Metabolic plasticity of central carbon metabolism protects mycobacteria

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Central carbon metabolism (CCM) that transforms carbon through glycolysis, gluconeogenesis, the pentose phosphate pathway, and the TCA pathway into energy is essential for the physiology of *Mycobacterium tuberculosis* (*Mtb*). In contrast to other pathogens, humans are the only known reservoir of *Mtb*, where it mainly resides in macrophages of the primary organ of infection, the lung. Growing evidence indicates that *Mtb* has evolved metabolic plasticity in response to a hypoxic, carbon-poor environment and resistance to the oxidative and nitrosative stresses of the human immune system by the CCM having interdependent physiological and pathogenic roles (1). In PNAS, Maksymiuk et al. (2) demonstrate that E1 of α -ketoglutarate dehydrogenase (KDH) in *Mtb* CCM, hydroxyoxoadipate synthase (HOAS), plays defensive roles in situational stresses when *Mtb* relies on glutamate metabolism and when *Mtb* is exposed to reactive nitrogen intermediates (RNIs).

The classic α -ketoglutarate dehydrogenase complex (KDH), which consists of three enzymes, catalyzes a high-flux reaction at the branch point in the TCA cycle between the production of reducing equivalents for energy generation and nitrogen metabolism to produce biosynthetic precursors for lipids, heme, and amino acids (3). Variant TCA cycles have been found in bacteria following adaptation to different environmental niches (4). For example, bacteria, such as *Helicobacter pylori*, that live in microaerophilic and anaerobic environments lack genes encoding KDH (5). Instead, they have a noncyclic branched pathway directed toward succinate in the reductive branch and α -ketoglutarate (α -KG) in the oxidative branch that are metabolically linked by α -KG oxidase activity. Some bacteria, such as *Escherichia coli*, operate a full TCA cycle under aerobic conditions but suppress KDH under anaerobic conditions to function as a branched TCA cycle (4).

Mtb, although a strictly aerobic bacillus, can survive long periods under hypoxia. A full TCA cycle is annotated in the *Mtb* genome

(6), but the annotations are not based on *Mtb* biochemical evidence. Furthermore, *Mtb* has a functional glyoxylate shunt, as evidenced by isocitrate lyase (Icl) activity, and an anaerobic-associated α -KG ferredoxin oxidoreductase (7), both of which could bypass the requirement for KDH activity. In fact, KDH activity was not detected in *Mtb* lysates (8) and E1 of KDH (Rv1248c) was typified as an α -KG decarboxylase that produced succinic semialdehyde (SSA) (9) in addition to a carbonylase on glyoxylate to form 2-hydroxy-3-oxoadipate (HOA) (10). *Mtb* has SSA dehydrogenases (GabD1 and GabD2) that can generate succinate from SSA, thereby completing a modified TCA cycle (9) (Fig. 1A). Maksymiuk et al. (2) investigate potential virulent functions of E1 as growing evidence to show that enzymes of *Mtb* CCM are not only functioning as annotated but also protect the pathogen via noncanonical functions. This protection was first observed with dihydrolipoamide acyl transferase (DlaT) and dihydrolipoamide dehydrogenase (Lpd), and then with Icl.

DlaT and Lpd are components of peroxynitrite reductase/peroxidase (PNR/P), which permits *Mtb* to survive exposure to RNIs. PNR/P consists of Lpd, DlaT, an alkyl hydroperoxide reductase subunit C (AhpC), and a thioredoxin-like protein (AhpD) (11) (Fig. 1B). In this system, AhpC catalyzes the NADH-driven reduction of peroxynitrite or hydroperoxide. The oxidized AhpC is reduced by AhpD that is regenerated by DlaT. Oxidized DlaT is reduced by Lpd to complete the catalytic cycle. However, DlaT is also critical for the intermediary metabolism of *Mtb*, because it is E2 of pyruvate dehydrogenase (PDH) that catalyzes the oxidation of pyruvate by NAD^+ to acetyl CoA, which feeds into the TCA cycle (8). Likewise, Lpd is E3 in the *Mtb* PDH complex, and it also serves as E1 of PNR/P, exhibiting both a canonical role in *Mtb* CCM as well as a protective role required for virulence. Lpd is also E3 in the branched chain keto acid dehydrogenase (BCKADH). BCKADH enables *Mtb*

Fig. 1. (A) *Mtb* TCA cycle showing the noncanonical functions of HOAS in the presence of glutamate. (B) Alternate four-component PNR/Ps to detoxify RNIs.

to metabolize branched chain amino acids in glucose-limiting conditions in vivo, but it can also prevent accumulation of pyruvate, branched chain amino acids, and keto acids, which have proved to be toxic to *Mtb* (12). Icl, in addition to its canonical function in assimilation of acetyl CoA and replenishment of TCA intermediates by dual roles in the glyoxylate shunt and methylcitrate cycles, protects *Mtb* from the oxidative stress of three mechanistically distinct antibiotics: isoniazid, rifampicin, and streptomycin (13).

