at the 16 position to yield small amounts of 16,17diOH-pregnenolone or 16,17diOH-progesterone, a reaction implicating the presence of the compound I intermediate when the 17OH-steroids are bound. Both ideas were reinforced by work from the Guengerich group who showed that human CYP17A1 was able to convert the synthetic 17α-hydroperoxy versions of pregnenolone and progesterone to the hydroxylase and lyase products as well as 16,17-dihydroxy steroids, in the absence of NADPH or cytochrome P450 reductase (Gonzalez et al., 2018).

A compound I-mediated mechanism has also been proposed for several other P450 enzymes that perform similar carbon-carbon bond cleavage reactions. For example, isotope labeling experiments suggested that Compound I is the key catalytic intermediate in the lyase reaction that is the final step of steroid aromatization to form estrogens (Yoshimoto and Guengerich, 2014). Additionally, recent studies using computational methodology supported compound I mediation of carbon-carbon bond cleavage by CYP11A1, although these studies suggested a slightly different pathway (Su et al., 2019).

3.4 Lyase Reaction by Peroxoanion

While investigating the aromatization process, with concomitant production of the aromatic ring of estrogen, Akhtar et al. showed that in the reaction in which the C-19 methyl group of an androgen is oxidized to formic acid, the peroxoanion appeared to be the key catalytic intermediate (Akhtar et al., 1982). This intermediate has since been suggested to also participate in lyase reactions performed by CYP2B4 (Vaz et al., 1996), CYP51 (Shyadehi et al., 1996), and CYP17A1 (Gregory et al., 2013; Lee-Robichaud et al., 1995). For this mechanism, the CYP17A1 nucleophilic peroxoanion would directly attack the 17OH-pregnenolone or 17OH-progesterone substrate to form a peroxohemiketal transition state (Figure 2, G). Subsequent fragmentation would result in cleavage of the C17-C20 bond, yielding the steroid ketone and acetate products (Figure 2, H). The difference between the compound I intermediate and the peroxoanion intermediate are two protonation events. Thus, it is important to note that an unusual inverse kinetic solvent isotope effect has been observed for the lyase reaction (Gregory et al., 2013), though the magnitude has varied in different reports for the different lyase substrates (Yoshimoto et al., 2016). That is, substitution of deuterated solvent causes an *increase* in the lyase reaction, which would be consistent with lyase mediation by the peroxoanion intermediate. This contrasts with CYP17A1-mediated hydroxylation reactions which are known to proceed via compound I, rely on protonation steps, and are *decreased* in the presence of deuterated solvent, which is a positive kinetic solvent isotope effect (Gregory et al., 2013; Yoshimoto et al., 2016). However, interpretation of these isotopic experiments is complex and there are other possible explanations. Other types of isotope labeling experiments have been suggested to

correspond most closely to those predicted from a peroxoanion intermediate. Pig microsomes incubated with a lyase substrate triply-deuterated at C21 in the presence of $^{18}O_2$ yielded acetate with incorporation of all three hydrogen atoms from the C21 methyl and one of the oxygens from $^{18}O_2$. This was interpreted as indicative of a peroxoanion intermediate because if compound I were involved, acetate would have incorporated only two of the three hydrogen atoms from the C21 methyl group (Akhtar et al., 1994). More recent high-resolution mass spectrometry studies with purified human CYP17A1 enzyme yielded similar experimental findings, but suggested mechanisms that would explain this result in terms of either a peroxo or compound I intermediate (Yoshimoto et al., 2016).

A second line of evidence for the peroxoanion intermediate was provided by monitoring the reaction progression via resonance Raman studies (Mak et al., 2018; Mak et al., 2015). Although products were not defined, cryo-trapped CYP17A1 reactions with the hydroxylase substrate pregnenolone detected the peroxoanion intermediate which then annealed to the hydroperoxy state proceeding to Compound I. Similar experiments with the favored lyase substrate 17OH-pregnenolone yielded an intermediate assigned as the peroxoanion, but which did not progress to the hydroperoxyl state, but rather to a new species (Mak et al., 2015). Later, similar studies with the progesterone hydroxylase substrate did not detect the peroxoanion intermediate (though it must occur), while the poor lyase substrate 17OH-progesterone generated both the peroxoanion and hydroperoxyl species (Mak et al., 2018). One explanation is that there is more efficient proton transfer to the peroxoanion intermediate when 17OH-progesterone is the substrate compared to the peroxoanion intermediate when 17OH-pregnenolone is the substrate. More efficient protonation of the peroxoanion species would reduce the lyase reaction, as observed for 17OH-progesterone.

Several studies suggest that the two lyase substrates interact differentially with the two oxygens of the peroxoanion intermediate. Resonance Raman data was consistent with the 17OH of 17OH-pregnenolone interacting with the proximal peroxoanion oxygen (closest to the iron), which would leave the distal or terminal nucleophilic oxygen of the bound O_2 available to attack the electrophilic C20 of the substrate. In contrast, the resonance Raman data was consistent with the 17OH of 17OH-progestereone interacting with the terminal or distal peroxoanion oxygen (Mak et al., 2018). These factors would be consistent with the observed higher CYP17A1 lyase activity for 17OH-pregnenolone over 17OH-progesterone and with earlier structural evidence on the heights of these two ligands in the active site (*vide infra* section 4.2).

3.5 Coordination of Hydroxylase and Lyase Reactions: Distributive versus Processive

Selective inhibition of the CYP17A1 lyase reaction is a holy grail in treating prostate cancer and other androgen and estrogen-associated diseases without disrupting CYP17A1-mediated hydroxylation to produce glucocorticoids. As a result, it is important to understand the coordination of these two reactions. In both the classical and backdoor pathways, hydroxylation precedes the lyase reaction, but their coordination can be either processive or distributive.

A processive arrangement would involve the hydroxylation product, which is also the lyase substrate, being retained within the CYP17A1 active site for the successive lyase reaction. In

the classic pathway, for example, pregnenolone would bind in the enclosed CYP17A1 active site, be converted to 17OH-pregnenolone, and then be converted to dehydroepiandrosterone. Only the final dehydroepiandrosterone product would be released from the CYP17A1 active site, not 17OH-pregnenolone. Such coordination of the two reactions would make it implicitly more difficult to inhibit the androgen-generating lyase reaction specifically, without also inhibiting the glucocorticoid-generating hydroxylation reaction. Specifically, it would be difficult for a competitive inhibitor to outcompete the already-bound hydroxylated steroid.

