The Tail of Mycolic Acids

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The FabH enzyme from *M. tuberculosis* binds the acyl tail of large substrates at the end of a buried hydrophobic tunnel. Sachdeva et al. (2008) use reactive chemical probes and X-ray crystallography to show that substrates can bind to an open state of FabH without threading through the tunnel.

Mycobacterium tuberculosis, a deadly human pathogen, is protected by a thick, waxy cell wall containing long-chain fatty acids. These fatty acids, called mycolic acids, are typically over 60 carbons in length and are known to be important for the growth, survival, and pathogenicity of *M. tuberculosis*. Mycolic acids confer unique physical features to *Mycobacteria*, such as low membrane permeability, that contribute to antibiotic resistance, as well as the ability to survive inside macrophages and evade immune surveillance. These properties result in persistent, long-term infections in humans and are responsible for the emergence of tuberculosis as one of the most widespread and deadly infectious diseases worldwide.

Not surprisingly, the biosynthesis of mycolic acids has been the focus of antitubercular drug discovery efforts. Two front-line drugs, isoniazid and ethionamide, inhibit fatty acid biosynthesis enzymes required for the production of mycolic acids. Other *M. tuberculosis* fatty acid biosynthesis enzymes could prove to be equally good drug targets. In this issue of *Chemistry & Biology*, Sachdeva and coworkers focus on FabH, another enzyme of fatty acid biosynthesis that has not yet been exploited as a tuberculosis drug target (Sachdeva et al., 2008). In this work, reactive chemical probes are cleverly employed to demonstrate that substrates can bind to an open state of *M. tuberculosis* FabH, bypassing a route previously thought to provide the only access to the active site.

Bacterial FabH enzymes typically accept acyl-CoenzymeA (CoA) substrates with small acyl groups such as acetyl-CoA (two carbons) and butyryl-CoA (four carbons). *M. tuberculosis* FabH (*Mt*FabH) is unusual in that medium chain length acyl groups of 14–20 carbons are preferred substrates (Brown et al., 2005). These acyl groups are produced by a Type I fatty acid synthase (FAS) and ultimately form the "tail" of the mycolic acid (Barry et al., 1998). Initially, the acyl tail is transferred from CoA to an active site cysteine (C112) on *Mt*FabH. *Mt*FabH then catalyzes the decarboxylative condensation of the acyl group with a second substrate, malonyl-ACP. The product of this reaction is further elongated by a Type II FAS. Thus, *Mt*FabH serves an important role as a link between Type I and Type II FASs in the production of mycolic acids (Choi et al., 2000).

FabH enzymes bind CoA and ACP substrates in a channel (the pantetheinate channel) leading from the enzyme surface to the active site cysteine residue. In *Mt*FabH, a second hydrophobic channel (the acyl channel) leads from the active site cysteine residue (C112) and forms a cavity that is only accessible through the pantetheinate channel. The crystal structure of *Mt*FabH active site mutant C112A was determined with bound lauroyl-CoA substrate (Musayev et al., 2005). The substrate was found in an "L"-shaped conformation with CoA occupying the pantetheinate channel and the 12 carbon lauroyl moiety occupying the acyl channel. Based on this structure, it appeared that the substrate would have to thread all the way through the pantetheinate channel to gain access to the acyl channel.

Sachdeva et al. (2008) report new findings that alter this perception of how *Mt*FabH binds substrates. Three inhibitors of *Mt*FabH are capable of covalent attachment to the active site cysteine, either by forming a thioester or a disulfide linkage. Sachdeva and coworkers present three crystal structures of *Mt*FabH with the bound inhibitors. In all three cases, electron density attributable to the covalently bound inhibitor is found in the acyl channel, even though one of the inhibitors contains a carboxylate group. It is difficult to imagine this charged compound threading through the hydrophobic substrate binding channels to reach the position observed in the crystal structure. The most exciting observations, however, involve a mutant *Mt*FabH in which a small residue lining the pantetheinate channel was substituted with a bulky phenylalanaine. This mutation, A246F, blocks the pantetheinate channel, severing access to the surface of the enzyme. Despite this blockage, two crystal structures clearly show covalently bound inhibitors occupying the acyl channel. As shown in Figure 1, the A246F mutation would block the compounds from reaching the active site in a "substrate threading" model of binding. However, if an "open state" is allowed, the inhibitors can enter the active site and covalently modify C112, as observed.

