Metabolomics of Central Carbon Metabolism in Mycobacterium tuberculosis

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ABSTRACT Metabolism is a biochemical activity of all cells, thought to fuel the physiologic needs of a given cell in a quantitative, rather than qualitatively specific, manner. Mycobacterium tuberculosis is a chronic facultative intracellular pathogen that resides in humans as its only known host and reservoir. Within humans, M. tuberculosis resides chiefly in the macrophage phagosome, the cell type and compartment most committed to its eradication. M. tuberculosis thus occupies the majority of its decades-long life cycle in a state of slowed or arrested replication. At the same time, M. tuberculosis has evolved its metabolic network to both maintain and propagate its survival as a species within a single host. Knowledge of the specific ways in which its metabolic network serves these distinct though interdependent functions, however, remains highly incomplete. In this article we review existing knowledge of M. tuberculosis’s central carbon metabolism as reported by studies of its basic genetic and biochemical composition, regulation, and organization, with the hope that such knowledge will inform our understanding of M. tuberculosis’s ability to traverse the stringent and heterogeneous niches encountered in the host.

Central carbon metabolism (CCM)—defined as the enzymatic transformation of carbon through glycolysis, the pentose phosphate pathway (PPP), the citric acid cycle, the glyoxylate shunt, the methylcitrate cycle, and gluconeogenesis—is a core feature of all cells that is used to provide energy, in the form of reducing equivalents and ATP, and essential biosynthetic precursors (Fig. 1). Remarkably, the same metabolic enzymes found in bacteria are also present in mammals, suggesting that the pathways of CCM have been fundamentally conserved. Intensive study has thus focused on the metabolic network of Escherichia coli as a model system with which to understand its basic principles. However, cells vary in their specific metabolic needs according to the selective pressures they encounter. Accordingly, growing evidence has established that metabolic enzymes are often capable of operating in a diverse array of configurations (1).

For organisms residing within polymicrobial ecosystems, it has generally been assumed that the objective goal of CCM is to meet the stoichiometric requirements needed to sustain maximal rates of replication with maximal efficiency. However, Mycobacterium tuberculosis is both microbially and ecologically unique. M. tuberculosis is a chronic intracellular pathogen that resides in humans as its only known host and reservoir, and within humans, the macrophage phagosome as its chief niche (2). M. tuberculosis has thus evolved, apart from other microbes, within an ultra-narrow ecological niche corresponding to the cell type and compartment most committed to its eradication. Within its host niche,
M. tuberculosis encounters diverse host-imposed stringencies, many of which are capable of inducing exit from the cell cycle. M. tuberculosis thus occupies the majority of its natural life cycle in a state of non- or slowly replicating physiology. Relieved of the requirement to double biomass, non- or slowly replicating M. tuberculosis has generally been perceived to have minimal metabolic activity. However, even nonreplicating bacilli face the challenge of preserving the integrity of essential cellular components and maintaining the ability to reenter the cell cycle to ensure transmission to a new host. Yet its physiology has been most extensively studied when replicating at maximal growth rates. Knowledge of the specific metabolic

FIGURE 1  Schematic representation of the CCM network of M. tuberculosis based on bioinformatic reconstruction and published literature. Reversible steps of glycolysis/gluconeogenesis are shown in purple, dedicated steps of glycolysis are shown in light blue, dedicated steps of gluconeogenesis are shown in pink, the PPP is shown in red, the TCA cycle is shown in green, the glyoxylate shunt is shown in orange, and the methylcitrate cycle is shown in dark blue. The dotted green line indicates a standing question of connectivity in the TCA cycle via ketoglutarate ferredoxin oxidoreductase. Common enzyme names are shown next to the reactions they catalyze. Gene symbols are shown in parentheses. doi:10.1128/microbiolspec.MGM2-0026-2013.f1
requirements and pathways used by both replicating and nonreplicating *M. tuberculosis* subpopulations thus represents major areas of unmet scientific need.

Like most bacteria, *M. tuberculosis* is capable of utilizing an array of organic substrates, including select carbohydrates, lipids, amino acids, and simple organic acids, to populate its CCM. Decades of studies in the optimization of batch culture conditions for *in vitro* growth of *M. tuberculosis* revealed that glycerol supports maximal growth rates and yields (3). In contrast, studies with *ex vivo* bacilli recovered from the lungs of infected mice showed that lipids appeared to function as *M. tuberculosis*’s preferred respiratory substrate (4). Multiple lines of evidence from *in vivo* studies, including gene expression analyses, as well as infection studies using defined mutant strains have further reinforced essential roles for lipid metabolism in both *M. tuberculosis* pathogenesis and persistence (1). Based on such studies, lipids such as fatty acids and cholesterol have come to be viewed as the predominant carbon and energy source for *M. tuberculosis* throughout infection, discounting a role for carbohydrates. Yet growing evidence has implicated an essential role for carbohydrate uptake and metabolism in the chronic phase of infection (5, 6).

In this article we review existing knowledge of *M. tuberculosis*’s CCM as reported by studies of its basic genetic and biochemical composition, regulation, and organization. This work is meant to build off a previous synthesis of the literature reported by Wheeler and Blanchard (7) and two recent minireviews by Rhee et al. (1) and Ehrt and Rhee (8) and thus focuses on complementing, rather than duplicating, information contained therein.

**CARBOHYDRATE METABOLISM**

Early work reported that *M. tuberculosis* was capable of utilizing both carbohydrate- and alcohol-based substrates during *in vitro* culture. However, in a systematic study of 24 substrates, Youmans and Youmans found that only glycerol, glucose, trehalose, and maltose (which may have contained trace amounts of trehalose, which is commonly used as a cryoprotectant) were capable of supporting its growth when provided as the primary carbon source in a chemically defined, basal medium (9). Gamble and Herrick further showed that the extent of glucose utilization by *M. tuberculosis* roughly paralleled its growth, while Loebel reported that metabolism of only glucose or glycerol was accompanied by increases in respiratory activity (10, 11). Youmans also reported the ability of gluconate, pyruvate, and lactate to support *in vitro* growth of *M. tuberculosis*, while Weinzirl and Knapton found that metabolism of only glycerol, but not glucose, lactose, or mannitol, led to acidification of the medium (12, 13). Together, these findings provided the first evidence for both an intact glycolytic pathway in *M. tuberculosis* and its coupling to an oxidative respiratory chain.

Work by Goldman, Murthy, and others subsequently adduced biochemical evidence for the presence of enzymes of the Embden-Meyerhof-Parnas (EMP) pathway and PPP in lysates of *M. tuberculosis* H37Rv (14, 15). Radiorespirometric studies using *M. tuberculosis* incubated in phosphate-buffered saline containing 14C-labeled glucose labeled at the 1, 2, 3-4, and 6 positions further showed the preferential recovery of respired 14CO2 from glucose labeled at the 3-4 positions, associated with an estimated ~94% metabolism through the EMP pathway that was accompanied by the detection of hexokinase, phosphofructokinase, fructose bisphosphate aldolase, and pyruvate kinase activities (16). The remaining 6% was attributed to metabolism through the PPP, owing to detection of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and absence of 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase activities associated with the Entner-Doudoroff pathway. Of interest, Youmans reported that while gluconate could support *M. tuberculosis* growth in a minimal Proskauer-Beck medium, pentose sugars could not, raising the possibility of a functional, though as yet unsubstantiated, Entner-Doudoroff pathway (9, 13). Analogous studies revealed the presence of robust 6-phosphogluconate dehydratase and KDPG aldolase activities in the lysates of the saprophytic species *Mycobacterium smegmatis*, albeit only when grown on glucose (17).