In contrast, in a completely distributive mechanism, the initial steroid substrate would bind in the active site to be converted to the 17-hydroxylated product, which would then be released from the CYP17A1 active site. CYP17A1 would then need to capture the 17OH-steroid within its active site for the lyase conversion to the final androgen product. For example, in the classical pathway, pregnenolone would bind to CYP17A1 and be converted to 17OH-pregnenolone, but the 17OH-pregnenolone would then be released from the CYP17A1 enzyme. The same, dissociated 17OH-pregnenolone molecule or some other 17OH-pregnenolone molecule present would then enter the active site of the same or a different CYP17A1 protein. The CYP17A1 enzyme can indeed bind 17-hydroxylated steroids directly and produce the corresponding lyase products (*i.e.* CYP17A1 binding is not selective for the unhydroxylated steroid), which suggests that the distributive process is a possibility, but does not rule out the processive process. Importantly for clinical applications, because the hydroxylated substrate needs to rebind, a distributive mechanism may provide an opportunity for selectively inhibiting the androgen-generating lyase reaction while still preserving the hydroxylation activity and downstream glucocorticoid production.

Detailed enzyme kinetic studies measuring ligand dissociation, substrate turnover rates, lag phase lengths for lyase product formation, and enzyme inhibition have suggested that CYP17A1 is primarily distributive, but has some processivity to it as well (Gonzalez and Guengerich, 2017). This likely means that some fraction of the time the hydroxylated substrate stays bound and directly converts to androgen, while most of the time it is released from the active site before further conversion. These studies also showed that pregnenolone is more processive than progesterone (Gonzalez and Guengerich, 2017).

4. STRUCTURE

CYP17A1 is embedded in the endoplasmic reticulum via two critical regions: an N-terminal transmembrane helix and a hydrophobic region on the distal side of the globular catalytic domain (Poulos and Johnson, 2015). The first CYP17A1 crystal structures obtained in 2012 show overall conservation of the general P450 enzyme fold with 12 major helices, typically labeled A-L (DeVore and Scott, 2012). To obtain these crystal structures, the transmembrane helix was truncated. This has become common practice for membrane P450 crystallization efforts, yielding catalytically-active protein still embedded in membrane, but improving enzyme solubility, oligomeric homogeneity, and yield (Scott and Godamudunage, 2018). Using this approach there are now multiple CYP17A1 structures in complex with all of the classical steroidogenic pathway substrates and a wide variety of inhibitors (DeVore and Scott, 2012; Fehl et al., 2018; Petrunak et al., 2014; Petrunak et al., 2017).

4.1 Structures with Inhibitors

The first CYP17A1 structures elucidated were with the steroid-based inhibitors abiraterone and galeterone and provided information useful for structure-based drug design purposes. Abiraterone and galeterone are pregnenolone derivatives with an extra double bond between C16 and C17 and either a pyridine (abiraterone) or benzimidazole (galeterone) substituting for the side chain (Figure 3). Both were developed clinically for the treatment of prostate cancer but their binding modes were unknown. The first reports of CYP17A1 structures (DeVore and Scott, 2012) revealed that abiraterone and galeterone are located in the active site with the respective nitrogens of the pyridine and imidazole rings directly forming coordinate covalent bonds with the heme iron. This also occurs in solution, as spectral changes distinctive for such an interaction (type II shift) were observed (DeVore and Scott, 2012; Garrido et al., 2014). This explains the inhibition, as the inhibitor nitrogens are occupying the position on the heme iron where O_2 would need to bind for catalysis. Titration experiments indicated that these inhibitors bind with very high affinity, with K_d values so low (nM) (DeVore and Scott, 2012; Garrido et al., 2014) that they are difficult to quantitate precisely. The low K_d value for abiraterone at least is due to a very slow off rate ($t_{1/2}$=1.8 hrs), as shown by rapid dilution studies (Garrido et al., 2014), leading some reports to call it an irreversible inhibitor. Some have also indicated that abiraterone is a slowly binding inhibitor and binds in multiple phases, invoking a requirement for CYP17A1 slow conformational changes (Cheong et al., 2020; Guengerich et al., 2019). There are also reports of the slow onset of abiraterone inhibition and improved inhibition by preincubating CYP17A1 with abiraterone for 30 minutes (Cheong et al., 2020; Jarman et al., 1998), but other studies have not required such extended preincubation for efficient inhibition of multiple activities (DeVore and Scott, 2012; Garrido et al., 2014; Petrunak et al., 2017). Regardless, since the ferric enzyme is complexed with inhibitors to generate the structures, these complexes are most relevant to the binding data, with the N-Fe bond strongly contributing to the affinity (Fehl et al., 2018). In both abiraterone and galeterone complexes, the planar body of the steroidal core plane is angled by 60° over the heme, extending over the I helix and with the C3 hydroxyl hydrogen bonded with N202 on the F helix (Figure 4) (DeVore and Scott, 2012). Despite having the same overall P450 fold and heme coordination, steroidal ligands in CYP19A1, CYP11A1, and CYP46A1 are all oriented in the opposite direction, with the steroidal plane directed over the K/L loop (Figure 4, orange) (DeVore and Scott, 2012).

Perhaps even more interestingly, these structures revealed available active site space adjacent to the steroid B ring and that this space was bordered by two charged active site residues, R239 and D298, providing obvious opportunities for improving inhibitor complementarity to the CYP17A1 active site. Based on this information modifications of abiraterone extending from the B ring C6 were explored to improve its selectivity for CYP17A1 over CYP21A2. CYP21A2 is another steroidogenic P450 acting on progesterone and 17OH-progesterone to generate mineralocorticoids and glucocorticoids, with off-target inhibition of the latter being a particularly relevant problematic side effect in prostate cancer patients treated with abiraterone. While a number of C6 substituents still effectively inhibited CYP17A1 and engaged R239 and D298 as desired, addition of an oxime improved the selectivity for CYP17A1 over CYP21A2 from 6.6-fold to 84-fold (Fehl et al., 2018). This

provides a useful example of structure-driven drug design which should improve clinical side effects. These compounds have been patented (Aube et al., 2015), but have not yet progressed to human trials.

Crystal structures were also obtained for CYP17A1 with the non-steroidal inhibitors orteronel and seviteronel. These compounds both contain a planar naphthalene core instead of the steroidal core, but retain a nitrogen heterocycle (Figure 3). They were developed in the search for lyase-selective inhibitors (Handratta et al., 2005; Rafferty et al., 2014). Similar to the steroidal inhibitor structures, their respective nitrogen heterocycles also coordinated the P450 heme (Petrunak et al., 2017) so they have a similar mode of inhibition. However, these ligands were particularly interesting because they participated in different active site interactions. Seviteronel has two difluoromethoxy side chains extending from its naphthalene core. For the tightest binding and best inhibiting *S*-seviteronel enantiomer, the planar naphthalene core occupied a similar space as the larger steroidal core in the complexes discussed above, but not only was one of the difluoromethoxy side chains directed toward N202 similar to the steroidal inhibitors, but the second difluormethoxy side chain was directed toward the polar R239 and new interactions were revealed with two other active site residues. Perhaps even more interesting, when CYP17A1 was incubated with racemic orteronel, it crystallized with both *R*-orteronel and *S*-orteronel, with differing binding orientations and into different CYP17A1 conformations. *R*-orteronel bound like the steroid-based inhibitors, with the naphthalene ring substituting for the steroidal core and its carboxamide side chain oxygen hydrogen bonding with N202 in the F helix. In contrast, *S*-orteronel angled toward the G-helix instead of the F-helix, with its carboxamide side chain engaging both R239 and N298 (Petrunak et al., 2017).

