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REVIEW

Protein targets for structure-based anti-*Mycobacterium tuberculosis* drug discovery

Zhiyong Lou[1] ✉, Xiaoxue Zhang[2]

[1] Laboratory of Structural Biology, Tsinghua University, Beijing 100084, China
[2] Higher Education Press, Beijing 100029, China
✉ Correspondence: louzy@xtal.tsinghua.edu.cn
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ABSTRACT

***Mycobacterium tuberculosis*, which belongs to the genus *Mycobacterium*, is the pathogenic agent for most tuberculosis (TB). As TB remains one of the most rampant infectious diseases, causing morbidity and death with emergence of multi-drug-resistant and extensively-drug-resistant forms, it is urgent to identify new drugs with novel targets to ensure future therapeutic success. In this regards, the structural genomics of *M. tuberculosis* provides important information to identify potential targets, perform biochemical assays, determine crystal structures in complex with potential inhibitor(s), reveal the key sites/residues for biological activity, and thus validate drug targets and discover novel drugs. In this review, we will discuss the recent progress on novel targets for structure-based anti-*M. tuberculosis* drug discovery.**

KEYWORDS *Mycobacterium tuberculosis*, crystal structure, drug discovery, target

INTRODUCTION

Mycobacterium tuberculosis, an intracellular bacterial pathogen, is the causative agent of tuberculosis (TB) and accounts for latently infects in around one-third population all over the world. Infection leads to fever, loss of weight, lung pain and coughing up blood/sputum. Co-infection of HIV and *M. tuberculosis* causes both TB and HIV to progress more rapidly (Lederer et al., 1975; Kurth et al., 2009). Although several antibiotics and the 'Directly Observed Treatment, Short-course' (DOTS) (Qureshi et al., 2010) have been used to effectively reduce the burden of TB, emergence of drug-resistant and drug-sensitive TB and co-infection with HIV result in increasing incidence of TB in recent years (Bocchino et al., 2000). Therefore, it is crucial to identify novel targets to develop new approaches and agents for anti-drug-resistant and -drug-sensitive *M. tuberculosis*.

M. tuberculosis belongs to the genus *Mycobacterium* and is a slow-growing aerobic rod-shaped bacterium. Comparing to other pathogenic viruses, *M. tuberculosis* has a unique cell wall, which is proved to be a significant virulence determinant (Godreuil et al., 2007; Rivers and Mancera, 2008a, b). The *M. tuberculosis* cell wall is formed by complicated structure, with peptidoglycans, mycolic acids, arabinogalactan, lipoarabinomannan and other components. The cell wall protects *M. tuberculosis* from the attack of immune system in infected host. In addition, the genome of *M. tuberculosis* encodes a series of pathways that are unique in *M. tuberculosis* but are absent in mammalian cells. These pathways become efficient targets to selectively inhibit the growth of *M. tuberculosis* with reduced side effects. Moreover, the genomic and structural genomic studies revealed several key enzymes that are crucial for *M. tuberculosis* growth, and also presented detailed structural information of these enzymes. Current data from 'TB Structural Genomics Consortium' shows that there are over 720 available TB protein structures, among which 266 are unique in TB (http://fold.doe-mbi.ucla.edu/TB/). These data suggest the potent targets to discover and/or design anti-*M. tuberculosis* drugs with high selectivity. This review discusses the novel targets for anti-*M. tuberculosis* drug discovery, particularly those with structures and complex structures containing potential inhibitors.

TARGETS IN CELL WALL BIOSYNTHESIS

The cell wall of *M. tuberculosis* is essential for its growth and survival in infected host, and thus, contributes to the resistance to most commonly-used antibiotics and che-

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motherapeutic agents. It has been revealed that the mycobacterial cell wall is composed of three covalently linked macromolecules, peptidoglycan, arabinogalactan and mycolic acids, which is a hallmark of mycobacteria (Chatterjee, 1997; Mdluli and Spigelman, 2006). Cell wall is characterized as a preferred source of molecular targets because the biosynthetic enzymes do not have homologues in mammalian system (Chatterjee, 1997; Mdluli and Spigelman, 2006).

Peptidoglycan biosynthesis

Peptidoglycan consists of alternating units of *N*-acetylglucosamine and *N*-gycolylmuramic acid and forms the backbone of the mycolyl-arabinogalactan-peptidoglycan (mAGP) (Kurth et al., 2009). The side chains of peptide are attached to muramic acid, and thus, crosslinked to arabinogalactan (AG) via a phosphodiester line to the position 6 of a proportion of muramic acid residues (Lederer et al., 1975; Chatterjee, 1997). In 2005, LeMagueres and colleagues reported the 1.9-Å crystal structure of alanine racemase from *M. tuberculosis* (Alr (Mtb)) and identified a conserved entryway into the active site (PDB code: 1XFC) (LeMagueres et al., 2005) (Fig. 1). These knowledge of detailed structural investigation and related biochemical analysis made this enzyme a challenging but important target for drug design against TB; furthermore, the related studies provided valuable insights about the precise mechanism of D-cycloserine inhibition against drug-resistant TB (Feng and Barletta, 2003; LeMagueres et al., 2005).

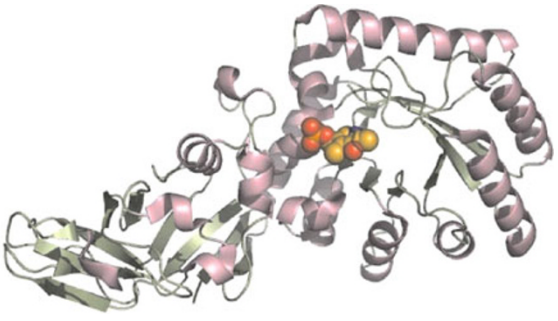

Figure 1. The crystal structure of *M. tuberculosis* alanine racemase with bound PLP.