Completion of the first *M. tuberculosis* genome sequence, coupled to homology-based sequence analysis, later provided an *in silico* inventory of *M. tuberculosis* CCM enzymes (18). This approach predicted the presence of candidates for all enzymes of both the EMP pathway and PPP, but also revealed unexpected apparent redundancies for some enzymes (such as its hexokinases, phosphofructokinases, etc.) while orphaning other previously detected activities (such as an alternative type I fructose bisphosphate aldolase).

Below, we provide a synthesis of the published literature concerning the specific enzymes of the *M. tuberculosis* glycolytic, gluconeogenic, and pentose phosphate pathways.
EMP PATHWAY AND GLUCONEOGENIC COUNTERPARTS

Glucokinase (E.C. 2.7.1.2) catalyzes the first committed step in glucose metabolism, the production of glucose-6-phosphate, an intermediate shared by both the EMP pathway and the PPP. The *M. tuberculosis* genome encodes two functional glucokinases: PPGK (*Rv2702* (18) and GLKA (*Rv0650*) (6)). Biochemical studies showed that PPGK catalyzes the direct phosphorylation of glucose using a broad range of phosphoryl donors with a preference for polyphosphate (PolyP) and, unlike most glucokinases, has a high affinity for its substrate, glucose (\(K_m = 0.28\) mM) (19, 20). glkA(*Rv0650*) encodes an annotated sugar kinase with only 22% amino acid identity to PPGK but 71% identity to a homolog from *M. smegmatis* which was shown to phosphorylate glucose (\(K_m = 9\) mM) using ATP as the phosphoryl donor (21). Direct biochemical characterization of the *M. tuberculosis* enzyme, however, remains lacking. Genetic studies showed that targeted deletion of both PPGK and GLKA selectively abolished *M. tuberculosis*’s ability to grow and metabolize glucose, but not acetate, in a manner that could be restored by expression of either ortholog, albeit at different levels of expression. PPGK/GLKA-deficient *M. tuberculosis* strains were further found to exhibit a 1 to 2 log\(_{10}\) defect in bacterial burden during the chronic phase of infection in a mouse model of tuberculosis (TB) that could be attributed to neither a defect in the metabolism of trehalose nor enhanced susceptibility to nutrient starvation, glucose intoxication, or oxidative stress (6). Together, these data indicate that *M. tuberculosis* expresses two functional glucokinases whose activities are jointly required for *in vitro* growth and metabolism in glucose-containing media and *in vivo* persistence in a mouse model of TB, but whose specific physiologic roles in glycolysis remain to be resolved.

Glucose-6-phosphate isomerase (PGI; E.C. 5.3.1.9) catalyzes the reversible conversion of glucose-6-phosphate into fructose-6-phosphate, the second step of the EMP, which is required for stabilization of the carbamion required for the action of fructose bisphosphate aldolase (discussed below) and is encoded by *pgi* (*Rv0946c*). *In vitro* studies of purified recombinant *M. tuberculosis* PGI have confirmed its activity as a metal-independent PGI with kinetic parameters similar to those reported for other bacterial and eukaryotic PGIs (\(K_m = 0.318\) mM for fructose-6-phosphate; \(K_\text{i} = 0.8\) mM for 6-phosphogluconate) (22). Insertional transposon mutagenesis studies have provided genetic evidence that PGI is essential for optimal growth *in vitro* (23, 24).

In addition to PGI, *M. tuberculosis* also encodes a phosphoglomutase (*pgm*; E.C. 5.4.2.2; *pgmA* (*Rv3068c*)), which can catalyze the reversible transfer of a phosphoryl group from the 1’ to the 6’ position on a glucose monomer (25). Glucose-1-phosphate is an intermediate of glycogen metabolism produced by the action of glycogen phosphorylase. PGM can thus generate glucose-6-phosphate as either a substrate or product for glycolytic or gluconeogenic metabolism, respectively. Insertional transposon mutagenesis data indicate that PGM is dispensable for optimal *in vitro* growth (23, 24).

Phosphofructokinase (PFK; E.C. 2.7.1.11) catalyzes the ATP-dependent phosphorylation of fructose-6-phosphate to produce fructose-1,6-bisphosphate, the first committed step of the EMP pathway, and is predicted to be encoded by two paralogous genes, *pfkA* (*Rv3010c*) and *pfkB* (*Rv2029c*), neither of which is predicted to be essential for *in vitro* and *in vivo* growth (5, 23, 24). Although both PFKA and PFKB are annotated as phosphofructokinases, each bears little sequence similarity to the other and belongs to a separate protein family, corresponding to the canonical PFK protein family and ribokinase superfamily, respectively. *In vitro* studies using hexahistidine-tagged recombinant forms of *M. tuberculosis* PFKA and PFKB confirmed that both were capable of phosphorylating fructose-6-phosphate, with PFKA exhibiting nearly 15 times as much activity as PFKB. Unlike the case for *E. coli* (where PFKB accounts for ∼10% of total PFK activity), however, a genetically engineered *M. tuberculosis* PFKA deletion mutant was found to be unable to grow on glucose *in vitro* and to lack detectable PFK activity, while a PFKB deletion mutant exhibited no apparent defect in growth on glucose with wild-type levels of PFK activity in lysates. Moreover, neither the growth nor enzymatic defects observed in the PFKA mutant could be rescued by confirmed expression of *pfkB* under the control of a constitutive strong promoter. A PFK-deficient *E. coli* mutant could conversely be complemented only when expressing *M. tuberculosis* PFKA and not PFKB.

Together, these findings establish that *M. tuberculosis* PFKA is both necessary and sufficient to support *in vitro* growth and metabolism of glucose through the EMP pathway. PFKA-deficient *M. tuberculosis* was also found to exhibit no defect in its ability to establish or maintain a chronic infection in mice, suggesting that either *M. tuberculosis* encodes a cryptic or compensatory PFK activity triggered by factors specifically encountered in the host, or that glycolysis is dispensable *in vivo* (26). Of note, *M. tuberculosis* PFKB shares 40% identity with...
E. coli PFKB, including the conserved catalytic motif GXGD that is indicative of ribokinase superfamily members that have been reported to phosphorylate a variety of substrates besides fructose-6-phosphate, such as fructose-1-phosphate in E. coli (27, 28) and tagatose-6-phosphate in both S. aureus (29) and E. coli, albeit with a lower efficacy than fructose-6-phosphate (30). It is thus plausible that M. tuberculosis PFKB may also phosphorylate sugar-based substrates other than fructose-6-phosphate. More extensive characterization of the kinetic properties and substrate specificities of M. tuberculosis’s PFKs and PFKA/PFKB-deficient M. tuberculosis thus await further investigation.