When comparing the CYP17A1 conformations binding *R*-orteronel and *S*-orteronel it became clear that there was a correlation between which enantiomer bound and conformational changes in the loop between the F and G helices. (Petrunak et al., 2017). These regions are of particular interest since they have frequently been implicated in ligand entry/exit from the P450 active site for both soluble bacterial enzymes (Li and Poulos, 1997; Ravichandran et al., 1993) and also for human membrane enzymes, which have an insertion in this area (Johnson and Stout, 2013; Scott et al., 2003; Scott et al., 2004). While most CYP17A1 structures demonstrate two distinct conformations of the F/G region just outside the active site proper, most ligands bind identically within the active site. However, not only do these two conformations correlate with selective binding of the two orteronel enantiomers, they also corresponded to the absence or presence of a second ligand at a peripheral binding site (Petrunak et al., 2017). In the CYP17A1 conformation that bound *S*-orteronel, there is electron density corresponding to peripheral ligand binding between the F/G helices and the N-terminus (Petrunak et al., 2017). The CYP17A1 conformation binding *R*-orteronel does not have this site available for ligand binding. The correlation between two conformations of the F/G loop access channel, the absence/presence of the second peripheral ligand, and the higher 8-fold selectivity of *R*-orteronel for inhibition of the lyase vs. hydroxylase reaction compared to the 4-fold selectivity for *S*-orteronel could be related to a partly distributive mechanism (*vide supra* section 3.5).

4.2 Structures with Substrates: Correlations with Functional Observations

Crystal structures of CYP17A1 bound to endogenous substrates have provided information about physiologically relevant interactions during enzyme catalysis (Petrunak et al., 2014). Structures were determined with both hydroxylase substrates pregnenolone and progesterone, as well as with the 17OH-pregnenolone and 17OH-progesterone products and lyase substrates (Petrunak et al., 2014). All four substrates bound with the steroid core in the same general orientation as the steroid based inhibitors and made various interactions with the same N202 residue in the roof of the active site (DeVore and Scott, 2012; Petrunak et al., 2014). The 3-keto progesterone-based compounds were hydrogen bond acceptors for the N202 side chain amide, while the 3-hydroxy pregnenolone-based compounds can be either hydrogen bond acceptors or donors for the amide or carboxyl N202 termini, respectively.

A potentially significant difference was observed in the structures of CYP17A1 bound to 17OH-pregnenolone. In different molecules of the crystal, this compound was bound at different heights above the heme (Figure 5). Some were closer to the heme with a longer distance to N202 residue on the F-helix that would not permit hydrogen bonding, while others were farther from the heme and within hydrogen bonding distance to N202 (Petrunak et al., 2014). This finding is consistent with the idea that in the conformation closer to the heme, 17OH-pregnenolone would interact more with the proximal oxygen of the peroxoanion intermediate (*vide supra* section 3.4), which would then be better able to perform the lyase reaction since the terminal oxygen is therefore available to attack C20 (Mak et al., 2018). This idea is also consistent with functional data where mutations of residue N202 altered the preference for 17OH-pregnenolone or 17OH-progesterone (Gregory et al., 2018). Thus, both structural and functional studies generally correspond.

Structural and functional studies have also shed insight into production of a minor 16OH-progesterone metabolite, as well as the dominant C17 hydroxylated product. Swart *et al.* noted that the ratio of these two products varied with species and that this also varied with the identity of the amino acid at position 105 (Swart et al., 2010; Swart et al., 1993). Leu at this position tends to produce less C16 hydroxylated product, while species with Ala at this position produced more of the C16 metabolite. The human CYP17A1 enzyme must have enough flexibility in progesterone positioning to place either the C16 or C17 closest to the iron oxo intermediate for reaction. CYP17A1 structures concur in that the side chain of 105 is directed into the active site in a way that could modulate substrate positioning to favor one metabolite over another.

4.3 Equilibria Between Multiple Conformational States: Conformational Selection

The crystal structures thus support the existence of at least two conformational states, particularly for the F/G loop and related differences in ligand binding both in the active site and in the peripheral site. The question remains how these conformations, and potentially others such as an open state whereby ligands access the otherwise buried active site, relate to functionality in solution and in the membrane. The kinetics of substrate binding to CYP17A1 are most consistent with conformational selection, defined as the protein existing in multiple conformational states prior to ligand binding and ligand then binding or “selecting” the most compatible conformation. Conformational selection is typified by two

key features that have been reported for CYP17A1 (Guengerich et al., 2019). First, the rate of ligand binding decreases with increasing substrate concentrations. Second, the rates increase with fixed steroid and increasing CYP17A1 concentrations. However this work did not exclude that ligand might also influence the CYP17A1 conformational state, as in the induced fit model (Guengerich et al., 2019). Solution NMR studies with abiraterone bound CYP17A1 have also supported multiple CYP17A1 conformational states, particularly in the F/G helices and the N-terminal portion of the I-helix, in response to cytochrome b_5 binding on the opposite site of the protein (*vide infra* section 5.3) (Estrada et al., 2014). Altogether, structural and functional experiments suggest that conformational selection is likely to play a large role in ligand binding. The potential of features outside the active site to modulate CYP17A1 function may provide additional opportunities for intervention in disease states.

5. CYTOCHROME b_5

5.1 Cytochrome b_5 Effects on CYP17A1 Function

As previously stated, microsomal P450 enzymes such as CYP17A1 are monooxygenases that require sequential delivery of two electrons to their catalytic heme for catalysis to occur. Microsomal P450 enzymes receive at least the first electron from cytochrome P450 reductase, but the second electron can be delivered by either cytochrome P450 reductase or cytochrome b_5. Although it is not well understood, the presence of cytochrome b_5 can accelerate, have no effect on, or impede catalysis for different human P450 enzymes. However, it is well documented that cytochrome b_5 plays a large role in CYP17A1 activity. While CYP17A1 hydroxylation activity is not appreciably affected by the presence or absence of cytochrome b_5, CYP17A1 lyase activity is essentially negligible in the absence of cytochrome b_5 but is strongly stimulated in its presence (Akhtar et al., 2011; Katagiri et al., 1995; Lee-Robichaud et al., 1995). This is an important developmental control mechanism. The onset of adrenarche correlates with increased expression of cytochrome b_5, which results in a corresponding increase in CYP17A1 lyase activity and therefore androgen production (Mapes et al., 1999; Suzuki et al., 2000; Turcu et al., 2014). Notably, cytochrome b_5 expression only occurs in the zona reticularis of the adrenal gland where CYP17A1 does both the hydroxylation and lyase reactions to produce androgens, but it is not expressed in the zona fasciculata where CYP17A1 performs only hydroxylation to generate glucocorticoids (Yanase et al., 1998). While this physiological control mechanism is thus well established, and b_5 is known to increase the fraction of electrons directed to product formation (Peng et al., 2016), the mechanism by which cytochrome b_5 facilitates lyase activity is still under discussion (Guengerich et al., 2019).