Arabinogalactan and lipoarabinomannan biosynthesis

Arabinogalactan polymer is composed exclusively of D-galactofuranoses and D-arabinofuranoses, which are extremely rare in nature, while other crucial components of mycobacterial cell wall are known to be embedded into the framework of mAGP (Chatterjee, 1997). In addition to the essential role in mycrobacterial cell wall, lipoarabinomannan (LAM) also exhibits a wide spectrum of immunomodulatory activity (Chatterjee, 1997). These observations suggest the possibilities to target arabinogalactan and LAM biosynthesis pathways to treat TB. For example, ribosyltransferase is key to catalyze decaprenyl-phosphoryl-D-atabinose synthesis (Huang et al., 2005); UDP-galactopyranose mutase (Kremer et al., 2001), galactofuranosyl transferase (Brunger et al., 1998), dTDP-6-deoxy-L-lyxo-4-hexulose reductase (RmlD) (Nakano et al., 2000), RmlB and RmlC are also essential for mycobacterial growth (Ma et al., 2001; Li et al., 2006). These enzymes can be the potential targets for anti-*M. tuberculosis* drug discovery.

Mycolic acid biosynthesis

M. tuberculosis produces three types of mycolic acids that differ primarily in the oxygen-containing substituents at the distal portion of the meromycolate branch (Chatterjee, 1997). Mycolic acids are large, α-alkyl branched and β-hydroxylated fatty acid. They are among the major determinants for the fluidity of mycobacterial cell wall and are also related to the sensitivity of mycobacterial species to hydrophobic antibiotics (Yuan et al., 1997). Because mycolic acids generally extend from C70 to C90, the correct folding and function of essential enzymes in type II fatty acid biosynthetic (FAS-II) pathway (Mdluli and Spigelman, 2006) that is responsible for large fatty acid synthesis are crucial for mycolic acid synthesis (Chatterjee, 1997). In contrast, FAS-I pathway generally synthesizes C16–C26 fatty acid. Mycobacterial FAS-I pathway shares the similarity with that in mammalian system, while FAS-II does not. Therefore, the distinct enzymes in FAS-II can be the potential targets for drug discovery.

Polyketide synthase Pks13, which is crucial for mycobacterial growth, catalyzes the final condensation step in mycolic acid synthesis in FAS-II pathway (Raman et al., 2005; Takayama et al., 2005; Bhatt et al., 2007). However, the full length structure of Pks13 protein is still unavailable. Nevertheless, several crystal structures of Pks13 homologues or active domain provide valuable insights about the mechanism of Pks13 enzyme activity (Maier et al., 2006; Tang et al., 2006). Acyl-AMP ligase (Trivedi et al., 2004; Portevin et al., 2005), FadD32 (Portevin et al., 2005; Leger et al., 2009) and the AccD4-containing acyl-coenzyme A (CoA) carboxylase (Portevin et al., 2005), are also proved to be essential for mycobacterial growth. Moreover, FabH (PDB code: 2QO1) (Scarsdale et al., 2001; He and Reynolds, 2002; Musayev et al., 2005), MabA (Cohen-Gonsaud et al., 2002; Ducasse-Cabanot et al., 2004) and InhA (PDB code: 1ZID) (Dessen et al., 1995; Sharma et al., 1998; Nunn et al., 2002; Ducasse-Cabanot et al., 2004; Oliveira et al., 2006) have indispensible roles in mycolic acid synthesis (Fig. 2A and 2B). As expected, large amount of compounds targeting these proteins were found to inhibit mycobacteria growth. In particular, isoniazid (INH), which targets *M. tuberculosis* InhA, is used as a first-line therapeutic drug in TB treatment.

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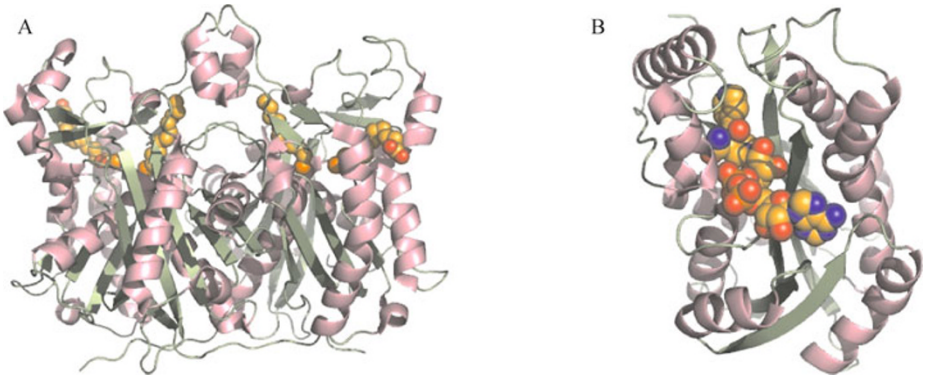

Figure 2. The crystal structure of FabH and InhA with their bound substrate or inhibitor.

TARGETS IN AMINO ACIDS AND COFACTORS BIOSYNTHESIS

Biosynthesis pathways of essential amino acids and cofactors are also crucial for the growth of *M. tuberculosis*, especially when the pathogen could not ingest enough nutrition from infected host. Therefore, the key enzymes in amino acid and cofactor biosynthesis pathways, which could be unique in mycobacteria, are believed to be effective drug targets.

The shikimate pathway, which is catalyzed by seven enzymes (AroB, AroC, AroE, AroG, AroK, AroK and AroQ), is essential for mycobacterial growth and is completely absent in mammalian cells. Therefore, the inhibitors to target the enzymes in shikimate pathway are hypersensitive. In addition, the crystallographic studies present detailed structural information and allow scientists to perform the structure-based drug design (Gourley et al., 1999; Rodriguez-Concepcion, 2004; Dias et al., 2006).

