Protein Cell 2011, 2(9): 691–695
DOI 10.1007/s13238-011-1100-8

Protein & Cell

PESPECTIVE

Biotin biosynthesis in *Mycobacterium tuberculosis*: physiology, biochemistry and molecular intervention

Wanisa Salaemae, Al Azhar, Grant W. Booker, Steven W. Polyak ✉

School of Molecular and Biomedical Sciences, University of Adelaide, South Australia 5005, Australia
✉ Correspondence: steven.polyak@adelaide.edu.au

Biotin is an important micronutrient that serves as an essential enzyme cofactor. Bacteria obtain biotin either through *de novo* synthesis or by active uptake from exogenous sources. *Mycobacteria* are unusual amongst bacteria in that their primary source of biotin is through *de novo* synthesis. Here we review the importance of biotin biosynthesis in the lifecycle of *Mycobacteria*. Genetic screens designed to identify key metabolic processes have highlighted a role for the biotin biosynthesis in bacilli growth, infection and survival during the latency phase. These studies help to establish the biotin biosynthetic pathway as a potential drug target for new anti-tuberculosis agents.

CLINICAL NEED FOR NEW ANTI-TB DRUGS

Tuberculosis (TB) is one of the most common causes of human mortality in the world. The continual rise in the number of TB patients highlights the critical demand for new approaches to treat this important infectious disease. It is estimated that there are now 9.8 million new cases of TB each year, more than at any other time in history (Dye and Williams, 2010). Our current inability to control this infectious disease stems from the loss of efficacy of vaccines and antibiotic pharmaceuticals that were once effective in the clinic. Replacement of old agents with new medicines required to treat drug resistant strains has been hampered by diminishing investment in antibiotic drug discovery and the lack of political desire to deliver therapies to those in most need (Koul et al., 2011; Lawn and Zumla, 2011). The complex lifecycle of *Mycobacterium tuberculosis*, the pathogenic bacilli responsible for TB, also contributes to the bacteria's extraordinary ability to evade antibiotic therapy (Russell et al., 2010). *M. tuberculosis* exists in both an active and latent state. Most antibiotics are effective against actively growing *Mycobacterium* as they target metabolic processes required for the primary progressive stage of infection (Baek et al., 2011; Koul et al., 2011). Conversely, dormant bacilli are more difficult to treat as they have evolved complex mechanisms that assist them to evade both antibiotics and the patient's immune system (Joshi et al., 2006; Ahmad, 2011). Hence, the bacteria can re-activate once antibiotic treatment has ceased or the immune system has been compromised, for example by co-infection with human immunodeficiency virus (HIV) (Kwan and Ernst, 2011; Lawn and Zumla, 2011). One novel strategy to treat TB is to pharmacologically target metabolic pathways essential in both the active and latent stages of *M. tuberculosis*. The biotin biosynthesis pathway is potentially an example of such a pathway. Here we review recent studies into this metabolic pathway with a view to establishing its disruption as a strategy for antibiotic drug discovery.

BIOTIN IS AN ENZYME COFACTOR

Biotin (aka vitamin H or B7) is an essential cofactor for two important biotin-dependent enzymes in *M. tuberculosis*, namely pyruvate carboxylase (PC) and acyl-CoA carboxylase (ACC). Here biotin is required for the transfer of a carboxyl anion onto a specific organic acid substrate. Pyruvate carboxylase plays an anapleurotic role in central carbon metabolism in many bacterial species by replenishing the TCA cycle with oxaloacetate (Eisenreich et al., 2010). The role of pyruvate carboxylase has not been extensively investigated in *Mycobacterium* compared with other bacteria, mainly due to the technical difficulties associated with studying metabolic pathways in an intracellular pathogen. Further work is needed to address our deficiencies in understanding in this area. Specifically, the mechanisms that permit the bacteria to survive and adapt to niche micro-environments inside host cells will need to be delineated