5.2 Direct Electron Transfer from Cytochrome b_5

One potential mechanism by which cytochrome b_5 might stimulate the CYP17A1 lyase activity is through direct electron transfer. The idea that cytochrome b_5 could directly transfer an electron to a P450 enzyme was first postulated in 1971 (Hildebrandt and Estabrook, 1971). Subsequent studies established that the redox potential of cytochrome b_5 is not sufficient for transfer of the first electron to the ferric P450 heme, but could donate the second electron after oxygen has bound (Guengerich, 1983; Guengerich et al., 1975; Iyanagi et al., 1974; Oprian and Coon, 1982; Sligar et al., 1979). Additionally, studies show that

cytochrome b_5 shares the same general binding site on CYP17A1 as cytochrome P450 reductase (Ahuja et al., 2013; Estrada et al., 2013; Gao et al., 2006).

In order to study if cytochrome b_5 facilitates the CYP17A1 lyase reaction by providing direct electron transfer, experiments utilized a form of cytochrome b_5 in which the heme is replaced with a redox-inactive manganese protoporphyrin IX (Duggal et al., 2016; Lee-Robichaud et al., 1998; Morgan and Coon, 1984). If the primary mechanism by which cytochrome b_5 accelerates the CYP17A1 lyase reaction is by facilitating electron transfer to the P450 heme, this redox-inactive form should not stimulate the lyase reaction. Unfortunately, the results from these experiments have been conflicting. Some show a loss of lyase activity, consistent with a role for cytochrome b_5 in facilitating electron transfer, while others show that the lyase activity is retained, suggesting electron transfer is not relevant (Duggal et al., 2016; Lee-Robichaud et al., 1998; Morgan and Coon, 1984). Thus, whether cytochrome b_5 facilitates the CYP17A1 lyase reaction by directly facilitating electron transfer is still under discussion.

5.3 Cytochrome b_5 Induced Conformational Changes

Another mechanism by which cytochrome b_5 might facilitate CYP17A1 lyase activity is through allosteric effects. The Auchus lab demonstrated that the lyase activity was facilitated by cytochrome b_5 missing its heme prosthetic group entirely, which would also be redox-inactive (Auchus et al., 1998). Such apo-cytochrome b_5 does retain much of its structure, but has a disordered heme-binding loop (Falzone et al., 2001). It is possible that the ordered region contacts the proximal CYP17A1 surface and allosterically affects the CYP17A1 conformation, but one would expect the now-disordered heme-binding surface to be making most of the contacts with CYP17A1. However, taken on their surface these experiments suggest that cytochrome b_5 may allosterically regulate the lyase reaction.

Solution NMR studies showed a conformational change occurs in CYP17A1 when cytochrome b_5 binds and that these conformations are substrate-dependent (Estrada et al., 2013). Specifically, pregnenolone binding in the presence of cytochrome b_5 induced a conformational change that was similar to that observed for 17OH-pregnenolone in the absence of cytochrome b_5, which would be consistent with the ability of cytochrome b_5 to allosterically modulate CYP17A1 to promote lyase activity (Estrada et al., 2014).

One would expect that the interaction of cytochrome b_5 would be stronger with CYP17A1 bound to the lyase substrate since cytochrome b_5 stimulates this reaction. However, solution NMR studies showed the opposite trend (Estrada et al., 2013). One current thought to explain this is that while cytochrome b_5 allosterically alters the CYP17A1 conformation, it also competes with the binding site for cytochrome P450 reductase and blocks the transport of the first electron (Estrada et al., 2013; Im and Waskell, 2011).

Thus, while it is clear that cytochrome b_5 has an important developmental and biochemical role in CYP17A1 lyase activity and that allosteric interactions likely come into play, the aggregate data in the literature do not rule out the possibility that cytochrome b_5 also does so by facilitating direct electron transport for catalysis. Continuing studies in this area remain

critical as a potential route to manipulate CYP17A1 hydroxylase and lyase activities individually for clinical advantage in a variety of disease states.

6. CYP17A1 ROLE IN DISEASE

Thus far this review has focused on how CYP17A1 functions and the roles that its structure and redox partner interactions have on its mechanism and function. These discussions are important because they inform ways in which CYP17A1 might be manipulated to affect its role in various diseases. CYP17A1 mutations vary widely in their metabolic variability and resulting clinical consequences (see supplement for (DeVore and Scott, 2012)). CYP17A1 has been heavily studied for its role in prostate cancer but is also implicated in several other diseases including breast cancer, glioblastoma, and Cushing's syndrome. The following section will look closer at the role of CYP17A1 in these diseases.

6.1 CYP17A1 in Prostate Cancer

The androgen receptor plays a critical role in prostate cancer. In normal prostate, androgens bind and activate the cytosolic androgen receptor. The androgen receptor then translocates to the nucleus where it promotes the production of proteins that play a role in prostate growth, such as prostate specific antigen (Fujita and Nonomura, 2019). Elevated and increasing levels of prostate specific antigen have been used as a biomarker of prostate cancer diagnostically, though there is debate on the clinical utility (Duffy, 2020). Once diagnosed, androgen-driven prostate cancer is typically initially treated with androgen deprivation therapy, but prostate cancer cells frequently adapt to survive under even castration levels of androgens, a state referred to as castration resistant prostate cancer (Fujita and Nonomura, 2019). These adaptations can include overexpressing or mutating the enzymes needed to generate the androgens testosterone and dihydrotestosterone (Fujita and Nonomura, 2019; LaTulippe et al., 2002) or the androgen receptor (Brooke and Bevan, 2009). Inhibiting CYP17A1 disrupts androgen production via both classic and backdoor pathways and in all tissues, and it has been found to be an effective treatment for castration resistant prostate cancer.

Abiraterone is the only CYP17A1 inhibitor approved by the U.S. F.D.A. It improved the overall rate of survival by approximately four months in patients who had previously received the previous standard of care, the chemotherapeutic microtubule stabilizer docetaxel (de Bono et al., 2011; Fizazi et al., 2012). Abiraterone was developed as a relatively selective CYP17A1 inhibitor that bound so tightly as to be characterized as irreversible by some authors (Potter et al., 1995; Rowlands et al., 1995). Abiraterone is now considered the standard of care for patients with castration resistant prostate cancer (Rice et al., 2019). While abiraterone had such success in patients, its inhibition is not selective for the lyase vs. hydroxylase activity of CYP17A1, and therefore mineralocorticoid production is also unintentionally inhibited (Potter et al., 1995; Rice et al., 2019). Recent pharmacokinetic-pharmacodynamic studies have suggested that, due to the nature of abiraterone's tight binding, current recommended clinical dosages might be unnecessarily high, and further studies should be done to investigate reduced dosages with potentially fewer mineralocorticoid related side effects (Cheong et al., 2020).