Fructose-1,6-bisphosphatase (E.C. 3.1.3.11) is a key enzyme of gluconeogenesis that catalyzes the dephosphorylation of fructose-1,6-bisphosphate (FBP) to fructose-6-phosphate. The M. tuberculosis genome is annotated to encode a single type II, metal-ion-dependent FBPase, GlpX (Rv1099c), whose structure and activity have been characterized in purified recombinant form, with kinetic parameters similar to those of other type II FBPases (31, 32). Though not essential for in vitro growth on glycolytic carbon sources, insertion transposon mutagenesis studies have implicated glpX as being essential for in vivo growth in a mouse model of tuberculosis (5, 23, 24).

FBP aldolase (E.C. 4.1.2.13) catalyzes the reversible aldol cleavage of FBP and condensation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) in glycolysis and gluconeogenesis, respectively. Though associated with structurally a conserved (βα)8 barrel fold, FBP aldolases are divided into two groups according to reaction mechanism (33). Class I FBP aldolases, found in both higher eukaryotes and bacteria, form a Schiff-base intermediate between the carbonyl substrate (FBP or DHAP) and an active site lysine residue (34). Class II FBP aldolases, by contrast, are absent from mammals and require a divalent metal ion to polarize the carbonyl group of the substrate (FBP or DHAP) and to stabilize the carbamion intermediate during catalysis (35–37). Early biochemical studies by Venkitasubramanian and colleagues reported that M. tuberculosis encoded both class I and class II FBP aldolases, as distinguished by their sensitivity to borohydride inactivation and EDTA, respectively. The former activity was detected only in well-oxygenated, fermentor-grown cultures, while the latter was primarily associated with hypoxic conditions (38–40). Unfortunately, homology-based sequence analyses identified only a single paralog, corresponding to a class I FBP aldolase encoded by fba (Rv0363c). Both transposon mutagenesis data and unsuccessful gene deletion efforts in the attenuated M. tuberculosis strain H37Ra have suggested that fba is essential for in vitro growth. Biochemical and structural studies have confirmed its Zn cofactor and tetrameric (dimer of dimers) structure with an apparent turnover number (21 s−1 at 28°C) higher than that reported for other class II FBP aldolases, such as from E. coli (10.5 s−1 at 30°C) (37), and apparent K_m (20 μM) approximately 10-fold lower than most FBP aldolases (33, 37, 41), resulting in one of the highest catalytic efficiencies (k_cat/K_m) for FBP cleavage. Efforts to develop cell-permeable, high-affinity inhibitors as potential novel TB drugs are thus under way.

Triose phosphate isomerase (TPI; E.C. 5.3.1.1) catalyzes the essential interconversion of DHAP and glyceraldehyde-3-phosphate and is one of the best-studied and catalytically most proficient enzymes ever characterized (42). In glycolysis, TPI channels these two products for the formation of pyruvate, while in gluconeogenesis, TPI ensures that both substrates are supplied in equimolar amounts to FBP aldolase. TPI is encoded by tpiA (Rv1438) and has been both structurally and kinetically characterized with features similar to those of other prototypic TPIs (43, 44).

In addition to its canonical roles in glycolysis and gluconeogenesis, TPI also serves an essential role in glycerol assimilation and/or glycerophospholipid metabolism. Early studies by Goldman demonstrated that cell-free extracts of M. tuberculosis H37Ra contained both glycerol dehydrogenase (E.C. 1.1.1.6) and dihydroxyacetone kinase (E.C. 2.7.1.29) activities and thus enable M. tuberculosis to assimilate glycerol into glycolysis and/or gluconeogenesis through its conversion to DHAP (45). Unfortunately, the genes encoding these orphan activities remain to be identified. Subsequent biochemical and genetic work provided additional evidence for the presence of a parallel pathway, consisting of glycerol kinase (E.C. 2.7.1.30; encoded by glpK [Rv3696c]) and both NAD(P)⁺ and quinol-dependent glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.114, 1.1.5.3; putatively encoded by gpdA1 [Rv0564c] and gpdA2 [Rv2982c] and by gldD1 [Rv2249c] and gldD2 [Rv3302c], respectively) orthologs, also capable of assimilating glycerol into glycolysis and/or gluconeogenesis through the production of DHAP. Not surprisingly, transposon mutagenesis data indicate that none of these genes is essential in isolation, though biochemical studies of these enzymes remain lacking (23, 24).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; E.C. 1.2.1.12) catalyzes the conversion of glyceraldehyde-3-phosphate to D-glycerate-1,3-bisphosphate. GAPDH is
encoded by a close sequence ortholog to other GAPDHs, gap (Rv1436), which is predicted to be essential by inser-
tional transposon mutagenesis studies and genomically
clustered with its TPI and phosphoglycerate kinase.

Unfortunately, specific biochemical data relating to M.
tuberculosis’s GAPDH and lower glycolytic enzymes—
including phosphoglycerokinase (E.C. 2.7.2.3; pgk
[Rv1437]), phosphoglyceromutase (E.C. 5.4.2.1; gpm1
[Rv0489, Rv3837c, Rv2419c]), enolase (E.C. 4.2.1.11;
eno [Rv1023]), pyruvate kinase (E.C. 2.7.1.40; pykA
[Rv1617]), and pyruvate phosphate dikinase (E.C.
2.7.9.1; ppdK [Rv1127c])—remain nearly absent. Not-
withstanding, M. tuberculosis appears to encode an in-
tact EMP pathway.

The EMP pathway is among the most conserved and
extensively studied metabolic pathways in all of biology.
Its main functions are to serve as a source of key
biosynthetic precursors and, in the absence of respira-

tory chain activity, furnish cellular energy in the form of
ATP. However, fundamental questions concerning its
physiologic role and regulation remain. For example,
unlike other bacteria, M. tuberculosis is not subject to
classical catabolite repression and is instead capable of
cocatabolizing multiple carbon sources (46). Moreover,
isotopic labeling studies specifically showed that M. tu-
brerculosis is capable of simultaneously metabolizing
glucose and acetate through the segregated operation of
its glycolytic and gluconeogenic pathways, a property
potentially well adapted to the nutrient-poor conditions
associated with the macrophage phagosome (1). How-
ever, a key question that arises is just what specific
functions and precursors M. tuberculosis’s EMP has
evolved to optimize production of? For organisms such
as M. tuberculosis that occupy nutrient- and energy-
poor niches and face little competition for resources, it is
possible that their EMP pathway may have evolved to
generate ATP at a higher yield at the expense of a lower
metabolic rate using biochemical, rather than genetic,
regulatory mechanisms.