Vitamin B2 (riboflavin) and B5 (pantothenate) are also essential for *M. tuberculosis*. Therefore, the four enzymes in pantothenate biosynthesis (Pan B–E) and lumazine synthase (LS) that catalyzes vitamin B5 and B2 synthesis, are characterized as attractive targets for anti-*M. tuberculosis* drug discovery (Cole et al., 2001; Sambandamurthy et al., 2002; Visca et al., 2002). Currently, several crystal structures are available and present the possibility for highly selective and sensitive anti-*M. tuberculosis* drug design (Cole et al., 2001; Chaudhuri et al., 2003; Wang and Eisenberg, 2003, 2006; Chetnani et al., 2009).

TARGETS IN DNA METABOLISM

In *M. tuberculosis*, there are two classes of ribonucleotide reductases (RNRs), which are currently named as Class Ib and Class II, respectively, and are responsible for catalyzing the formation of deoxyribonucleotides from ribonucleotides. Class Ia is found in mammalian cells and certain bacteria, such as *E. coli* (Georgieva et al., 2008). Due to their essential roles, RNRs are characterized as the potential targets for antibacterial drug discovery. Class I enzymes are composed of

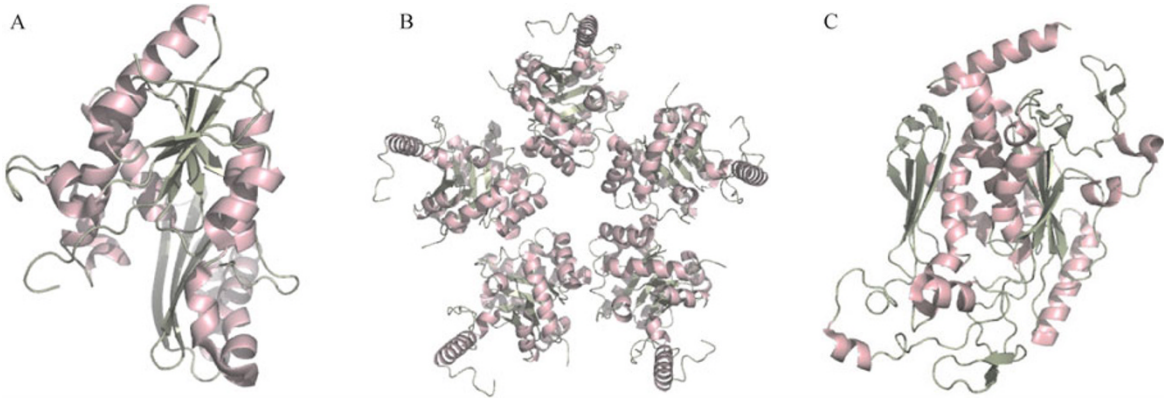

Figure 3. The crystal structures of the (A) pantothenate synthetase (PDB code: 2A88), (B) hydroxymethyltransferase (PDB code: 1OY0) and (C) chorismate synthase (PDB code: 1ZTB).

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the two homodimeric proteins, R1 and R2. Protein R1 contains substrate binding site, whereas R2 has a stable free radical on a tyrosine residue, neighboring an antiferromagnetically coupled high spin Fe(III)-Fe(III) site in each polypeptide of the R2 dimer, which is crucial for the enzyme activity (Sjoberg et al., 1978; Fontecave et al., 1992; Jordan et al., 1996). Although two genes encode RNR subunit in *M. tuberculosis*, R2-2 is generally believed to be the enzymatically active form (Meganathan, 2001; Marques et al., 2008). Uppsten and colleagues reported the crystal structure for the small subunit of *M. tuberculosis* RNR and identified the clear active center, which helps elucidate the mechanism of its activity (PDB code: 1UZR) (Uppsten et al., 2004) (Fig. 4). As the small subunit of *M. tuberculosis* RNR was reported to be hypersensitive to the class I RNR inhibitor hydroxyurea (Mowa et al., 2009), the structural knowledge provides a new direction to discover anti-*M. tuberculosis* inhibitors.

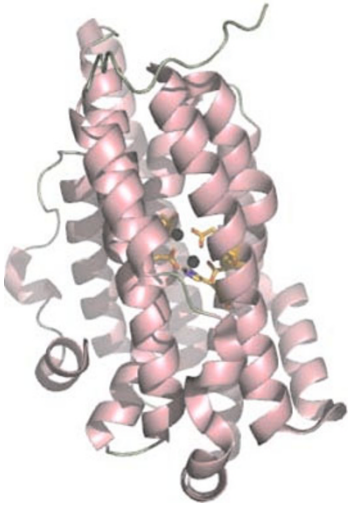

Figure 4. The crystal structure of the *M. tuberculosis* small subunit of RNR.

DNA ligase can link together two DNA strands that have double-strand break. DNA ligases are classified as either NAD+- or ATP-dependent, depending upon their specific cofactor (Vispe and Satoh, 2000). Comparing to the universal ATP-dependent ligases, NAD+-dependent ligases (LigA) are only found in certain viruses and bacteria, including *M. tuberculosis*. Srivastava and colleagues recently reported the crystal structure of *M. tuberculosis* LigA with bound AMP and provided it as a novel target for anti-*M. tuberculosis* drug discovery (Srivastava et al., 2005) (Fig. 5). Moreover, it is found that several nucleoside analogue and other compounds are effectively LigA inhibitors (Srivastava et al., 2005; Srivastava et al., 2007). In particular, glycofuranosylated diamine-based inhibitors could distinguish between the two types of ligases, and showed anti-TB activity (Gong et al., 2004).

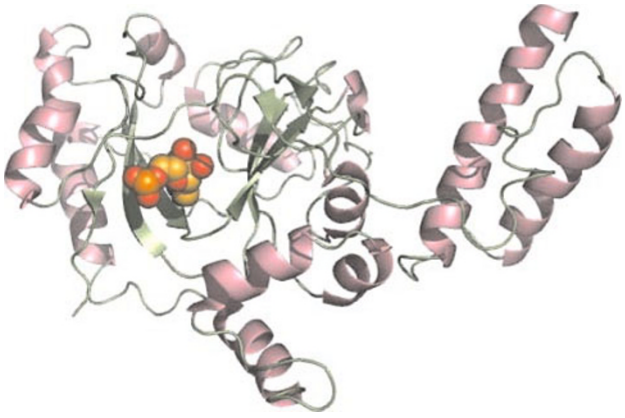

Figure 5. The crystal structure of *M. tuberculosis* LigA with bound ATP.