(Eisenreich et al., 2010). In contrast, ACC has been the subject of greater research focus primarily due to its potential as an antibiotic drug target (Wright and Reynolds, 2007; Chan and Vogel, 2010; Parsons and Rock, 2011). ACC catalyses the carboxylation of various acyl CoA substrates, such as acetyl CoA, propionyl CoA and butyryl CoA (Arabolaza et al., 2010; Gago et al., 2011). The products of these reactions feed into the fatty acid synthesis and polyketide synthesis pathways, resulting in the production of mycolic acids and multimethyl-branched fatty acids present in the cell envelope (Takayama et al., 2005; Gago et al., 2011). These pathways are especially important in *Mycobacterium* sp., as the cell envelope contains a complex lipid bi-layer composed primarily of mycolic acid. It has been estimated that 10% of the *Mtb* genome is devoted to fatty acid biosynthesis (Minnikin et al., 2002). The cell membrane greatly enhances the bacterium's ability to resist chemical damage, survive in hostile environments, and limits its susceptibility to many antibiotics (Niederweis et al., 2010). Therefore, the metabolic enzymes responsible for the synthesis of membrane lipids represent promising targets for the development of new anti-mycobacterial drugs. Clinical validation for this approach is provided by the anti-TB drug isoniazid that targets fatty acid biosynthesis (Lu and Tonge, 2008). The important metabolic functions of both PC and ACC are critically dependent upon the availability of biotin as a coenzyme.

BIOTIN BIOSYNTHETIC PATHWAY

Many micro-organisms, plants and fungi can synthesize biotin *de novo* (Cronan and Lin, 2011). In contrast, mammals are biotin auxotrophs that obtain the micronutrient from intestinal micro-flora, dietary sources and recycling (Said, 2009). The absence of an analogous metabolic pathway in mammals makes biotin biosynthesis an attractive prospect for antibiotic discovery. The final four steps in the pathway are conserved amongst the biotin-producing organisms. The universal biosynthetic pathway, shown in Fig. 1, converts a pimeloyl-thioester to biotin through the activity of four enzymes, namely 7-keto-8-aminopelargonic acid synthase (KAPAS, encoded by *bioF*), 7,8-diaminopelargonic acid synthase (DAPAS, encoded by *bioA*), dethiobiotin synthetase (DTBS, encoded by *bioD*), and biotin synthase (BS, encoded by *bioB*) (reviewed (Cronan and Lin, 2011)). Briefly, KAPAS converts a pimelate moiety to 7-keto-8-aminopelargonic acid (KAPA) using L-alanine as an amino donor. The KAPA is subsequently converted to 7, 8-diaminopelargonic acid (DAPA) by the activity of DAPAS requiring S-adenosylmethionine (SAM) as an amino donor. Next, the conversion of DAPA into dethiobiotin (DTB) is catalyzed by DTBS that requires CO_2 and ATP to close the ureido ring. Finally, the sulfur ring of biotin is closed by biotin synthase requiring one sulfur atom and two electrons transferred from flavodoxin, SAM and nicotinamide adenine dinucleotide phosphate (NADPH) (Berkovitch et al., 2004). As will be discussed below, genetic studies that disrupt *bioF*, *A*, *D* and *B* assist in establishing the biotin biosynthesis pathway as a target for the development of new anti-TB agents.