Based on their observations, Sachdeva and coworkers propose a model in which a concerted hinge motion exposes the active site allowing acyl-CoA substrates to easily access the acyl channel. A similar event may facilitate the release of products. This model of substrate binding builds on previous structural and biochemical observations. In the absence of ligands, *E. coli* FabH has been structurally characterized in a partially disordered state that appears to become more ordered upon ligand binding (Qiu et al., 2001). Similarly, modification of the active site cysteine in one monomer of *E. coli* FabH seems to reduce the reactivity of the other monomer as if a structurally ordered state was triggered, limiting access to the active sites (Alhamadsheh et al., 2007). Taken together, these results suggest that substrates may bind to a pre-existing open state of FabH without traversing

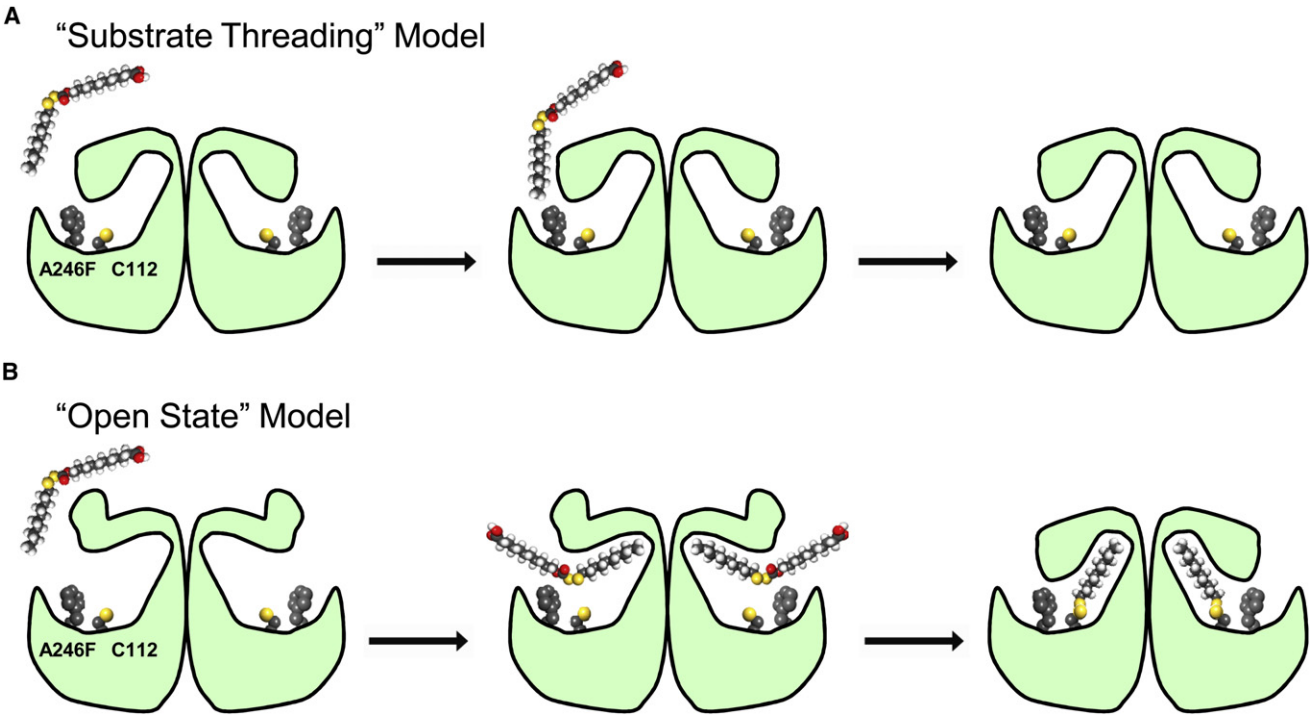

Figure 1. Two Models of Substrate Binding to MtFabH
(A) The "substrate threading" model. Substrates must traverse through the pantetheinate channel to reach the acyl channel. The A246F mutation will block the pantetheinate channel with a bulky phenylalanine side chain, preventing the substrate from reaching the active site.
(B) The "open state" model proposed by Sachdeva et al. (2008). Concerted conformational changes expose the channels and allow substrates to enter the active site directly. The phenylalanine side chain of mutant A246F does not block the substrate (compound III is shown) from entering the active site and reacting with C112.

through the pantetheinate channel. Ultimately, a more detailed understanding of conformational changes in *Mt*FabH will aid in the discovery of novel therapeutics to combat tuberculosis.

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