Thymidine kinase (TK), also known as a phosphotransferase, is found in most living cells. There are two forms of TKs in mammalian cells (TK1 and TK2), while several bacteria express their own TKs. As TKs have key functions in DNA synthesis, and therefore, in cell division, they are the targets of many anti-bacteria drugs. Recently, Li de la Sierra and colleagues reported the crystal structure of the *M. tuberculosis* TMP kinase (PDB code: 1G3U) (Li de la Sierra et al., 2001). They also identified a magnesium-binding site and a characteristic LID region. Comparing to the homologues in *E. coli* and yeast, the binding site of TMP in *M. tuberculosis* shows distinct differences. The observations about the interaction network, involving highly conserved side-chains of the protein, the magnesium ion, a sulphate ion and TMP itself, present *M. tuberculosis* TK as a good target to design selective inhibitors (Li de la Sierra et al., 2001) (Fig. 6). Based on this work, a serial of *M. tuberculosis* TK inhibitors were identified (Fioravanti et al., 2003; Haouz et al., 2003; Vanheusden et al., 2003, 2004), among which 30-azidodeoxythymidine monophosphate is a special one—it is a competitive inhibitor of *M. tuberculosis* TK, but is a substrate for TKs in human and other species (Fioravanti et al., 2005).

TARGETS IN GLYOXYLATE BYPASS

Glyoxylate shunt pathway is a carbon assimilatory pathway that allows the net synthesis of C4 dicarboxylic acids from C2 compounds. The first step of glyoxylate shunt is catalyzed by isocitrate lyase (ICL). There are two *M. tuberculosis* ICL proteins, a smaller one ICL1 and a larger one ICL2, which will cause complete impairment of *M. tuberculosis* replication and rapid elimination from infected lungs (Munoz-Elias and McKinney, 2005; Gould et al., 2006; Munoz-Elias et al., 2006). The structure of *M. tuberculosis* ICL was reported by Sharma and colleagues, and presents the precise inhibitory mechanism of the potent anti-*M. tuberculosis*

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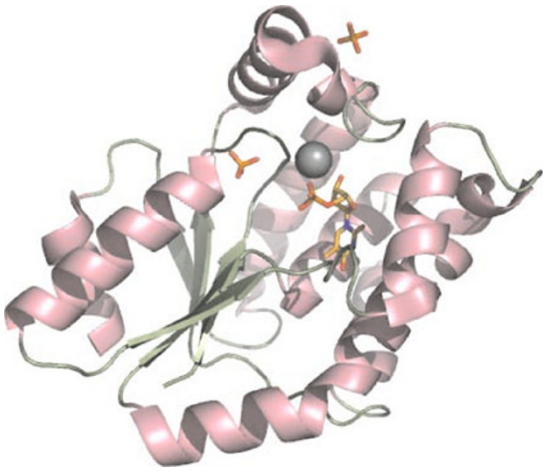

Figure 6. The crystal structure of *M. tuberculosis* LigA with bound magnesium ion, a sulphate ion mimicking and TMP.

3-nitropropionate and 3-bromopyruvate (Sharma et al., 2000) (Fig. 7).

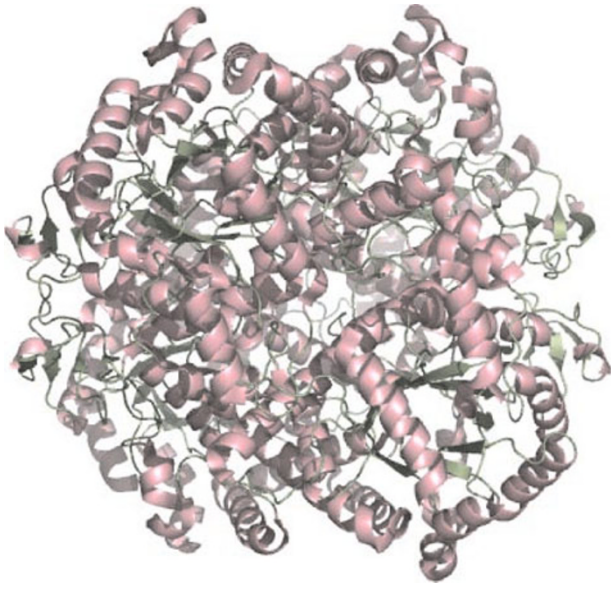

Figure 7. The crystal structure of *M. tuberculosis* ICL (PDB code: 1F8M).

TARGETS IN REGULATORY PROTEINS

The regulatory proteins, such as ArgP (Zhou et al.), GlnD and GlnE (McCoy et al., 2007; Carroll et al., 2008), and IdeR (Rodriguez-Concepcion, 2004) could act as transcription factors, regulators for other proteins, and cofactors, etc, and are essential for *M. tuberculosis* growth. Inhibition of regulatory proteins would have additive effects of disrupting a whole network of *M. tuberculosis* lifecycle and thus prevent the growth in infected host. However, the reported structural investigation is still very limited till now, and current drug discovery strategy to target these proteins is mainly based on biochemical assay.

PERSPECTIVES

Currently, several novel targets that are involved in the crucial steps in *M. tuberculosis* lifecycle and could be important for anti-*M. tuberculosis* drug discovery have been reported. Although some effective and highly selective inhibitors for these targets were identified or designed, the precise mechanism remains unclear at molecular level.