Whilst the final four steps are highly conserved, precursors that feed into this pathway can be acquired through different means. New insights into the mechanisms employed by the model bacteria *Escherichia coli* have come to light, and have been reviewed recently (Cronan and Lin, 2011). Briefly, the 3-carbon chain present in malonyl CoA is extended by four additional carbon units by the fatty acid biosynthetic pathway (Fig. 1). The BioC methyltransferase is required to move the biotin precursor into the fatty acid synthesis pathway, whereas the esterase BioH facilitates escape of pimeloyl-thioester so BioF can use it as a substrate in the conserved pathway (Lin et al., 2010). The identification of BioC and BioH homologues in available *Mycobacteria* genomes suggests that this pathway is also employed by this species (Yu et al., 2011). A recent study highlighted the importance of the biotin precursor pathway in *Mycobacteria*. Deletion of Rv1882c, a putative short chain dehyrodogenase in the fatty acid biosynthesis pathway, yielded an *M. tuberculosis* strain with attenuated growth on blood agar and in murine macrophages *in vitro*. Supplementing the growth media with high concentrations of biotin (> 1 μmol/L) restored growth rates. Unlike wildtype *Mycobacterium marinum*, a Rv1882c deletion mutant strain also failed to colonize the livers of zebrafish and were completely cleared from the fish two weeks post infection (Yu et al., 2011). These data suggest that the intracellular supply of biotin is insufficient for *Mycobacterium* survival, and the bacteria are critically dependent upon *de novo* synthesis.

BIOTIN BIOSYNTHESIS IS REQUIRED IN INFECTION AND LATENCY

Genetic studies have played an important role in establishing the importance of biotin synthesis in *Mycobacterium* at various stages of its life-cycle. Access to genome-wide, insertional mutagenic libraries of *Mycobacteria* has facilitated phenotypic screening to identify key metabolic pathways required under a variety of conditions (Sassetti et al., 2001, 2003). For example disruption of *bioA* attenuated the growth of *M. smegmatis* on carbon-depleted media, imitating the nutrient deprived state experienced by stationary phase bacteria during latency (Keer et al., 2000). As the ability of *Mycobacteria* to grow inside macrophages is related to its virulence and pathogenicity (Russell, 2001), manipulation of *in vitro* growth conditions can also imitate conditions experienced *in vivo*. Non-stimulated macrophages model initial and latent infections, whereas macrophages stimulated with interferon gamma imitates an on-going immune response (Rengarajan et al., 2005). *bioF* and *bioB* mutants were identified in a genetic screen investigating genes