THE PPP

Genomic analysis of all sequenced M. tuberculosis strains
has revealed a fundamental conservation of orthologs for
all genes of both the oxidative and nonoxidative branches.
The canonical PPP metabolizes glucose to produce re-
ducing equivalents (in the form of NADPH), nucleotide
precursors (in the form of pentoses), and triose inter-
mediates to fuel lower glycolysis, depending on the
needs of the cell. The pathway itself consists of two
different phases. One is an irreversible oxidative phase
in which glucose-6-phosphate is converted to ribulose-
5-phosphate by oxidative decarboxylation to generate
NADPH and/or intermediates of the Entner-Doudoroff
pathway discussed above. The other is a reversible
nonoxidative phase in which phosphorylated sugars are
interconverted to generate xylulose-5-phosphate,
ribulose-5-phosphate, and ribose-5-phosphate, from
which intermediates of lower glycolysis or phosphoribosyl
pyrophosphate (PRPP), a key cofactor required for syn-
thesis of histidine and purine/pyrimidine nucleotides,
are formed.

In contrast to the EMP pathway, considerably less is
known about the biochemical activities and metabolic
roles of M. tuberculosis’s PPP enzymes. Apart from in-
sertional transposon mutagenesis data (which suggest
that only its transketolase and transaldolase are essen-
tial), biochemical knowledge of its enzymes is specifi-
cally limited to its glucose-6-phosphate dehydrogenase,
ribose-5-phosphate isomerase, and transketolase, which
are reviewed below (23, 24).

Glucose-6-phosphate dehydrogenase (G6PD; E.C.
1.1.1.49) converts glucose-6-phosphate into 6-phos-
phonoglucono-δ-lactone and is the rate-limiting enzyme
of the PPP. Unlike most bacteria, including those
containing the unusual flavin F420 cofactor, all myco-
bacterial species, including M. tuberculosis, encode
two paralogous G6PD activities: a canonical NADP-
dependent glucose-6-phosphate dehydrogenase (47, 48),
which is found in most organisms ranging from bacteria
to yeasts to humans (and in M. tuberculosis is encoded by
two orthologs, zwf1 [Rv1121] and zwf2 [Rv1447c]),
and an evolutionarily distinct, F420-dependent G6PD
(which in M. tuberculosis also consists of two orthologs
coded by fgd1 [Rv0407] and fgd2 [Rv0132c]) (48).
Enzymatic assays using cell-free lysates suggested that
the NADP and F420-dependent activities were similar,
leaving their specific roles and apparent redundancy
unresolved (49).

Ribose-5-phosphate isomerase (E.C. 5.3.1.6) cata-
ylizes the reversible isomerization of ribulose-5-phos-
phate to δ-ribose-5-phosphate (50) and is encoded by
two nonhomologous genes, rpiA and rpiB. The rpiA type
is most common and is found in all three domains of life.
rpiBs, in contrast, have so far been restricted to the
genomes of some bacteria and protozoa. M. tuberculosis
encodes a single RpiB (Rv2465) that was crystallized and
shown to catalyze the isomerization of ribulose-5-phos-
phate and δ-ribose-5-phosphate with an efficiency very
similar to that of the E. coli RpiB (50). Somewhat sur-
prisingly, however, M. tuberculosis’s RpiB was not
predicted to be essential for in vitro growth (23, 24).
Transketolase (TKT; E.C. 2.2.1.1) is encoded by tkt (Rv1449) and catalyzes the conversion of sedoheptulose-7-phosphate to d-ribose-5-phosphate. In vitro studies of purified recombinant M. tuberculosis TKT showed that, despite significant sequence differences from the TKTs of yeast, E. coli, maize, and spinach, M. tuberculosis TKT exhibits very similar kinetic constants and structurally consists of the same overall fold and domain topology as other TKTs (51). Compared to human TKT, M. tuberculosis TBTKT was also found to exhibit a broad substrate specificity for a range of phosphorylated sugars (52). Given that the apparent nonessentiality of its ribose-5-phosphate isomerase (24), TKT may play a key role in linking the nonoxidative part of the PPP to biosynthesis of pentose sugars.

Like its EMP, the M. tuberculosis PPP remains almost wholly undescribed. Key questions pertain to both the operation and regulation of its oxidative and nonoxidative branches as well as the differential biological roles of NADP and F420-dependent G6PD.

METABOLISM OF SHORT-CHAIN CARBOXYLIC ACIDS

Short-chain carboxylic acid metabolism, including pyruvate metabolism, the tricarboxylic acid (TCA) cycle, and its variants, serves as a hub for CCM, because it integrates glycolysis, β-oxidation, gluconeogenesis, energy metabolism, and many biosynthetic pathways. Early studies of carbon source utilization in M. tuberculosis indicated that cultured bacilli are capable of driving the near-complete respiratory oxidation of glucose and glycerol to CO₂ (53). Evidence of a conserved CCM network followed with several reports of glycolytic (14, 45), pyruvate dehydrogenase (PDH) (54), and TCA cycle (55–60) activities from crude and partially purified cellular extracts. Studies by Edson et al. regarding central carbon flux in Mycobacterium butyricum demonstrated that ¹⁴C-labeled glycerol could be converted to α-ketoglutarate through pyruvate as an intermediate (61), solidifying a model for canonical oxidative connectivity between glycolysis and the TCA cycle in mycobacteria.

PDH COMPLEX

PDH, a ternary complex consisting of a thiamine pyrophosphate-dependent acetyl-transferring PDH (E1 subunit, E.C. 1.2.4.1), a dihydrolipoamide-residue acetyltransferase (E2 subunit, E.C. 2.3.1.12), and a flavin adenine dinucleotide-dependent dihydrolipoyl dehydrogenase (E3 subunit, E.C. 1.8.1.4), catalyzes the unidirectional oxidative decarboxylation of pyruvate to acetyl coenzyme A (CoA) with the reduction of NAD⁺ to NADH. Preliminary annotation of the M. tuberculosis H37Rv genome sequence predicted that the E1 component of PDH was redundantly encoded by Rv2241 and Rv2496c/Rv2497c, the E2 component by Rv2495c, and the E3 component by Rv0462, Rv0794c, and/or Rv3303c (18).

Biochemical assessment of purified recombinant PDH E3 candidate Rv0462 (Lpd) revealed that this protein is a multifunctional dihydrolipoamide:NAD⁺ oxidoreductase for the M. tuberculosis PDH complex, as well as for branched-chain keto acid dehydrogenase (BCKAD) and peroxynitrite reductase/peroxidase (PNR/P) complexes (62–65). This unique multifunctionality links CCM with oxidative stress defense in M. tuberculosis. Consistent with its dual role in pyruvate and branched-chain keto acid metabolism, deletion of Rv0462 was found to preclude growth of M. tuberculosis on carbohydrates and branched-chain amino acids (65). Moreover, owing to the essential role of Rv0462 in PNR/P activity, this mutant strain demonstrated hypersensitivity to oxidative stressors such as acidified nitrite and H₂O₂ (65). This compound phenotype also resulted in dramatic attenuation in a murine model of infection (65). Unlike that observed for Rv0462, enzymatic studies of purified recombinant Rv0794c and Rv3303c revealed that these E3 paralogs were nonfunctional in lipoylamine reduction (62). However, as an indication of their dispensability in CCM, high-throughput mutational analysis of M. tuberculosis H37Rv has indicated that Rv0794c and Rv3303c are nonessential for normal growth in laboratory culture (24).