ATP biosynthesis is one of the most crucial bioreactions in *M. tuberculosis* lifecycle. Impaired function of ATP synthase may lead to ATP depletion and imbalance in pH homeostasis, both causing decreased survival (Deckers-Hebestreit and Altendorf, 1996; Hasan et al., 2006). Recently, an ATP synthase inhibitor (R207910), which targets F0 subunit of ATP synthase, atpE, was reported to contain high potency on suppressing both drug-sensitive and drug-resistant *M. tuberculosis in vitro* (Andries et al., 2005). In addition, the comparison of ATP synthase sequences from different species further supports the rationale to consider the specificity of antibacterial spectrum and the safety profile when targeting ATP synthase (Andries et al., 2005).

Menaquinone is reported to be essential for *M. tuberculosis* growth (Weinstein et al., 2005; Yano et al., 2006; Teh et al., 2007). Because menaquinone biosythesis pathway is absent in humans, it becomes an interesting target for anti-*M. tuberculosis* drug discovery. Although menaquinone biosythesis is extensively studied in *E. coil* and is relevant to some other enzymes, such as MenA–F (Meganathan, 2001), only type-II NADH-menaquinone oxidoreductase (NDH-2) is a potential target for structure-based drug discovery. However, there is only one reported structure of NDH-2 homologue, which is from *Pyrococcus furiosus Pfu-1140779-001* (PDB code: 1XHC). To elucidate the atomic coordinate of *M. tuberculosis* NDH-2 is an important future direction for anti-*M. tuberculosis* drug discovery.

In summary, the novel targets for developing anti-*M. tuberculosis* drugs shed the light for treating drug-sensitive and drug-resistant *M. tuberculosis*. Further knowledge is required to reveal the atomic coordinate and complexes with inhibitors or substrates of these novel targets in *M. tuberculosis* lifecycle. This could be helpful to understand the precise inhibitory mechanism and to discover and design novel compounds to treat TB.

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ABBREVIATIONS

AG, arabinogalactan; CoA, acyl-coenzyme A; DOTS, Directly Observed Treatment, Short-course; FAS-II, type II fatty acid biosynthetic; ICL, isocitrate lyase; INH, isoniazid; LAM, ipoarabinomannan; LigA, NAD+-dependent ligases; LS, lumazine synthase; mAGP, mycolyl-arabinogalactan-peptidoglycan; NDH-2, NADH-menaquinone oxidoreductase; RmlD, dTDP-6-deoxy-L-lyxo-4-hexulose reductase; RNRs, ribonucleotide reductases; TK, Thymidine kinase

REFERENCES

Andries, K., Verhasselt, P., Guillemont, J., Gohlmann, H.W., Neefs, J. M., Winkler, H., Van Gestel, J., Timmerman, P., Zhu, M., Lee, E., *et al*. (2005). A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science 307, 223–227.

Bhatt, A., Molle, V., Besra, G.S., Jacobs, W.R., Jr., and Kremer, L. (2007). The Mycobacterium tuberculosis FAS-II condensing enzymes: their role in mycolic acid biosynthesis, acid-fastness, pathogenesis and in future drug development. Mol Microbiol 64, 1442–1454.

Bocchino, M., Sanduzzi, A., and Bariffi, F. (2000). Mycobacterium tuberculosis and HIV co-infection in the lung: synergic immune dysregulation leading to disease progression. Monaldi Arch Chest Dis 55, 381–388.

Brunger, A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., Grosse-Kunstleve, R.W., Jiang, J.S., Kuszewski, J., Nilges, M., Pannu, N.S., *et al*. (1998). Crystallography & NMR system: A new software suite for macromolecular structure determination. Acta Crystallogr D Biol Crystallogr 54, 905–921.

Carroll, P., Pashley, C.A., and Parish, T. (2008). Functional analysis of GlnE, an essential adenylyl transferase in Mycobacterium tuberculosis. J Bacteriol 190, 4894–4902.

Chatterjee, D. (1997). The mycobacterial cell wall: structure, biosynthesis and sites of drug action. Curr Opin Chem Biol 1, 579–588.

Chaudhuri, B.N., Sawaya, M.R., Kim, C.Y., Waldo, G.S., Park, M.S., Terwilliger, T.C., and Yeates, T.O. (2003). The crystal structure of the first enzyme in the pantothenate biosynthetic pathway, ketopantoate hydroxymethyltransferase, from M tuberculosis. Structure 11, 753–764.

Chetnani, B., Das, S., Kumar, P., Surolia, A., and Vijayan, M. (2009). Mycobacterium tuberculosis pantothenate kinase: possible changes in location of ligands during enzyme action. Acta Crystallogr D Biol Crystallogr 65, 312–325.

Cohen-Gonsaud, M., Ducasse, S., Hoh, F., Zerbib, D., Labesse, G., and Quemard, A. (2002). Crystal structure of MabA from Mycobacterium tuberculosis, a reductase involved in long-chain fatty acid biosynthesis. J Mol Biol 320, 249–261.

Cole, S.T., Eiglmeier, K., Parkhill, J., James, K.D., Thomson, N.R., Wheeler, P.R., Honore, N., Garnier, T., Churcher, C., Harris, D., *et al*. (2001). Massive gene decay in the leprosy bacillus. Nature 409, 1007–1011.

Deckers-Hebestreit, G., and Altendorf, K. (1996). The F0F1-type ATP synthases of bacteria: structure and function of the F0 complex. Annu Rev Microbiol 50, 791–824.

Dessen, A., Quemard, A., Blanchard, J.S., Jacobs, W.R., Jr., and Sacchettini, J.C. (1995). Crystal structure and function of the isoniazid target of Mycobacterium tuberculosis. Science 267, 1638–1641.

Dias, M.V., Borges, J.C., Ely, F., Pereira, J.H., Canduri, F., Ramos, C. H., Frazzon, J., Palma, M.S., Basso, L.A., Santos, D.S., *et al*. (2006). Structure of chorismate synthase from Mycobacterium tuberculosis. J Struct Biol 154, 130–143.