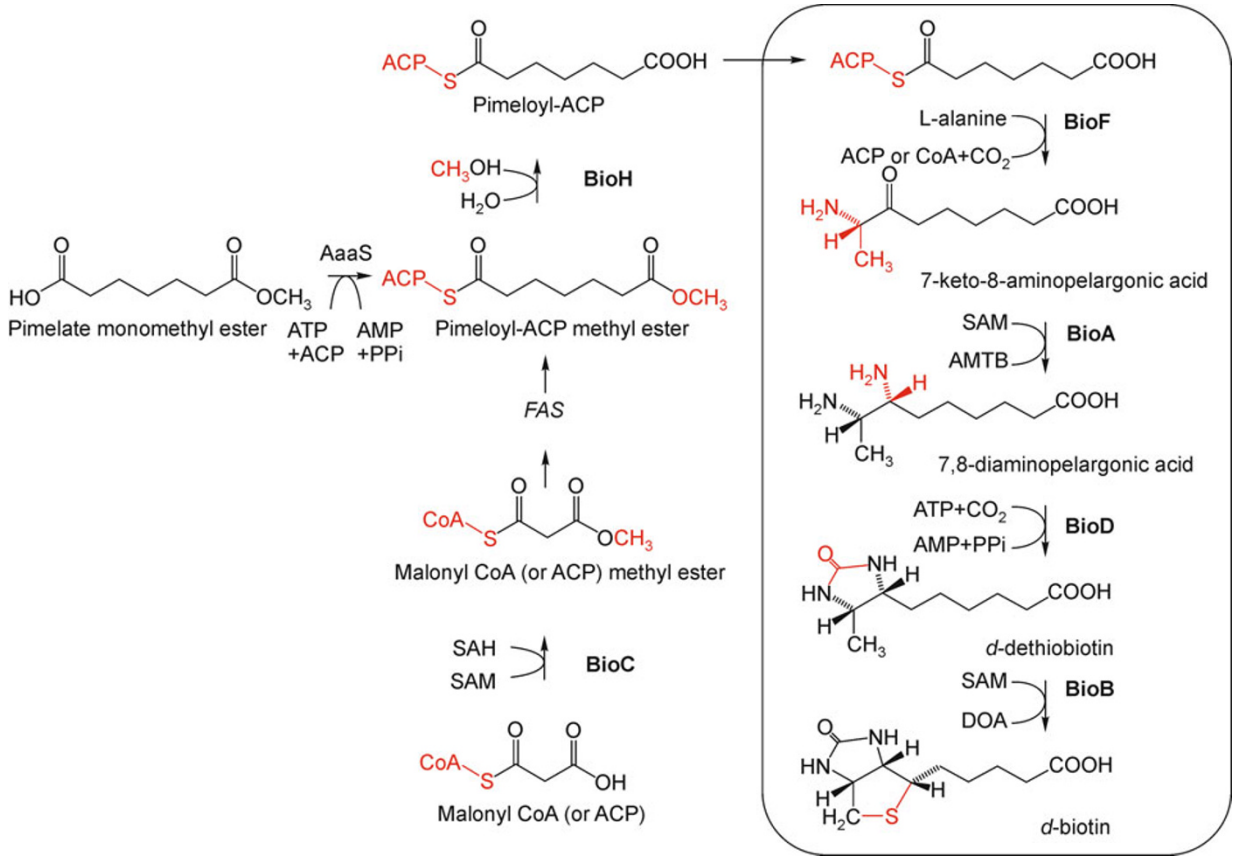

Figure 1. Putative biotin biosynthesis pathway in *M. tuberculosis*. The conserved metabolic pathway (boxed) and synthesis of the precursors to this pathway, are shown. Intermediates in the biotin biosynthesis pathway are represented, with modifications made to the chemical structures during catalysis highlighted in red. This pathway is proposed to exist in *M. tuberculosis* due to the presence of BioC and BioH homologues in the bacilli's genome that play key roles in the pathway determined for *E. coli* (Lin et al., 2010). BioF can accept either pimeloyl-CoA or pimeloyl-ACP as a substrate. Figure adapted from (Seki, 2006; Mann et al., 2009) and (Lin et al., 2010). Copyright 2006,2009 Wiley Periodicals, Inc (withpermission).

required for prolonged infection of primary murine macrophages (Rengarajan et al., 2005). Both mutant strains showed attenuated growth in un-stimulated and stimulated macrophages, suggesting a critical role for *de novo* biotin synthesis during and post infection.

Biotin biosynthesis in *Mycobacteria* has also been investigated in mouse models of the infectious disease. Genome-wide genetic screens performed in mice, designed to isolate *M. tuberculosis* genes required for *in vivo* virulence, identified *bioF*, *A* and *B* (Sassetti and Rubin, 2003). These mutant strains showed dramatically reduced growth rates in the murine infection model. Particularly noteworthy was *bioF*. An in-frame deletion of *bioF* in *M. tuberculosis* resulted in rapid clearance of the mutant strain in the early stages of infection, and showed poor survival in mouse lung and spleen (Sassetti and Rubin, 2003). Interestingly, the genome sequence of *M. tuberculosis* contains two *bioF* genes (*bioF1*, Rv1569 and $bioF_2$, Rv0032). However, only *bioF1* was identified in any of the above screens, suggesting no redundancy between the two alleles. This is supported by targeted gene knockout studies that showed deletion of *bioF1* has a bacteriocidal phenotype unless grown *in vitro* on media supplemented with high concentrations of biotin (Dey et al., 2010).