Other CYP17A1 inhibitors have also been investigated for castration resistant prostate cancer such as seviteronel (VT 464 or INO-464) (Rafferty et al., 2014), galeterone (TOK-001 or VN/124–1) (Handratta et al., 2005), and orteronel (TAK700) (Kaku et al., 2011). Both seviteronel and orteronel were developed to preferentially inhibit CYP17A1 lyase activity over hydroxylase activity to try to reduce mineralocorticoid inhibition (Rafferty et al., 2014; Yamaoka et al., 2012), but do not seem to do so very effectively (Petrunak et al., 2017). Additionally, seviteronel and galeterone were found to directly interact with the androgen receptor and act as antagonists (Norris et al., 2017). Regardless, none of these CYP17A1 inhibitors made it through clinical trials. Seviteronel was discontinued due to toxicity (Madan et al., 2020) and is no longer being pursued (personal communication, Dr. Keith Schmidt, National Cancer Institute). Galeterone (ClinicalTrials.gov identifier: NCT02438007) and orteronel were discontinued when they did not meet their primary endpoints for progression free survival and overall survival, respectively (Fizazi et al., 2015; Njar; Saad et al., 2015).

6.2 Breast Cancer

Breast cancer progression could be impacted by androgens in two ways. First, in females CYP17A1-generated androgens are converted into estrogens. In the estrogen-receptor positive breast cancer patient, reducing androgen levels could result in downstream estrogen starvation and thus a positive clinical effect. Certainly, testosterone levels in breast cancer patients correlate with poor prognosis (Harvell et al., 2008; Micheli et al., 2007). Secondly, in certain types of breast cancer the androgen receptor is expressed to the same extent or higher than the estrogen receptor or progesterone receptor, and its overexpression is correlated with tamoxifen resistance (Amicis et al., 2010; Kuenen-Boumeester et al., 1996; Moinfar et al., 2003; Vera-Badillo et al., 2014). In females, it is hypothesized that the androgen receptor can replace estrogen-dependent signaling to stimulate transcription of steroid responsive genes to drive breast cancer (Robinson et al., 2011; Vera-Badillo et al., 2014). Both concepts suggest that inhibition of CYP17A1 might be productive in improving breast cancer prognosis.

As a result, CYP17A1 inhibitors have been clinically investigated to treat breast cancer (Bardia et al., 2018; O'Shaughnessy et al., 2016; Rampurwala et al., 2017). Abiraterone did not improve progression free survival in patients as compared to treatment with exemastane (O'Shaughnessy et al., 2016). Seviteronel's toxicity (Madan et al., 2020) combined with the fact that it did not perform better than abiraterone precluded it from further studies (personal communication, Dr. Keith Schmidt, National Cancer institute). However, orteronel was well tolerated in Phase I clinical trials (Rampurwala et al., 2017), and a Phase II clinical trial is still listed as ongoing (ClinicalTrials.gov identifier: NCT01990209).

6.3 Cushing's Syndrome

Cushing's syndrome is a steroidogenic disease in which patients have an excess of cortisol. This hypercortisolism drives metabolic changes and can cause bone loss, high blood pressure, and occasionally type 2 diabetes. It is also a rare but important cause of androgen excess. Inhibition of CYP17A1 reduces the production of both androgens and cortisol and is

thus beneficial to patients with Cushing's syndrome experiencing hypercortisolism (Attard et al., 2012).

Some evidence indicates that a particular allele of CYP17A1 may correlate with Cushing's syndrome treatment success. A silent mutation in the coding region has been identified that does not change the severity of disease, but is associated with better normalization of cortisol levels using ketoconazole and metyrapone (Valassi et al., 2017). Through various mechanisms, this allele could result in altered CYP17A1 expression levels and thus activity in these patients, thereby modulating their therapeutic response. Because of this, an active clinical trial is listed to study the steroidal CYP17A1 inhibitor abiraterone's ability to normalize urinary free cortisol excretion in Cushing's Syndrome patients (ClinicalTrials.gov identifier: NCT03145285).

6.4 Glioblastoma

Glioblastoma or glioblastoma multiforme is an aggressive form of brain cancer comprising 15% of all brain tumors (Young et al., 2015). In addition to its androgenic roles elsewhere in the body, in brain tissues dehydroepiandrosterone is an important protective neurosteroid. In some glioblastomas CYP17A1 is overexpressed (Chuang et al., 2017). CYP17A1-generated dehydroepiandrosterone has then been shown to protect glioma cells against chemo-induced apoptosis, resulting in drug resistance (Bastianetto et al., 1999; Camporez et al., 2011; Chuang et al., 2017). Specifically, the glioblastoma drug temozolomide normally leads to apoptosis by inducing DNA damage and the production of reactive oxygen species (Kang et al., 2012; Yoshimoto et al., 2012). It is thought that dehydroepiandrosterone protects from such apoptosis because it regulates several antioxidant enzymes through unknown signaling pathways (Bastianetto et al., 1999). Since this mechanism requires CYP17A1 activity to produce dehydroepiandrosterone, inhibition of CYP17A1 may promote the efficacy of temozolomide.

In addition to glioblastoma protection mediated by dehydroepiandrosterone, there is a second mechanism by which CYP17A1 may affect glioblastomas. Studies suggest that CYP17A1 is required for stabilization and localization of the secretion-associated Ras-related GTPase SAR1 (Lin et al., 2019). SAR1 is one of five proteins that make coat vesicles that move proteins from the ER to the golgi apparatus (Fang et al., 2015; Nakagawa et al., 2017; Sato and Nakano, 2007). Without appropriate localization of such proteins, the ER accumulates unfolded proteins, resulting in ER stress (Fang et al., 2015; Nakagawa et al., 2017), and ultimately in tumor cell death. Early studies suggested that inhibition of CYP17A1 by abiraterone disrupts a direct protein-protein interaction between SAR1 and CYP17A1 (Lin et al., 2019), which could be related to the conformational selection concept (*vide supra* section 4.3).

Either or both mechanisms would be consistent with the finding that the CYP17A1 inhibitor abiraterone showed tumor suppressive effects on glioblastomas both *in vitro* in cell based assays and *in vivo* in mouse models (Lin et al., 2019). A clinical trial is currently listed as ongoing for seviteronel in glioblastoma patients that are androgen receptor positive to monitor the objective tumor response (ClinicalTrials.gov identifier: NCT03600467).