Ducasse-Cabanot, S., Cohen-Gonsaud, M., Marrakchi, H., Nguyen, M., Zerbib, D., Bernadou, J., Daffe, M., Labesse, G., and Quemard, A. (2004). *In vitro* inhibition of the Mycobacterium tuberculosis beta-ketoacyl-acyl carrier protein reductase MabA by isoniazid. Antimicrob Agents Chemother 48, 242–249.

Feng, Z., and Barletta, R.G. (2003). Roles of Mycobacterium smegmatis D-alanine:D-alanine ligase and D-alanine racemase in the mechanisms of action of and resistance to the peptidoglycan inhibitor D-cycloserine. Antimicrob Agents Chemother 47, 283–291.

Fioravanti, E., Adam, V., Munier-Lehmann, H., and Bourgeois, D. (2005). The crystal structure of Mycobacterium tuberculosis thymidylate kinase in complex with 3'-azidodeoxythymidine monophosphate suggests a mechanism for competitive inhibition. Biochemistry 44, 130–137.

Fioravanti, E., Haouz, A., Ursby, T., Munier-Lehmann, H., Delarue, M., and Bourgeois, D. (2003). Mycobacterium tuberculosis thymidylate kinase: structural studies of intermediates along the reaction pathway. J Mol Biol 327, 1077–1092.

Fontecave, M., Nordlund, P., Eklund, H., and Reichard, P. (1992). The redox centers of ribonucleotide reductase of Escherichia coli. Adv Enzymol Relat Areas Mol Biol 65, 147–183.

Georgieva, E.R., Narvaez, A.J., Hedin, N., and Graslund, A. (2008). Secondary structure conversions of Mycobacterium tuberculosis ribonucleotide reductase protein R2 under varying pH and temperature conditions. Biophys Chem 137, 43–48.

Godreuil, S., Renaud, F., Van de Perre, P., Carriere, C., Torrea, G., and Banuls, A.L. (2007). Genetic diversity and population structure of Mycobacterium tuberculosis in HIV-1-infected compared with uninfected individuals in Burkina Faso. Aids 21, 248–250.

Gong, C., Martins, A., Bongiorno, P., Glickman, M., and Shuman, S. (2004). Biochemical and genetic analysis of the four DNA ligases of mycobacteria. J Biol Chem 279, 20594–20606.

Gould, T.A., van de Langemheen, H., Munoz-Elias, E.J., McKinney, J. D., and Sacchettini, J.C. (2006). Dual role of isocitrate lyase 1 in the glyoxylate and methylcitrate cycles in Mycobacterium tuberculosis. Mol Microbiol 61, 940–947.

Gourley, D.G., Shrive, A.K., Polikarpov, I., Krell, T., Coggins, J.R., Hawkins, A.R., Isaacs, N.W., and Sawyer, L. (1999). The two types of 3-dehydroquinase have distinct structures but catalyze the same overall reaction. Nat Struct Biol 6, 521–525.

Haouz, A., Vanheusden, V., Munier-Lehmann, H., Froeyen, M., Herdewijn, P., Van Calenbergh, S., and Delarue, M. (2003). Enzymatic and structural analysis of inhibitors designed against Mycobacterium tuberculosis thymidylate kinase. New insights into the phosphoryl transfer mechanism. J Biol Chem 278, 4963–4971.

Hasan, S., Daugelat, S., Rao, P.S., and Schreiber, M. (2006). Prioritizing genomic drug targets in pathogens: application to Mycobacterium tuberculosis. PLoS Comput Biol 2, e61.

He, X., and Reynolds, K.A. (2002). Purification, characterization, and identification of novel inhibitors of the beta-ketoacyl-acyl carrier

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protein synthase III (FabH) from Staphylococcus aureus. Antimicrob Agents Chemother 46, 1310–1318.

Huang, H., Scherman, M.S., D'Haeze, W., Vereecke, D., Holsters, M., Crick, D.C., and McNeil, M.R. (2005). Identification and active expression of the Mycobacterium tuberculosis gene encoding 5-phospho-{alpha}-d-ribose-1-diphosphate: decaprenyl-phosphate 5-phosphoribosyltransferase, the first enzyme committed to decaprenylphosphoryl-d-arabinose synthesis. J Biol Chem 280, 24539–24543.

Jordan, A., Pontis, E., Aslund, F., Hellman, U., Gibert, I., and Reichard, P. (1996). The ribonucleotide reductase system of Lactococcus lactis. Characterization of an NrdEF enzyme and a new electron transport protein. J Biol Chem 271, 8779–8785.

Kremer, L., Dover, L.G., Morehouse, C., Hitchin, P., Everett, M., Morris, H.R., Dell, A., Brennan, P.J., McNeil, M.R., Flaherty, C., *et al*. (2001). Galactan biosynthesis in Mycobacterium tuberculosis. Identification of a bifunctional UDP-galactofuranosyltransferase. J Biol Chem 276, 26430–26440.

Kurth, D.G., Gago, G.M., de la Iglesia, A., Bazet Lyonnet, B., Lin, T. W., Morbidoni, H.R., Tsai, S.C., and Gramajo, H. (2009). ACCase 6 is the essential acetyl-CoA carboxylase involved in fatty acid and mycolic acid biosynthesis in mycobacteria. Microbiology 155, 2664–2675.

Lederer, E., Adam, A., Ciorbaru, R., Petit, J.F., and Wietzerbin, J. (1975). Cell walls of Mycobacteria and related organisms; chemistry and immunostimulant properties. Mol Cell Biochem 7, 87–104.

Leger, M., Gavalda, S., Guillet, V., van der Rest, B., Slama, N., Montrozier, H., Mourey, L., Quemard, A., Daffe, M., and Marrakchi, H. (2009). The dual function of the Mycobacterium tuberculosis FadD32 required for mycolic acid biosynthesis. Chem Biol 16, 510–519.