BIOTIN TRANSPORTER PROTEINS

Based on the studies above, the literature strongly suggests that *Mycobacteria's* primary source of biotin is via *de novo* biosynthesis. In other words, the bacilli do not posses a biotin transport system to scavenge biotin from exogenous sources. Many other bacteria do possess this ability by utilizing a biotin transport protein. The most characterized example is BioY (Rodionov et al., 2009). This transporter works with an energy coupling system to actively move biotin across the bacterial cell membrane in an ATP-dependent manner (Hebbeln et al., 2007; Rodionov et al., 2009). Genome annotation studies have failed to identify homologues of the *bioY* gene in the *M. tuberculosis* genome (Rodionov et al., 2002; Hebbeln

et al., 2007). Supporting the observation that *Mycobacteria* require *de novo* biotin synthesis are reports that chemical inhibition of the biotin biosynthetic enzymes also impedes the growth of *Mycobacteria in vitro*. This has been investigated using two natural compounds isolated from culture filtrates of *Streptomyces* species, namely amiclenomycin and actithiazic acid (Ogata et al., 1973; Okami et al., 1974). The BioA inhibitor, amiclenomycin, is a narrow-spectrum antibiotic with activity against *Mycobacteria* sp., but not other bacteria or fungi that can scavenge exogenous biotin (Kitahara et al., 1975). Its anti-TB activity can be reversed by high concentrations of external biotin, above 0.01 μg/mL (Sandmark et al., 2002; Mann et al., 2005), which is at least 10-fold greater than the concentration found in normal human plasma (Mock and Malik, 1992). This implies that the water-soluble biotin might enter through the bacilli membrane using mechanisms that are not yet identified, but only in supra-physiological concentrations of the nutrient. Similarly, the BioA inhibitor actithiazic acid also displays narrow spectrum activity against *Mycobacteria* (Ogata et al., 1973). Together, the restricted antibiotic spectrum is consistent with the genetic studies demonstrating *de novo* biotin biosynthesis is essential in *Mycobacterium* sp., but not other eubacteria.

FUTURE DIRECTIONS

In this review we have highlighted key studies that demonstrate biotin biosynthesis is an important metabolic process in *Mycobacteria*. Why the bacilli have evolved such dependence upon their own synthesis, rather than scavenging from exogenous sources, is puzzling. This is especially so when one considers that biotin biosynthesis is an energetically expensive exercise requiring at least six enzymes and seven ATP equivalents to generate one biotin molecule (Abdel-Hamid and Cronan, 2007). It is possible that the quantity of bioavailable biotin present inside mammalian host cells where the bacilli reside is so scarce that *de novo* synthesis is a sound survival strategy. By applying genetics with modern research tools, such as proteomics and metabolomics, researchers can begin to dissect how metabolic enzymes and pathways are regulated in response to the niche microenvironments encountered inside host cells. In particular, this work will allow us to better understand the contribution of the biotin-dependent enzymes, pyruvate carboxylase and acyl CoA carboxylase, in metabolic adaptation. Additionally, the work highlighted in this review helps to establish the biotin biosynthesis pathway as a potential target for the development of new anti-TB agents. Especially important are studies that suggest this pathway is critical in the active growth phase as well as latency. Whilst the genetic validation studies are encouraging, the lack of pharmacological validation demands more work in this area. Towards this end, the recent availability of X-ray structures for BioA and BioD are likely to assist efforts in structure guided inhibitor design (Dey et al., 2010).

ACKNOWLEDGEMENTS

This work was supported by the National Health and Medical Research Council of Australia and the Australian Academy of Science International Linkage Scheme. WS was a recipient of the Royal Thai Government Scholarship.

ABBREVIATIONS

ACP, acyl carrier protein; AaaS, Acyl-ACP synthetase; AMTB, S-adenosyl-2-oxo-4-methylthiobutyric acid; DOA, 5′-deoxyadenosine; FAS, fatty acid biosynthesis pathway; SAH, S-adenosylhomocysteine; SAM, S-adenosyl-L-methionine

REFERENCES

Abdel-Hamid, A.M., and Cronan, J.E. (2007). In vivo resolution of conflicting in vitro results: synthesis of biotin from dethiobiotin does not require pyridoxal phosphate. Chem Biol 14, 1215–1220.

Ahmad, S. (2011). Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. Clin Dev Immunol 2011, 814943.