7. CONCLUSION

CYP17A1 plays a critical role in steroidogenesis, acting as the enzymatic gateway to glucocorticoid and androgen synthesis. Regardless of whether the steroidogenic pathway follows the classical or backdoor pathway, CYP17A1 is absolutely required for the synthesis of the most potent androgen, dihydrotestosterone. In both pathways, CYP17A1 performs two types of reactions: hydroxylation and the carbon-carbon bond cleavage, or lyase reaction. While the chemistry of the hydroxylation reactions is well understood, the chemistry of the lyase reaction may differ and is under significant discussion. Recent structural evidence demonstrates that both hydroxylase and lyase substrates bind similarly in a single active site, but with some variation in positioning of lyase substrates that is consistent with some of the mechanistic proposals and functional observations. CYP17A1 structures with steroidal inhibitors have helped direct drug optimization, particularly in terms of selectivity, while those with nonsteroidal inhibitors have defined new opportunities for both optimization of active site interactions, as well as provided clues about other opportunities for CYP17A1 modulators to act outside the active site. Finally, both structural and functional evidence suggest that CYP17A1 exists in a dynamic equilibrium of two or more conformations. Such conformational selection may help explain the selectivity of the lyase reaction, as well as the role that cytochrome b_5 has in facilitating the lyase reactions. These structural and functional studies are ultimately important because they improve understanding of the physiological role of CYP17A1 and provide opportunities to modulate it to treat disease. CYP17A1 is critically important in the treatment of castration resistant prostate cancer, for which the CYP17A1 inhibitor abiraterone has proven a highly effective drug. However, CYP17A1 inhibition is also being investigated for the treatment of breast cancer, Cushing's syndrome, and glioblastoma (ClinicalTrials.gov identifiers: NCT01990209, NCT03145285, NCT03600467).

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ABBREVIATIONS

CYP17A1	cytochrome P450 17A1
CPR	cytochrome P450 reductase
cyt b_5	cytochrome b_5

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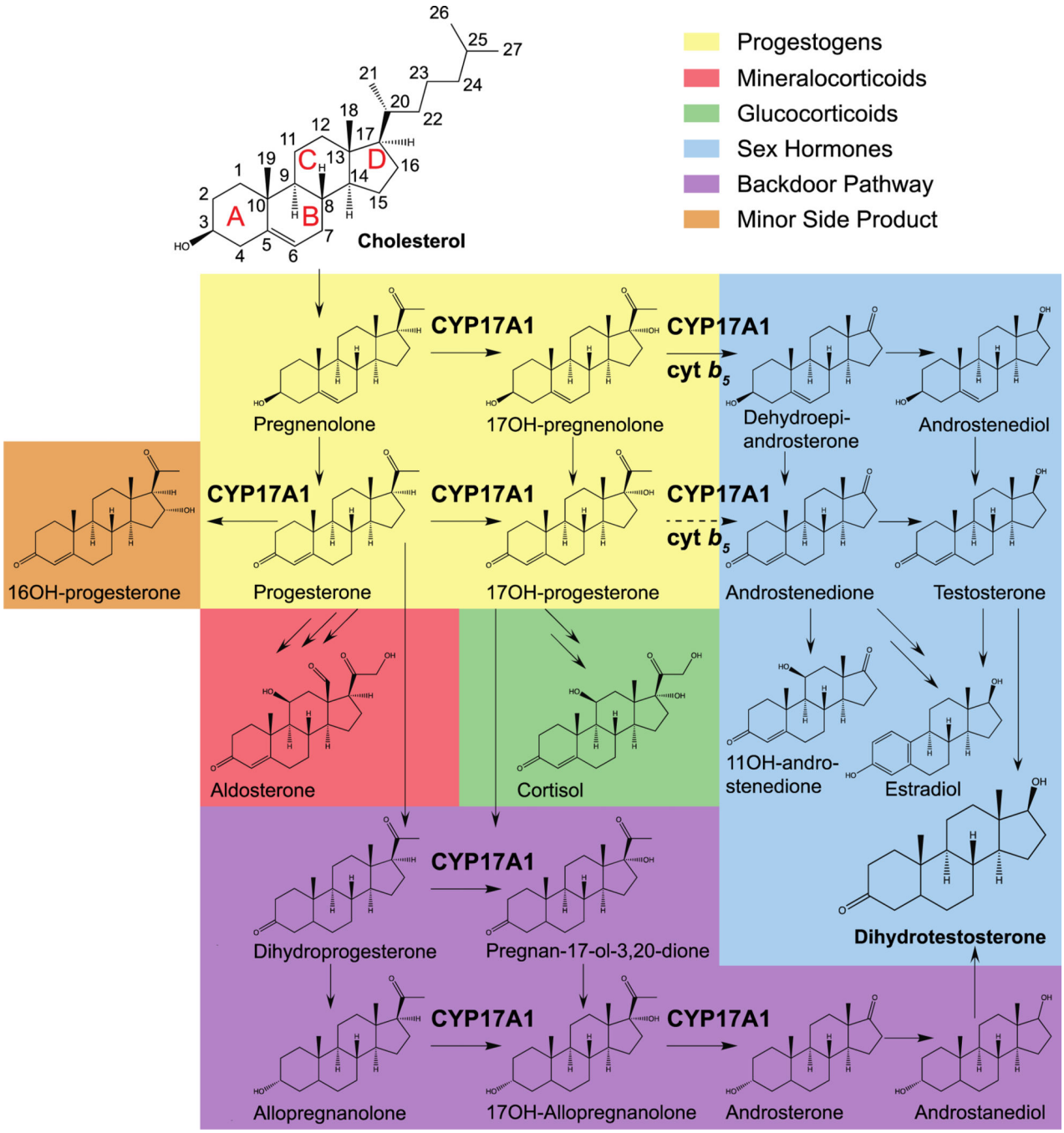

Figure 1: The classical and backdoor pathways of steroidogenesis both require CYP17A1 to produce dihydrotestosterone.

The classical pathway of steroid production converts cholesterol into progestogens (yellow) and then subsequently into glucocorticoids (green), mineralocorticoids (salmon), and sex hormones (blue). CYP17A1 17α-hydroxylation is required to generate 17OH-pregnenolone and 17OH-progesterone, while subsequent CYP17A1 lyase action generates sex hormones dehydroepiandrosterone and androstenedione (broken arrow indicates relatively low activity), with the latter subsequently converted to estradiol, 11OH-androstenedione, or testosterone and then dihydrotestosterone. The backdoor pathway (purple) utilizes most of

the same enzymes in a different order, generating different intermediates such as allopregnanolone to ultimately yield dihydrotestosterone.