Assessment of the biochemical activity of purified recombinant PDH E2 candidate Rv2495c and α-ketoglutarate dehydrogenase (KDH) E2 candidate Rv2215 (DlaT) demonstrated that the latter is indeed the E2 component of both PDH (64) and of PNR/P (63), whereas the former is the E2 component of BCKAD (65). Like Rv0462, Rv2215 has been shown to be critical for optimal growth of M. tuberculosis in vitro (24) and for growth and survival in murine bone marrow–derived macrophages, mice, and guinea pigs (23, 65–67). Based on its role in CCM and oxidative stress defense, Rv2215 has been the target of small molecule screening efforts that have revealed the selective activity of a set of drug-like compounds, rhodanines, that synergize with host immune effector functions to kill nonreplicating M. tuberculosis (67).
Enzymatic studies using a partially purified protein from \textit{M. tuberculosis} H37Rv, and purified recombinant protein, have established Rv2241 (AceE) as the sole E1 component of PDH, consistent with its initial annotation \cite{64}. In contrast, the Rv2496c/Rv2497c complex has been shown to function as a component of BCKAD, rather than PDH \cite{65}. Like the other subunits of PDH, Rv2241 is essential for normal growth in standard laboratory medium \cite{24}, but, unlike the E2 and E3 components, is dispensable for growth and survival of \textit{M. tuberculosis} in a murine model of infection \cite{5}. These data demonstrate that formation of acetyl-CoA from pyruvate may not be critical for \textit{M. tuberculosis} pathogenesis, consistent with the observation that virulent \textit{Mycobacterium bovis} lacks connectivity between glycolysis and pyruvate metabolism due to an inactive pyruvate kinase (PykA) \cite{68, 69}.

**TCA CYCLE**

In many aerobic organisms, a large proportion of acetyl-CoA generated from PDH enters the TCA cycle and is further oxidized to generate reducing equivalents, typically in the form of NAD(P)H and FADH$_2$, with the concurrent production of CO$_2$. These reducing equivalents can then participate in various oxidoreductive processes throughout central metabolism. In the presence of a suitable terminal electron acceptor, respiratory reoxidation of these reducing equivalents can be used in the generation of an electrochemical gradient across the cytoplasmic membrane. As described in reference 112, the potential energy of this gradient can be tapped into for the synthesis of ATP and for other processes that are driven by chemiosmosis. Beyond this role in providing an abundant source of reducing power, the TCA cycle is also critical for providing essential substrates for biosynthesis of many amino acids, cofactors, and nucleotides. This biosynthetic role for the TCA cycle mandates input of additional four-carbon units for sustained flux, a role that is often satisfied by pyruvate carboxylase, which catalyzes the ATP-dependent carboxylation of pyruvate to oxaloacetate.

While orthologs for TCA cycle enzymes are represented throughout the three domains of life, it is becoming increasingly apparent that the utility, structure, and enzymatic components of this central metabolic pathway can vary considerably from organism to organism. While the identities of enzymes of the TCA cycle and linked pathways, such as the glyoxylate shunt, have been inferred from the complete genome sequence of \textit{M. tuberculosis} \cite{18}, functional roles for many of these paralogs have yet to be established. Indeed, despite evidence for oxidative decarboxylating enzymes of the TCA cycle, it is currently unclear whether there is significant carbon flow from isocitrate to succinyl-CoA via this pathway under physiological conditions. Further, until recently, it was unclear whether the glyoxylate shunt played an ancillary role to the TCA cycle or whether flux through the glyoxylate shunt represented the majority of traffic through the cycle. Given that the true enzymatic function, mechanisms of regulation, and directionality of metabolic flux cannot be reliably determined by homology-based modeling, the physiologic roles of most steps in the \textit{M. tuberculosis} TCA cycle and related pathways await confirmation by functional analysis.

**Citrate Synthase**

Citrate synthase (E.C. 2.3.3.1) serves as the major entry point for carbon into the TCA cycle by catalyzing the sequential aldol condensation of oxaloacetate with acetyl-CoA to form citryl-CoA and hydrolysis to form citrate and free CoA. Ochoa et al. were the first to describe the presence of citrate synthase activity in cellular extracts of \textit{M. tuberculosis} \cite{55}. Homology searches have indicated that \textit{M. tuberculosis} encodes two paralogs of citrate synthase: \textit{citA} and \textit{gltA2} (Rv0889c and Rv0896). Based on its apparent essentiality for growth \textit{in vitro}, \textit{gltA2} is thought to encode the major citrate synthase of \textit{M. tuberculosis} central metabolism \cite{23}. However, to date, biochemical characterization of the GltA2 protein and assessment of its specific role in \textit{M. tuberculosis} physiology has yet to be reported. In contrast to \textit{gltA2}, \textit{citA} appears to be nonessential \cite{23, 24}; thus, it is unclear whether this gene encodes a functional citrate synthase and whether the corresponding gene product plays a role in CCM.

In addition to citrate synthase, \textit{M. tuberculosis} also encodes a paralog of the β-subunit of citrate lyase (E.C. 4.1.3.6, encoded by \textit{citE} or Rv2498c) that is predicted to catalyze the reverse reaction of citryl-CoA to oxaloacetate and acetyl-CoA. While CitE appears to be important for growth of \textit{M. tuberculosis} within macrophages \cite{23}, its functional role in CCM has yet to be determined. Based on the proximity and positive correlation of gene expression of \textit{citE} with genes for a putative acyl-CoA transferase (Rv2503c and Rv2504c), acyl-CoA carboxylase (Rv2501c and Rv2502c), and acyl-CoA dehydrogenase (Rv2500c), it is highly anticipated that the function of \textit{M. tuberculosis} CitE is linked to fatty acid synthesis through the formation of some species of acyl-CoA \cite{70}. Because \textit{M. tuberculosis} does not appear to
utilize exogenous citrate (71, 72), a source for citrate to support such a pathway remains unclear. In some anaerobic microbes, such as Chlorobium tepidum, citrate lyase is associated with operation of a reductive TCA cycle that enables CO₂ assimilation and balancing of the cellular reduction/oxidation potential (73). While evidence for a full reductive TCA cycle in M. tuberculosis has not been reported, it is intriguing that the citE gene cluster is induced under anaerobic conditions (74–77).

Aconitase
Following citrate synthesis, the next step in the TCA cycle is the sequential dehydration of citrate to cis-aconitate and rehydration to isocitrate, which is catalyzed by the iron-sulfur cluster protein aconitase (E.C. 4.2.1.3). High-throughput gene essentiality screens indicate that acn (Rv1475c) is essential for growth of M. tuberculosis in culture (23, 24). Sequence analysis and biochemical characterization of purified recombinant M. tuberculosis aconitase indicates that this enzyme has the typical features of a dual function bacterial aconitase A/iron-responsive protein (AcnA/IRP) family member (78). Like other bacterial AcnA/IRPs, the M. tuberculosis protein contains a labile 4Fe-4S cluster that is essential for catalyzing interconversion of citrate and isocitrate. When this cluster is impaired due to oxidative damage or iron deficiency, the protein adopts the ability to bind RNA hairpins containing a sequence-specific loop of C-A-G-C/U-G. Gel shift assays have demonstrated that target RNAs for the M. tuberculosis apo-aconitase include messages for human ferritin, M. tuberculosis thioredoxin, and the iron regulator encoded by ideR. Based on this IRP activity, it is likely that M. tuberculosis apo-aconitase plays a role in the response to iron limitation and/or oxidative stress, yet assessment of this role awaits further analysis.