Arabolaza, A., Shillito, M.E., Lin, T.W., Diacovich, L., Melgar, M., Pham, H., Amick, D., Gramajo, H., and Tsai, S.C. (2010). Crystal structures and mutational analyses of acyl-CoA carboxylase beta subunit of Streptomyces coelicolor. Biochemistry 49, 7367–7376.

Baek, S.H., Li, A.H., and Sassetti, C.M. (2011). Metabolic regulation of mycobacterial growth and antibiotic sensitivity. PLoS Biol 9, e1001065.

Berkovitch, F., Nicolet, Y., Wan, J.T., Jarrett, J.T., and Drennan, C.L. (2004). Crystal structure of biotin synthase, an S-adenosylmethionine-dependent radical enzyme. Science 303, 76–79.

Chan, D.I., and Vogel, H.J. (2010). Current understanding of fatty acid biosynthesis and the acyl carrier protein. Biochem J 430, 1–19.

Cronan, J.E., and Lin, S. (2011). Synthesis of the α,ω-dicarboxylic acid precursor of biotin by the canonical fatty acid biosynthetic pathway. Curr Opin Chem Biol 15, 407–413.

Dey, S., Lane, J.M., Lee, R.E., Rubin, E.J., and Sacchettini, J.C. (2010). Structural characterization of the Mycobacterium tuberculosis biotin biosynthesis enzymes 7,8-diaminopelargonic acid synthase and dethiobiotin synthetase. Biochemistry 49, 6746–6760.

Dye, C., and Williams, B.G. (2010). The population dynamics and control of tuberculosis. Science 328, 856–861.

Eisenreich, W., Dandekar, T., Heesemann, J., and Goebel, W. (2010). Carbon metabolism of intracellular bacterial pathogens and possible links to virulence. Nat Rev Microbiol 8, 401–412.

Gago, G., Diacovich, L., Arabolaza, A., Tsai, S.C., and Gramajo, H. (2011). Fatty acid biosynthesis in actinomycetes. FEMS Microbiol Rev 35, 475–497.

Hebbeln, P., Rodionov, D.A., Alfandega, A., and Eitinger, T. (2007). Biotin uptake in prokaryotes by solute transporters with an optional ATP-binding cassette-containing module. Proc Natl Acad Sci U S A 104, 2909–2914.

Joshi, S.M., Pandey, A.K., Capite, N., Fortune, S.M., Rubin, E.J., and Sassetti, C.M. (2006). Characterization of mycobacterial virulence genes through genetic interaction mapping. Proc Natl Acad Sci U S A 103, 11760–11765.

Keer, J., Smeulders, M.J., Gray, K.M., and Williams, H.D. (2000).

Mutants of Mycobacterium smegmatis impaired in stationary-phase survival. Microbiology 146, 2209–2217.

Kitahara, T., Hotta, K., Yoshida, M., and Okami, Y. (1975). Biological studies of amiclenomycin. J Antibiot (Tokyo) 28, 215–221.

Koul, A., Arnoult, E., Lounis, N., Guillemont, J., and Andries, K. (2011). The challenge of new drug discovery for tuberculosis. Nature 469, 483–490.

Kwan, C.K., and Ernst, J.D. (2011). HIV and tuberculosis: a deadly human syndemic. Clin Microbiol Rev 24, 351–376.

Lawn, S.D., and Zumla, A.I. (2011). Tuberculosis. Lancet 378, 57–72.

Lin, S., Hanson, R.E., and Cronan, J.E. (2010). Biotin synthesis begins by hijacking the fatty acid synthetic pathway. Nat Chem Biol 6, 682–688.

Lu, H., and Tonge, P.J. (2008). Inhibitors of FabI, an enzyme drug target in the bacterial fatty acid biosynthesis pathway. Acc Chem Res 41, 11–20.

Mann, S., Colliandre, L., Labesse, G., and Ploux, O. (2009). Inhibition of 7,8-diaminopelargonic acid aminotransferase from Mycobacterium tuberculosis by chiral and achiral anologs of its substrate: biological implications. Biochimie 91, 826–834.