LeMagueres, P., Im, H., Ebalunode, J., Strych, U., Benedik, M.J., Briggs, J.M., Kohn, H., and Krause, K.L. (2005). The 1.9 Å crystal structure of alanine racemase from Mycobacterium tuberculosis contains a conserved entryway into the active site. Biochemistry 44, 1471–1481.

Li de la Sierra, I., Munier-Lehmann, H., Gilles, A.M., Barzu, O., and Delarue, M. (2001). X-ray structure of TMP kinase from Mycobacterium tuberculosis complexed with TMP at 1.95 Å resolution. J Mol Biol 311, 87–100.

Li, W., Xin, Y., McNeil, M.R., and Ma, Y. (2006). rmlB and rmlC genes are essential for growth of mycobacteria. Biochem Biophys Res Commun 342, 170–178.

Ma, Y., Stern, R.J., Scherman, M.S., Vissa, V.D., Yan, W., Jones, V.C., Zhang, F., Franzblau, S.G., Lewis, W.H., and McNeil, M.R. (2001). Drug targeting Mycobacterium tuberculosis cell wall synthesis: genetics of dTDP-rhamnose synthetic enzymes and development of a microtiter plate-based screen for inhibitors of conversion of dTDP-glucose to dTDP-rhamnose. Antimicrob Agents Chemother 45, 1407–1416.

Maier, T., Jenni, S., and Ban, N. (2006). Architecture of mammalian fatty acid synthase at 4.5 Å resolution. Science 311, 1258–1262.

Marques, M.A., Neves-Ferreira, A.G., da Silveira, E.K., Valente, R.H., Chapeaurouge, A., Perales, J., da Silva Bernardes, R., Dobos, K. M., Spencer, J.S., Brennan, P.J., *et al*. (2008). Deciphering the proteomic profile of Mycobacterium leprae cell envelope. Proteomics 8, 2477–2491.

McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C., and Read, R.J. (2007). Phaser crystallographic software. J Appl Crystallogr 40, 658–674.

Mdluli, K., and Spigelman, M. (2006). Novel targets for tuberculosis drug discovery. Curr Opin Pharmacol 6, 459–467.

Meganathan, R. (2001). Biosynthesis of menaquinone (vitamin K2) and ubiquinone (coenzyme Q): a perspective on enzymatic mechanisms. Vitam Horm 61, 173–218.

Mowa, M.B., Warner, D.F., Kaplan, G., Kana, B.D., and Mizrahi, V. (2009). Function and regulation of class I ribonucleotide reductase-encoding genes in mycobacteria. J Bacteriol 191, 985–995.

Munoz-Elias, E.J., and McKinney, J.D. (2005). Mycobacterium tuberculosis isocitrate lyases 1 and 2 are jointly required for in vivo growth and virulence. Nat Med 11, 638–644.

Munoz-Elias, E.J., Upton, A.M., Cherian, J., and McKinney, J.D. (2006). Role of the methylcitrate cycle in Mycobacterium tuberculosis metabolism, intracellular growth, and virulence. Mol Microbiol 60, 1109–1122.

Musayev, F., Sachdeva, S., Scarsdale, J.N., Reynolds, K.A., and Wright, H.T. (2005). Crystal structure of a substrate complex of Mycobacterium tuberculosis beta-ketoacyl-acyl carrier protein synthase III (FabH) with lauroyl-coenzyme A. J Mol Biol 346, 1313–1321.

Nakano, Y., Suzuki, N., Yoshida, Y., Nezu, T., Yamashita, Y., and Koga, T. (2000). Thymidine diphosphate-6-deoxy-L-lyxo-4-hexulose reductase synthesizing dTDP-6-deoxy-L-talose from Actinobacillus actinomycetemcomitans. J Biol Chem 275, 6806–6812.

Nunn, C.M., Djordjevic, S., Hillas, P.J., Nishida, C.R., and Ortiz de Montellano, P.R. (2002). The crystal structure of Mycobacterium tuberculosis alkylhydroperoxidase AhpD, a potential target for antitubercular drug design. J Biol Chem 277, 20033–20040.

Oliveira, J.S., Pereira, J.H., Canduri, F., Rodrigues, N.C., de Souza, O.N., de Azevedo, W.F., Jr., Basso, L.A., and Santos, D.S. (2006). Crystallographic and pre-steady-state kinetics studies on binding of NADH to wild-type and isoniazid-resistant enoyl-ACP (CoA) reductase enzymes from Mycobacterium tuberculosis. J Mol Biol 359, 646–666.

Portevin, D., de Sousa-D'Auria, C., Montrozier, H., Houssin, C., Stella, A., Laneelle, M.A., Bardou, F., Guilhot, C., and Daffe, M. (2005). The acyl-AMP ligase FadD32 and AccD4-containing acyl-CoA carboxylase are required for the synthesis of mycolic acids and essential for mycobacterial growth: identification of the carboxylation product and determination of the acyl-CoA carboxylase components. J Biol Chem 280, 8862–8874.

Qureshi, H., Arif, A., Alam, E., and Qadir, N. (2010). Integration of informal medical practitioners in DOTS implementation to improve case detection rate. J Pak Med Assoc 60, 33–37.

Raman, K., Rajagopalan, P., and Chandra, N. (2005). Flux balance analysis of mycolic acid pathway: targets for anti-tubercular drugs. PLoS Comput Biol 1, e46.

Rivers, E.C., and Mancera, R.L. (2008a). New anti-tuberculosis drugs in clinical trials with novel mechanisms of action. Drug Discov Today 13, 1090–1098.

Rivers, E.C., and Mancera, R.L. (2008b). New anti-tuberculosis drugs with novel mechanisms of action. Curr Med Chem 15, 1956–1967.

Rodriguez-Concepcion, M. (2004). The MEP pathway: a new target for the development of herbicides, antibiotics and antimalarial

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drugs. Curr Pharm Des 10, 2391–2400.