Isocitrate Dehydrogenase
Once formed, isocitrate can continue through the TCA cycle via oxidative decarboxylation to α-ketoglutarate catalyzed by isocitrate dehydrogenase (ICD; E.C. 1.1.1.42). M. tuberculosis encodes two orthologs of isocitrate dehydrogenase, ICD-2 (encoded by Rv0066c) and ICD-1 (encoded by Rv3339c). Recombinant proteins of each ortholog have been expressed, purified, and biochemically characterized as exclusively NADP⁺-dependent enzymes (79–81). The precise reaction sequence has been elaborated for ICD-1, in which NADP⁺ binds, followed by binding, oxidation, and decarboxylation of isocitrate and sequential release of CO₂, α-ketoglutarate, and NADPH. While deletions of the gene for each ICD ortholog have not been reported, transposon mutagenesis has demonstrated that each allele is dispensable for growth (23, 24, 82). Because M. tuberculosis can bypass the oxidative decarboxylation steps of the TCA cycle by diverting isocitrate through the glyoxylate shunt, it is unclear whether the nonessentiality of each ICD is due to redundancy or general nonessentiality for this node in CCM.

α-Ketoglutarate Decarboxylase
Depending on the metabolic demands of a cell, carbon flow can continue through the TCA cycle from isocitrate to α-ketoglutarate via a second oxidative decarboxylation. Most often, the unidirectional oxidative decarboxylation of α-ketoglutarate to succinyl-CoA is coupled to the reduction of NAD⁺ and is catalyzed by KDH, a ternary complex that is closely related to PDH. This complex consists of a succinyl-transferring KDH (E.C. 1.2.4.2, E1 subunit), a dihydrolipoyllysine-residue succinyltransferase (E.C. 2.3.1.61, E2 subunit), and a dihydrolipoyl dehydrogenase (E.C. 1.8.1.4, E3 subunit). From the complete genome sequence of M. tuberculosis H37Rv, it was predicted that the E1 component of KDH was encoded by Rv1248c, E2 by Rv2215 (dlaT), and E3 by Rv0462 (lpdC), Rv0794c, and/or Rv3303c (18). However, consistent with previous reports of CoA-independent α-ketoglutarate decarboxylase (KGD) activity in M. tuberculosis cellular extracts (60), the predicted E1 component Rv1248c was demonstrated to have a nonoxidative KGD activity that yielded succinic semialdehyde rather than succinyl-CoA (64, 83). This activity was independent of the various M. tuberculosis E2 and E3 subunits and relied on ferric cyanide for re-oxidation in cell free assays (83).

Subsequent work found that this KGD-mediated formation of succinic semialdehyde was the product of a slow side reaction whose physiologic role remains unknown (84). Using a novel approach termed activity-based metabolomic profiling, it was later demonstrated that Rv1248c was most proficient in catalyzing the synthesis of 2-hydroxy-3-oxoadipate from α-ketoglutarate and glyoxylate in reaction mixtures as well as in whole cells (84, 85). Interestingly, Rv1248c was also shown to be capable of functioning as a succinyltransferring KDH when supplied with DlaT and Lpd as E2 and E2 components, respectively (86). Moreover, this KDH activity was shown to be allosterically activated by acetyl-CoA and inhibited by the forkhead-associated domain protein GarA (85, 86), the latter of which regulates many enzymes linked to glutamate metabolism in mycobacteria (87). In addition, M.


\textit{M. tuberculosis} strains deleted for \textit{Rv1248c} show conditional growth defects in medium lacking fatty acids (88). These growth defects could be partially alleviated by inclusion of succinate in the growth medium. While these observations are consistent with a role for \textit{Rv1248c} in succinate production, it is important to note that the metabolic basis for these phenotypes and their relevance for \textit{in vivo} growth remains unresolved.

Many microaerophilic and strictly anaerobic organisms utilize an alternative enzyme for interconversion of \(\alpha\)-ketoglutarate and succinyl-CoA, \(\alpha\)-ketoglutarate:ferrodoxin oxidoreductase (KOR; E.C. 1.2.7.3). KOR and other \(\alpha\)-keto acid:ferrodoxin oxidoreductase family members are composed of a CoA-coordinating \(a'\)-subunit (KorA) and a TPP and iron-sulfur cluster containing \(\beta\)-subunit (KorB). In contrast to KGD, KOR is often utilized for redox recycling of succinyl-CoA to \(\alpha\)-ketoglutarate in a \(\mathrm{CO}_2\)-assimilating reverse TCA cycle. Yet the presence of paralogs for this oxidoreductase in many aerobic organisms suggests that KOR might play a greater role in oxidative metabolism than previously thought. Corresponding activity for KOR was recently described for \textit{M. tuberculosis} and was found to be dependent upon the \textit{Rv2455c} (\textit{korB}) \textit{Rv2454c} (\textit{korA}) gene cluster (88). A mutant strain deleted for \textit{korAB} was defective for growth in the absence of exogenously supplied \(\mathrm{CO}_2\), suggesting that KOR can function in the oxidative direction during aerobic growth \textit{in vitro}. Interestingly, a mutant strain deleted for both \textit{korAB} and \textit{Rv1248c} showed a slow growth phenotype relative to the wild-type strain. Thus, if KOR and \textit{Rv1248c} are involved in conversion of \(\alpha\)-ketoglutarate to succinyl-CoA, this function is nonessential for growth of \textit{M. tuberculosis} in laboratory culture. Further investigation is required to determine the extent of KOR’s contribution to flux through the TCA cycle and to determine whether this function plays a role in \textit{M. tuberculosis} pathogenesis.

\textbf{Succinyl-CoA Synthetase}

Succinyl-CoA synthetase (SCS; E.C. 6.2.1.5) is a heterodimeric enzyme responsible for the interconversion of succinyl-CoA and succinate. When functioning as a component of the oxidative TCA cycle, SCS-dependent conversion of succinyl-CoA to succinate yields ATP via substrate-level phosphorylation of ADP. Conversely, in organisms that drive a reductive TCA cycle, SCS can function in synthesis of succinyl-CoA at the expense of ATP. Tian et al. recently reported the detection of succinyl-CoA hydrolytic activity in crude cellular extracts of \textit{M. tuberculosis} \textit{H37Rv} using the colorimetric readout of adduct formation between thionitrobenzoate and free CoA (83). However, it is not clear whether this activity was distinguished from SCS-independent hydrolysis of succinyl-CoA. Thus, it remains to be determined whether \textit{M. tuberculosis} expresses abundant SCS activity and, if so, whether this enzyme functions in the TCA cycle or perhaps serves as a source of succinyl-CoA from succinate derived from other pathways such as the glyoxylate shunt. Despite this uncertainty, genes for the putative SCS subunits (SucCD encoded by \textit{Rv0951/Rv0952}) have been described as essential for growth of \textit{M. tuberculosis} \textit{H37Rv} in laboratory culture, consistent with a critical role for SCS in CCM (23, 24).