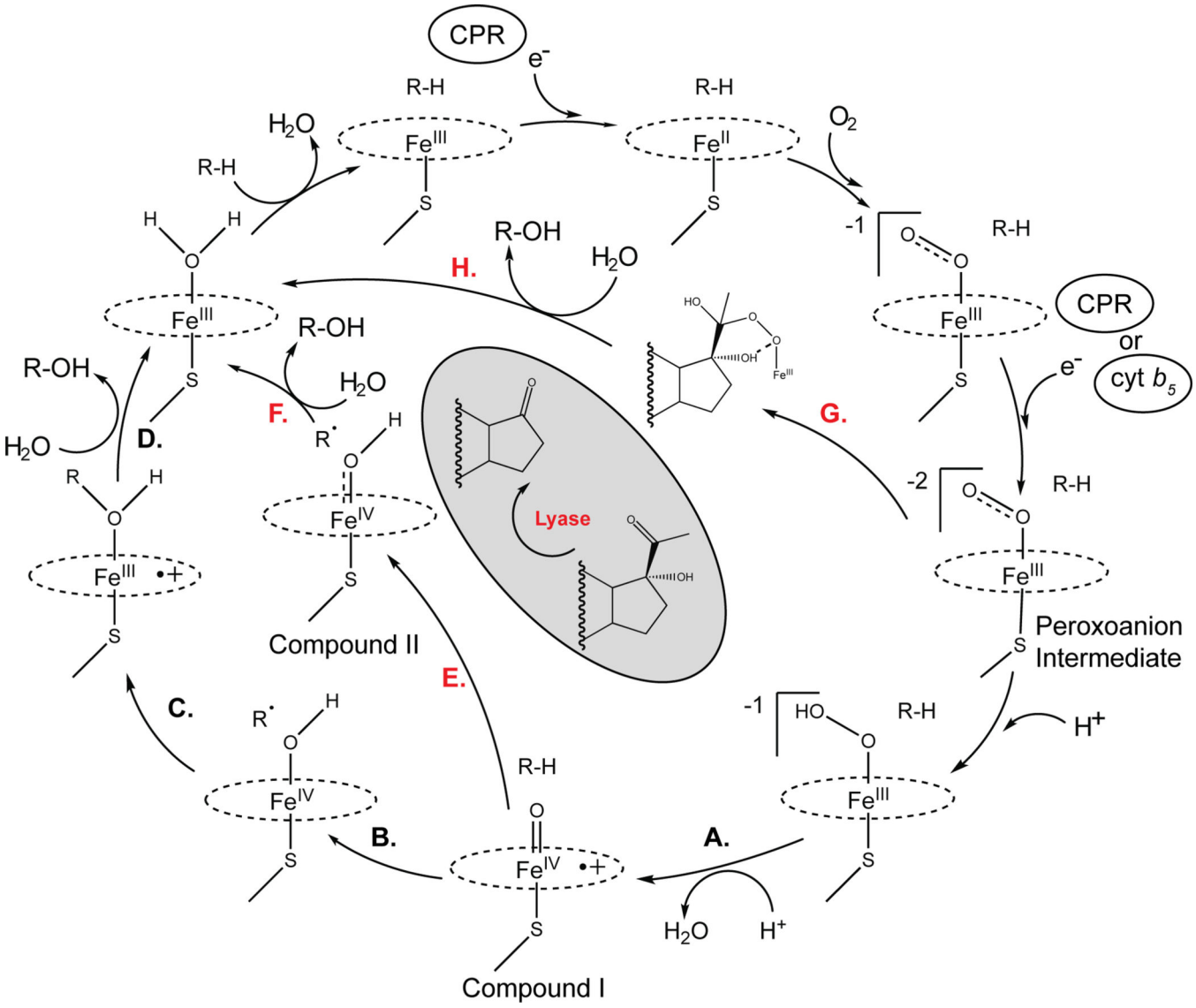

Figure 2: CYP17A1 hydroxylation reaction follows the typical P450 catalytic cycle with the key intermediate being compound I, but the lyase reaction intermediate may proceed via either compound I or the peroxoanion.

Substrate (RH) binding in the active site displaces a water molecule bound to the ferric (Fe^{3+}) heme (top left), permitting the heme to subsequently accept an electron from cytochrome P450 reductase (CPR). The now ferrous (Fe^{2+}) heme binds molecular oxygen (O_2) and accepts a second electron, from either CPR or cytochrome b_5 (cyt b_5) to form a peroxoanion. For a typical P450 hydroxylation reaction, this peroxoanion species is protonated twice, cleaving the O-O bond, and releasing water (A) and forming the ferryl compound I. Compound I attacks the substrate, abstracting a hydrogen (B). Rebound then occurs (C). Finally, the product is released from the active site and water rebinds the ferric heme to complete the catalytic cycle (D). For the CYP17A1 lyase reaction, however, two different catalytic intermediates have been suggested. First, compound I could abstract a hydrogen from the substrate forming compound II and a substrate radical (E), prompting cleavage of the carbon-carbon bond (grey circle) and release of the lyase product (F). The

second proposed lyase mechanism does not involve compound I. Instead, the peroxoanion intermediate itself directly attacks the substrate to form a peroxohemiketal transition state (G) and release the lyase product (H).

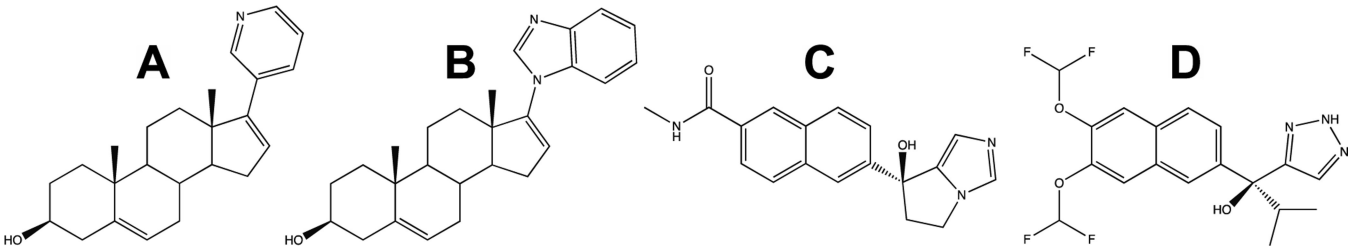

Figure 3: Structures of CYP17A1 inhibitors.

Steroid based inhibitors A) abiraterone and B) galeterone both contain the pregnenolone core with an extra double bond between the C16 and C17 and either a pyridine (A) or benzimidazole (B) substituent at the C17 position. Non-steroidal inhibitors C) orteronel and D) seviteronel both contain a naphthalene core instead of the steroidal core, but both contain a nitrogen heterocycle.