Sambandamurthy, V.K., Wang, X., Chen, B., Russell, R.G., Derrick, S., Collins, F.M., Morris, S.L., and Jacobs, W.R., Jr. (2002). A pantothenate auxotroph of Mycobacterium tuberculosis is highly attenuated and protects mice against tuberculosis. Nat Med 8, 1171–1174.

Scarsdale, J.N., Kazanina, G., He, X., Reynolds, K.A., and Wright, H. T. (2001). Crystal structure of the Mycobacterium tuberculosis beta-ketoacyl-acyl carrier protein synthase III. J Biol Chem 276, 20516–20522.

Sharma, V., Grubmeyer, C., and Sacchettini, J.C. (1998). Crystal structure of quinolinic acid phosphoribosyltransferase from Mmycobacterium tuberculosis: a potential TB drug target. Structure 6, 1587–1599.

Sharma, V., Sharma, S., Hoener zu Bentrup, K., McKinney, J.D., Russell, D.G., Jacobs, W.R., Jr., and Sacchettini, J.C. (2000). Structure of isocitrate lyase, a persistence factor of Mycobacterium tuberculosis. Nat Struct Biol 7, 663–668.

Sjoberg, B.M., Reichard, P., Graslund, A., and Ehrenberg, A. (1978). The tyrosine free radical in ribonucleotide reductase from Escherichia coli. J Biol Chem 253, 6863–6865.

Srivastava, S.K., Dube, D., Kukshal, V., Jha, A.K., Hajela, K., and Ramachandran, R. (2007). NAD+-dependent DNA ligase (Rv3014c) from Mycobacterium tuberculosis: novel structure-function relationship and identification of a specific inhibitor. Proteins 69, 97–111.

Srivastava, S.K., Tripathi, R.P., and Ramachandran, R. (2005). NAD+-dependent DNA Ligase (Rv3014c) from Mycobacterium tuberculosis. Crystal structure of the adenylation domain and identification of novel inhibitors. J Biol Chem 280, 30273–30281.

Takayama, K., Wang, C., and Besra, G.S. (2005). Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis. Clin Microbiol Rev 18, 81–101.

Tang, Y., Kim, C.Y., Mathews, II, Cane, D.E., and Khosla, C. (2006). The 2.7-Angstrom crystal structure of a 194-kDa homodimeric fragment of the 6-deoxyerythronolide B synthase. Proc Natl Acad Sci U S A 103, 11124–11129.

Teh, J.S., Yano, T., and Rubin, H. (2007). Type II NADH: menaquinone oxidoreductase of Mycobacterium tuberculosis. Infect Disord Drug Targets 7, 169–181.

Trivedi, O.A., Arora, P., Sridharan, V., Tickoo, R., Mohanty, D., and Gokhale, R.S. (2004). Enzymic activation and transfer of fatty acids as acyl-adenylates in mycobacteria. Nature 428, 441–445.

Uppsten, M., Davis, J., Rubin, H., and Uhlin, U. (2004). Crystal structure of the biologically active form of class Ib ribonucleotide reductase small subunit from Mycobacterium tuberculosis. FEBS Lett 569, 117–122.

Vanheusden, V., Munier-Lehmann, H., Froeyen, M., Busson, R., Rozenski, J., Herdewijn, P., and Van Calenbergh, S. (2004). Discovery of bicyclic thymidine analogues as selective and high-affinity inhibitors of Mycobacterium tuberculosis thymidine monophosphate kinase. J Med Chem 47, 6187–6194.

Vanheusden, V., Munier-Lehmann, H., Froeyen, M., Dugue, L., Heyerick, A., De Keukeleire, D., Pochet, S., Busson, R., Herdewijn, P., and Van Calenbergh, S. (2003). 3'-C-branched-chain-substituted nucleosides and nucleotides as potent inhibitors of Mycobacterium tuberculosis thymidine monophosphate kinase. J Med Chem 46, 3811–3821.

Visca, P., Fabozzi, G., Milani, M., Bolognesi, M., and Ascenzi, P. (2002). Nitric oxide and Mycobacterium leprae pathogenicity. IUBMB Life 54, 95–99.

Vispe, S., and Satoh, M.S. (2000). DNA repair patch-mediated double strand DNA break formation in human cells. J Biol Chem 275, 27386–27392.

Wang, S., and Eisenberg, D. (2003). Crystal structures of a pantothenate synthetase from M. tuberculosis and its complexes with substrates and a reaction intermediate. Protein Sci 12, 1097–1108.

Wang, S., and Eisenberg, D. (2006). Crystal structure of the pantothenate synthetase from Mycobacterium tuberculosis, snapshots of the enzyme in action. Biochemistry 45, 1554–1561.

Weinstein, E.A., Yano, T., Li, L.S., Avarbock, D., Avarbock, A., Helm, D., McColm, A.A., Duncan, K., Lonsdale, J.T., and Rubin, H. (2005). Inhibitors of type II NADH:menaquinone oxidoreductase represent a class of antitubercular drugs. Proc Natl Acad Sci U S A 102, 4548–4553.

Yano, T., Li, L.S., Weinstein, E., Teh, J.S., and Rubin, H. (2006). Steady-state kinetics and inhibitory action of antitubercular phenothiazines on mycobacterium tuberculosis type-II NADH-menaquinone oxidoreductase (NDH-2). J Biol Chem 281, 11456–11463.

Yuan, Y., Crane, D.C., Musser, J.M., Sreevatsan, S., and Barry, C.E., 3rd (1997). MMAS-1, the branch point between cis- and trans-cyclopropane-containing oxygenated mycolates in Mycobacterium tuberculosis. J Biol Chem 272, 10041–10049.

Zhou, X., Lou, Z., Fu, S., Yang, A., Shen, H., Li, Z., Feng, Y., Bartlam, M., Wang, H., and Rao, Z. Crystal structure of ArgP from Mycobacterium tuberculosis confirms two distinct conformations of full-length LysR transcriptional regulators and reveals its function in DNA binding and transcriptional regulation. J Mol Biol 396, 1012–1024.

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