\textbf{Succinate:Menaquinone Oxidoreductase}

Succinate:quinone oxidoreductase (E.C. 1.3.5.1) couples the reversible oxidation of succinate to fumarate with the reduction of membrane-soluble quinones to quinols (menaquinone and menaquinol in the mycobacteria [89, 90]). When functioning in oxidative metabolism, succinate:quinone oxidoreductase is commonly referred to as succinate dehydrogenase (SDH) and serves as a direct link between the TCA cycle and electron transport. SDH complexes generally consist of an enzymatic flavin adenine dinucleotide subunit SdhA, an iron-sulfur cluster subunit SdhB, an integral membrane \(b\)-type cytochrome subunit SdhC, and often an integral membrane anchor subunit SdhD.

SDH activity has been confirmed in \textit{M. tuberculosis} \textit{H37Rv} by following the succinate-dependent reduction of potassium ferricyanide by crude cellular extracts (60), as well as by following the succinate-dependent reduction of iodonitrotetrazolium by the membrane fraction of lysed bacilli (91). While the genes primarily responsible for encoding this activity have yet to be substantiated by direct evidence, SDH of \textit{M. tuberculosis} is thought to be redundantly encoded by operons \textit{Rv0247c-Rv0249c} and \textit{Rv3316-Rv3319}. In these operons, \textit{Rv0247c} (\textit{sdhB2}) and \textit{Rv3319} (\textit{sdbhB}) encode the putative iron-sulfur cluster subunits, \textit{Rv0248c} (\textit{sdhA2}) and \textit{Rv3318} (\textit{sdbhA}) encode the flavin adenine dinucleotide-containing catalytic subunits, \textit{Rv0249c} (\textit{sdbhC2}) and \textit{Rv3316} (\textit{sdbhC}) encode the integral membrane cytochrome B subunits, and \textit{Rv3317} (\textit{sdbhD}) encodes an integral membrane anchor. While the \textit{Rv3316-Rv3319} operon has been predicted to be nonessential in both \textit{H37Rv} and CDC1551 in high-throughput mutagenesis screens (23, 24, 82), essentiality data for the \textit{Rv0247c-Rv0249c} operon have proven to be more variable, with indications of \textit{in vitro} essentiality of all components in one report (23) and essentiality for growth in the spleen of C57BL/6J mice in another (5).
In addition to SDH, *M. tuberculosis* also expresses fumarate reductase (FRD) activity that is likely encoded by the *Rv1552-Rv1555* operon. In this operon, *Rv1552* encodes the enzymatic subunit FrdA, *Rv1553* encodes the iron-sulfur cluster subunit FrdB, *Rv1554* encodes the cytochrome subunit FrdC, and *Rv1555* encodes the anchor subunit FrdD. While these genes are nonessential for growth of *M. tuberculosis* in vitro and in vivo (23, 24, 82, 91), their expression is induced by more than 200-fold under hypoxic nonreplicating conditions (91). DNA binding studies have revealed that this regulation likely occurs through a cyclic AMP-mediated response with the binding of Crp (Rv3676) to the promoter region of *Rv1552* (92). Taken together with the observation that genes for the oxidative arm of the TCA cycle are downregulated under hypoxia, it has been suggested that FRD functions in a reductive half cycle that is designed to compensate for the lack of a sufficient exogenous terminal electron acceptor (91). By using stable isotope tracking methods, it has been demonstrated that *M. tuberculosis* can drive the reductive conversion of pyruvate to succinate, yet FrdA is nonessential for this pathway, likely due to the ability of SDH to compensate in the absence of FrdA (91). With this metabolic redundancy, it is difficult to probe the role for FRD in supporting persistence of *M. tuberculosis* in vivo, and thus we await further examination.

**Fumarase**

Fumarase (FUM; E.C. 4.2.1.2) catalyzes the reversible hydration of fumarate to malate and comes in two general varieties, the class I iron-sulfur cluster-dependent type and the class II iron-sulfur cluster-independent type. Sequence analysis indicates that *M. tuberculosis* encodes a class II FUM (encoded by *Rv1098c*) and unlike many bacteria lacks a class I enzyme. Robust FUM activity from *M. tuberculosis* H37Rv cellular extracts has been reported (60, 83). The activity and structure of purified recombinant *M. tuberculosis* FUM have recently been characterized (93). Similar to other class II orthologs, this enzyme assembles into a homotetramer and shows absolute conservation of active site residues including the essential catalytic serine at position 318 (93). While *Rv1098c* deletion mutant strains have yet to be described, high-throughput assessments of gene essentiality indicate that this gene is required for growth of *M. tuberculosis* in culture (23, 24).

**Malate Dehydrogenase**

Malate dehydrogenase catalyzes the final step in the TCA cycle with the oxidation of malate to oxaloacetate. Malate dehydrogenase comes in two forms, one that is soluble and transfers reducing equivalents to NAD*^+* (MDH; E.C. 1.1.1.37) and another that is a flavin-dependent membrane-associated enzyme that ultimately transfers reducing equivalents to quinones (MQO; E.C. 1.1.5.4). Malate dehydrogenase activity has been detected in *M. tuberculosis* H37Rv cell-free extracts (60, 83). Yet because *M. tuberculosis* is predicted to encode both MDH (*Rv1240*) and MQO (*Rv2852c*), the principal enzyme for this activity has yet to be determined. Of note, overexpression of MQO in *M. smegmatis* influences susceptibility to isoniazid through modulation of the NADH/NAD*^+* ratio (94). Consistent with the possibility that these enzymes serve a semi-redundant function in CCM, *Rv1240* and *Rv2852c* are independently nonessential for growth in laboratory culture (23, 24, 82).

**GLYOXYLATE SHUNT AND THE METHYLCITRATE CYCLE**

In addition to serving as a source of reducing equivalents and ATP, the TCA cycle also serves as a key source of essential biosynthetic precursors. However, classical operation of the TCA cycle under conditions of aerobic respiration results in the oxidative decarboxylation of every acetyl-CoA unit consumed, without a net assimilation of additional carbon units. The use of TCA cycle intermediates for biosynthetic purposes thus requires the activity of anaplerotic reactions that replenish consumed intermediates and sustain their respiratory and/or bio-energetic functions.