In their quest for noncanonical functions of *Mtb* HOAS, E1 of KDH, Maksymiuk et al. (2) find that HOAS was nonessential in standard in vitro cultures but required for *Mtb* persistence in mice. To understand the loss of virulence, in vitro growth of the

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HOAS mutant and wild type (wt) was assessed on 192 individual carbon sources. The only substrate on which the HOAS mutant did not grow relative to the wt was glutamate. Coincidentally, *Mtb* deficient in DltA, E2 of KDHC, did not grow in the presence of glutamate either, suggesting KDHC becomes essential when *Mtb* encounters glutamate. Despite not growing in glutamate, both the HOAS and DltA mutants remained viable. Although KDH activity had not been detected in *Mtb* lysates (8), Wagner et al. (14) detected low in vitro KDH basal activities when KDH was activated with acetyl-CoA, which was confirmed by Maksymiuk et al. (2). Minimal effects of HOAS deletion in *Mtb* on in vitro growth and low KDH enzymatic activity may be due to the presence of bypass shunts in *Mtb*.

When the HOAS or DltA mutants were grown on glutamate and acetate, metabolomics demonstrated markedly increased levels of α -KG and SSA. SSA can also arise from transamination between α -KG and GABA catalyzed by 4-aminobutyrate transaminase (GabT), in addition to nonoxidative decarboxylation of α -KG. Maksymiuk et al. (2) demonstrate that exogenously added SSA inhibited growth of all of the strains. SSA can be oxidized by SSA dehydrogenases GabD1 and GabD2 (9), but GabD1, which is more efficient, is inhibited by high concentrations of SSA (15) in addition to glyoxylate (16). In the presence of glutamate, HOAS deficiency in *Mtb* also leads to increased levels of glyoxylate because glyoxylate is the cosubstrate in the carboligation reaction to form HOA. Thus, when glutamate is supplied to the HOAS mutant, there is increased GabT activity and higher levels of SSA, which, along with increased glyoxylate levels, will inhibit GabD1 and result in accumulation of growth-inhibitory SSAs. Thus, HOAS protects *Mtb* from toxic SSA during glutamate anaplerosis.

Maksymiuk et al. (2) find that HOAS was essential for *Mtb* to persist in iNOS-expressing mice. Because the DltA and HOAS mutants exhibited similar metabolomics, and DltA plays a role in PNR/P against RNIs in vitro (11), the authors investigated if RNIs contributed to the lower virulence of the HOAS mutant in inducible nitric oxide synthase-expressing mice. In most antioxidant systems, electrons for the reductase reaction are provided by NADPH or NADH drawn from CCM as in *Mtb* PNR/P.

However, NADH, NADPH, and Lpd are all known targets of RNIs (17, 18). Therefore, the authors proposed that in the absence of Lpd and NADH, when *Mtb* is exposed to RNIs, the DltA/AhpD/AhpC system could use

As illustrated by Maksymiuk et al., future *Mtb* studies need to focus on protein function in conditions mimicking the in vivo environment of *Mtb* infection.

electrons from the oxidative decarboxylation of 2-ketoacids to reduce peroxide or peroxynitrite. They demonstrated that HOAS or AceE of the PDH complex could participate in a four-enzyme peroxidase system, HOAS or AceE/DltA/AhpD/AhpC, where α -KG or pyruvate was used as the source of reductive electrons to generate succinyl CoA and acetyl CoA, respectively. Thus, *Mtb* could potentially use components and metabolites of the CCM for defense against RNIs.

As illustrated by Maksymiuk et al. (2), future *Mtb* studies need to focus on protein

function in conditions mimicking the in vivo environment of *Mtb* infection because there is increasing evidence for enzymes with canonical annotations for CCM also being involved in pathogen virulence. These findings could be harnessed in antituberculosis drug development. Although drug development shies away from using enzymes of *Mtb* CCM as potential targets due to the presence of orthologous enzymes in humans, their roles in *Mtb* pathogenicity under in vivo host conditions could be targeted. For example, in nutrient-deprived conditions with high nitrosative stress, should glutamine be the only carbon source available for energy production and viability in a persistent state, potential inhibitors of HOAS may promote accumulation of growth-inhibitory SSA, and make *Mtb* more susceptible to host-generated RNIs. Furthermore, HOAS activity is regulated allosterically by acetyl CoA and by GarA, whose activity, in turn, is controlled by PknG and PknB (14). It is possible that regulation and allosteric mechanisms of CCM enzymes are different from regulation and allosteric mechanisms of the host orthologs, and can thus enable development of mechanism-based inhibitors that have improved potency and specificity against the pathogen's homologs (19).

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