Mann, S., Marquet, A., and Ploux, O. (2005). Inhibition of 7,8-diaminopelargonic acid aminotransferase by amiclenomycin and analogues. Biochem Soc Trans 33, 802–805.

Minnikin, D.E., Kremer, L., Dover, L.G., and Besra, G.S. (2002). The methyl-branched fortifications of Mycobacterium tuberculosis. Chem Biol 9, 545–553.

Mock, D.M., and Malik, M.I. (1992). Distribution of biotin in human plasma: most of the biotin is not bound to protein. Am J Clin Nutr 56, 427–432.

Niederweis, M., Danilchanka, O., Huff, J., Hoffmann, C., and Engelhardt, H. (2010). Mycobacterial outer membranes: in search of proteins. Trends Microbiol 18, 109–116.

Ogata, K., Izumi, Y., and Tani, Y. (1973). The controlling action of actithiazic acid on the biosynthesis of biotin-vitamers by various microorganisms Agr. Biol Chem 37, 1079–1085.

Okami, Y., Kitahara, T., Hamada, M., Naganawa, H., and Kondo, S. (1974). Studies on a new amino acid antibiotic, amiclenomycin. J Antibiot (Tokyo) 27, 656–664.

Parsons, J.B., and Rock, C.O. (2011). Is bacterial fatty acid synthesis a valid target for antibacterial drug discovery? Curr Opin Microbiol Aug 20. [Epub ahead of print].

Rengarajan, J., Bloom, B.R., and Rubin, E.J. (2005). Genome-wide requirements for Mycobacterium tuberculosis adaptation and survival in macrophages. Proc Natl Acad Sci U S A 102, 8327–8332.

Rodionov, D.A., Hebbeln, P., Eudes, A., ter Beek, J., Rodionova, I.A., Erkens, G.B., Slotboom, D.J., Gelfand, M.S., Osterman, A.L., Hanson, A.D., *et al.* (2009). A novel class of modular transporters for vitamins in prokaryotes. J Bacteriol 191, 42–51.

Rodionov, D.A., Mironov, A.A., and Gelfand, M.S. (2002). Conservation of the biotin regulon and the BirA regulatory signal in Eubacteria and Archaea. Genome Res 12, 1507–1516.

Russell, D.G. (2001). Mycobacterium tuberculosis: here today, and here tomorrow. Nat Rev Mol Cell Biol 2, 569–577.

Russell, D.G., Barry, C.E. 3rd, and Flynn, J.L. (2010). Tuberculosis: what we don't know can, and does, hurt us. Science 328, 852–856.

Said, H.M. (2009). Cell and molecular aspects of human intestinal biotin absorption. J Nutr 139, 158–162.

Sandmark, J., Mann, S., Marquet, A., and Schneider, G. (2002). Structural basis for the inhibition of the biosynthesis of biotin by the antibiotic amiclenomycin. J Biol Chem 277, 43352–43358.

Sassetti, C.M., Boyd, D.H., and Rubin, E.J. (2001). Comprehensive identification of conditionally essential genes in mycobacteria. Proc Natl Acad Sci U S A 98, 12712–12717.

Sassetti, C.M., Boyd, D.H., and Rubin, E.J. (2003). Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol 48, 77–84.

Sassetti, C.M., and Rubin, E.J. (2003). Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci U S A 100, 12989–12994.

Seki, M. (2006). Biological significance and development of practical synthesis of biotin. Med Res Rev 26, 434–482.

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.

Wright, H.T., and Reynolds, K.A. (2007). Antibacterial targets in fatty acid biosynthesis. Curr Opin Microbiol 10, 447–453.

Yu, J., Niu, C., Wang, D., Li, M., Teo, W., Sun, G., Wang, J., Liu, J., and Gao, Q. (2011). MMAR_2770, a new enzyme involved in biotin biosynthesis, is essential for the growth of Mycobacterium marinum in macrophages and zebrafish. Microbes Infect 13, 33–41.