During growth on glycolytic carbon sources, this anaplerotic function can be metabolically served by the catalytic activities of phosphoenolpyruvate (PEP) carboxylase/carboxykinase (PCKA) (E.C. 4.1.1.32), pyruvate carboxylase (PCA) (E.C.6.4.1.1), and/or malic enzyme (MEZ) (E.C.1.1.1.38), encoded by *pckA* (*Rv0211*), *pca* (*Rv2967c*), and *mez* (*Rv2332*), respectively. Activity of cellular and purified PckA and PCA has been characterized for *M. smegmatis* (95, 96) but not *M. tuberculosis*, while biochemical studies of MEZ remain lacking. Of these three enzymes, only *pca* is predicted to be essential for *in vitro* growth of strains H37Rv and CDC1551 (24, 82). Looking ahead, key unanswered questions concerning these enzymes will pertain to resolving their *in vitro* and *in vivo* regulation and directionality (24, 82).

During growth on fatty acids, metabolite replenishment is accomplished by the linked enzymatic in-activation of isocitrate dehydrogenase and genetic upregulation of the glyoxylate shunt, which generates one molecule of malate from two molecules of...
acetyl-CoA via the successive actions of isocitrate lyase (ICL) (EC 4.1.3.1) and malate synthase (EC 2.3.3.9). The *M. tuberculosis* genome encodes an intact glyoxylate shunt that consists of two paralogous ICLs (encoded by *icl* [Rv0467] and *aceAa/aceAb* [Rv1915-Rv1916]) and a single malate synthase (encoded by *glcB* [Rv1837c]). Despite exhibiting only 27% sequence identity to one another, both ICLs share a highly conserved active site and catalytic activity that function to generate glyoxylate and succinate from cleavage of a single molecule of isocitrate, albeit with distinct catalytic efficiencies (Km = 0.19 mM ICL1; 1.14 mM ICL2; kcat = 5.24 s\(^{-1}\) ICL1; 1.38 s\(^{-1}\) ICL2) (97). Consistent with its canonical role in anaerobiosis, *M. tuberculosis* mutants lacking both ICLs were shown to be incapable of growing on fatty acids in vitro and either establishing or maintaining a chronic infection in mice (98, 99). Moreover, ICL-deficient *M. tuberculosis* strains appear to be the most profoundly attenuated strains reported to date (99).

The *glcB*-encoded malate synthase G of *M. tuberculosis* catalyzes the Claisen-like condensation of acetyl-CoA and glyoxylate to enable the net assimilation of two acetyl-CoA units into the TCA cycle via the production of malate. The GlcB enzyme is an 80-kDa monomeric protein homologous (with ~60% identity) to the malate synthase (AceB) of *Corynebacterium glutamicum* (100) and *E. coli* (101) but distinct from a second group of malate synthases called malate synthase A found in *E. coli* K-12 (102), *Yersinia pestis* (103), *Vibrio cholerae* (104), yeast, and higher plants. Biochemical studies of the *M. tuberculosis* GlcB further reported evidence of sequential binding of glyoxylate followed by acetyl-CoA and ordered release of CoA followed by malate with the following rate constants (Kgly = 30 μM, Kacetyl-CoA = 10 μM; kcat = 23 s\(^{-1}\)). Consistent with its genomic non-redundancy, insertional transposon mutagenesis studies have predicted the *glcB* of *M. tuberculosis* to be essential for optimal growth in vitro (5).

In addition to their role in anaerobiotic metabolism of even-numbered short-chain fatty acids, *M. tuberculosis* ICLs were also found to be essential for in vitro growth on odd-numbered short-chain fatty acids. This finding led to both structural and enzymatic evidence of ICL1 (but not ICL2) as a bifunctional ICL and methylisocitrate lyase (MCL). MCL is an enzyme of the methylcitrate cycle, the dominant pathway for metabolism of propionyl-CoA in eubacteria, for which the *M. tuberculosis* genome encodes orthologs of all enzymes except MCL (18, 97, 99). The ICL1 of *M. tuberculosis* is thus a dual substrate enzyme that participates in two separate pathways of fatty acid metabolism using a single active site chemistry.

Studies in *E. coli* showed that ICL also participated in the recently discovered PEP-glyoxylate cycle, which supports complete oxidation of carbohydrates to CO\(_2\) during growth on limiting concentrations of glucose and balanced conversion of acetyl-CoA into CO\(_2\) and pyruvate/PEP during growth on lipids (105). Evidence for such a cycle in *M. tuberculosis*, however, remains lacking. Nonetheless, a recent study by Beste et al. provided evidence of an analogous pathway for pyruvate dissimilation involving *M. tuberculosis* ICLs and carbon dioxide (106).

From a historical perspective, early studies by Wayne showed that *M. tuberculosis*, modeled to enter into a state of nonreplicative quiescence, exhibited a hypoxia- rather than fatty acid–induced increase in ICL activity that was linked to an increase in glycine dehydrogenase activity and thought to facilitate recycling of accumulated NADH reducing equivalents (107). More recent work, however, showed that this increase was tied to the biochemical ability of ICL to produce succinate as a multifunctional metabolic end product, rather than an intermediate, used to more broadly sustain membrane potential, respiratory (or ATP generating) activity, and carbon flow during entry into, residence in, and exit from hypoxic quiescence (108). Such studies thus highlight the potential multiplicity of biochemical functions and physiologic roles encoded by canonical metabolic enzymes not foreseen by genetic or bioinformatic approaches.

**CONCLUDING REMARKS**

Despite the established connectivity between glycolysis and the TCA cycle in *M. tuberculosis*, Segal and Bloch made the striking observation that while glyceraldehyde, glucose, and fatty acids could stimulate respiration in *vivo*, only lipids could stimulate measurable respiration in *ex vivo*–derived bacilli (109). This result was the first indication that *M. tuberculosis* might preferentially use β-oxidation, rather than glycolysis, as a substrate entry pathway for CCM in *vivo*. The enzymatic basis for this ability to utilize β-oxidation units, in the form of acetyl-CoA, was later revealed with the detection of the glyoxylate shunt enzymes, ICL and malate synthase, in partially purified extracts of cultured *M. tuberculosis* (60, 110).

Through the use of site-directed mutagenesis, the importance of lipid utilization and its essential connectivity to gluconeogenesis for *in vivo* growth of *M. tuberculosis* has recently been established with the observation that
independent mutant strains lacking the genes for ICL and phosphoenolpyruvate carboxykinase were defective for growth and persistence in a murine model of infection (98, 99, 105, 111). These observations, coupled with the observation that virulent M. bovis lacks integration between glycolysis and the TCA cycle due to a deficiency in pyruvate kinase (encoded by pykA or Rv1617) activity (68), have called into question whether carbohydrates, such as glucose, are relevant carbon sources for growth and survival of M. tuberculosis in vivo. Interestingly, it was recently revealed that while hexokinase, required for glucose utilization, is dispensable for the acute phase of M. tuberculosis infection, this enzyme plays an essential role in persistence during the chronic phase of infection, thereby indicating that acquisition and utilization of glucose are important for the fitness of M. tuberculosis in vivo (6). A major area of interest in the coming years will be to assess the specific roles of various CCM pathways and their connectivity in different microenvironments in vivo. Carbon transformations aside, a broader question to be addressed is how M. tuberculosis coordinates the need for biosynthetic intermediates with the accompanying alterations in respiratory equivalents and the extent to which M. tuberculosis can dissociate one from the other.

REFERENCES