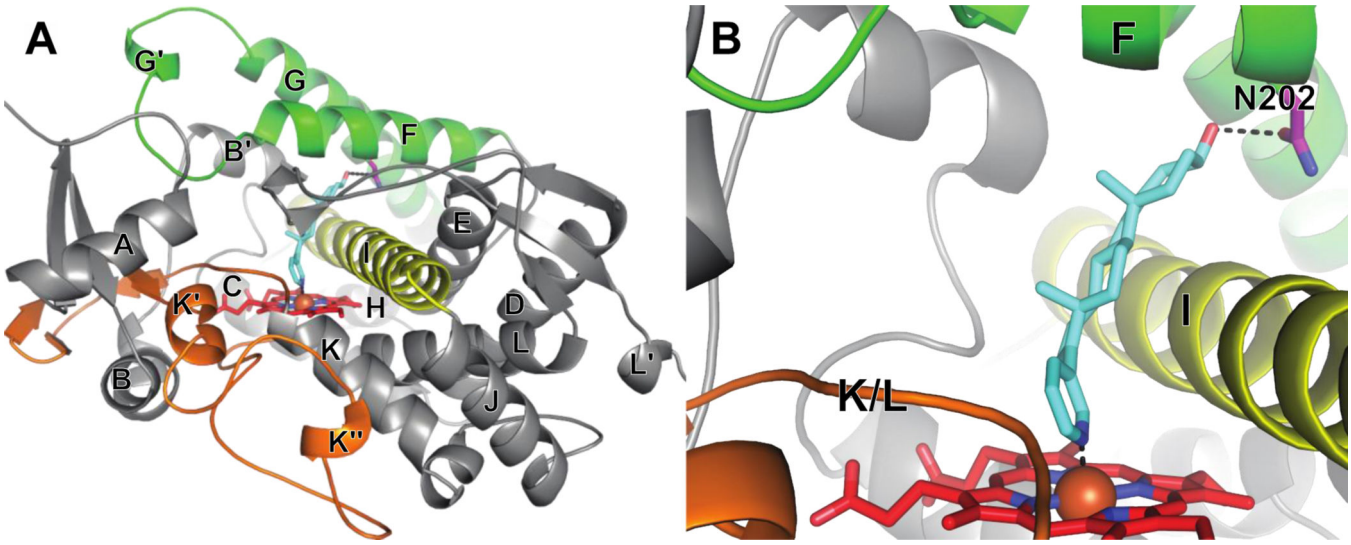

Figure 4: X-ray structure of CYP17A1 with steroidal inhibitor abiraterone (PDB 3RUK).

A) CYP17A1 has a typical P450 fold composed of multiple alpha helices (A-L) and four beta sheets enclosing the catalytic heme (red sticks) within a single globular domain. Ligands such as abiraterone (cyan sticks) thus bind in a buried active site on one side of the heme. The F/G region (green ribbons) composes part of the active site roof and is thought to be dynamic to permit substrate access. B) A closer look at the active site shows that abiraterone coordinates the heme iron (orange sphere) with the steroidal core canted over the I helix (yellow) and with its C3 hydroxyl interacting with N202 (magenta sticks) in the F helix. Contrastingly, steroid ligands bind in the opposite direction for other steroidogenic P450 enzymes, instead orienting over the K/L loop (orange).

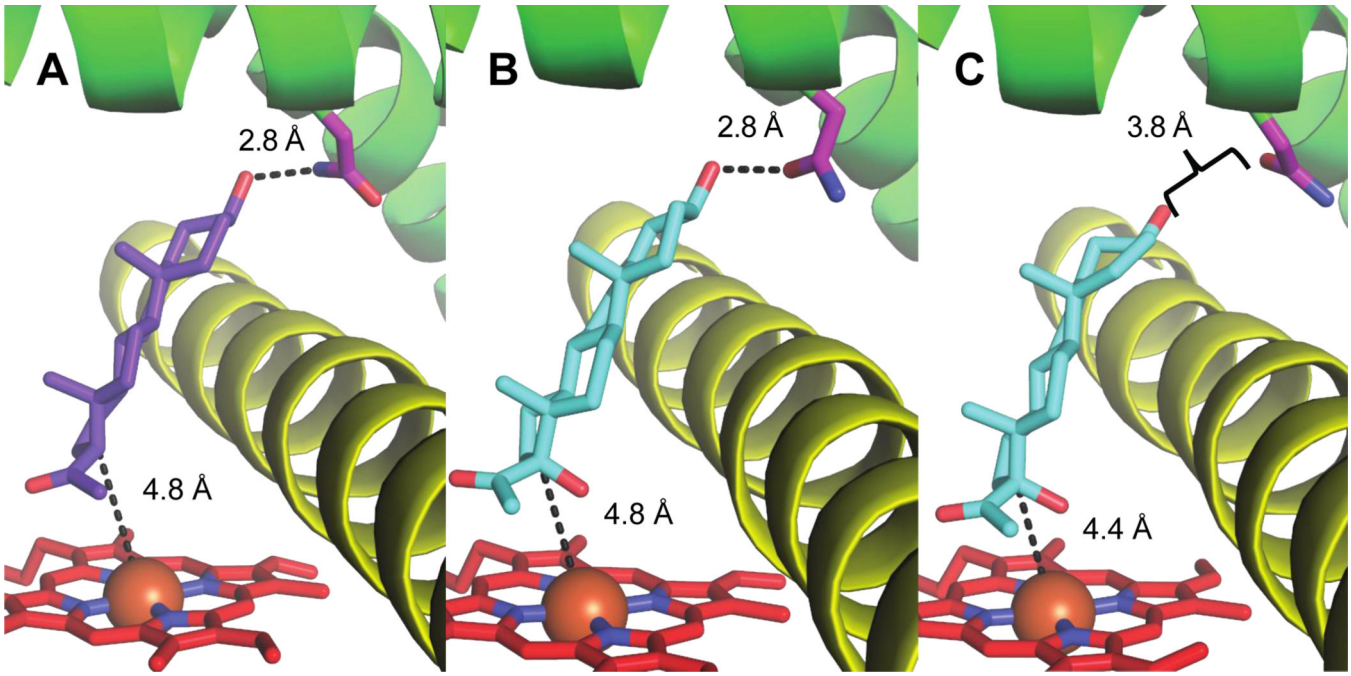

Figure 5: X-ray crystal structures of CYP17A1 with pregnenolone (PDB 4NKW) and 17OH-pregnenolone (PDB 4NKZ) demonstrate different distances to the heme iron (orange sphere) and N202 (magenta sticks).

(A) Pregnenolone (purple) binds to CYP17A1 with the C3 hydroxyl group hydrogen bonded to N202 at 2.8 Å, while the distance from the site of metabolism and the heme iron is 4.8 Å. The hydroxylation product and lyase substrate 17OH-pregnenolone (cyan sticks) has variation in its positioning. (B) One conformation shows distances similar to that of the hydroxylation substrate. This best positions the ligand 17OH to interact with the distal oxygen of a peroxoanion intermediate, which could impair the lyase chemistry. In contrast, the most different conformation observed (C) the C3 hydroxyl is too far from the N202 residue for a hydrogen bond (3.8 Å) and the distance from the heme is reduced to 4.4 Å. This distance best positions the ligand 17OH to interact with the *proximal* oxygen of a peroxoanion intermediate, which could facilitate the lyase chemistry.