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Targeting Bacterial Central Metabolism for Drug Development

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Current antibiotics, derived mainly from natural sources, inhibit a narrow spectrum of cellular processes, namely DNA replication, protein synthesis, and cell wall biosynthesis. With the worldwide explosion of drug resistance, there is renewed interest in the investigation of alternate essential cellular processes, including bacterial central metabolic pathways, as a drug target space for the next generation of antibiotics. However, the validation of targets in central metabolism is more complex, as essentiality of such targets can be conditional and/or contextual. Bearing in mind our enhanced understanding of prokaryotic central metabolism, a key question arises: can central metabolism be bacteria's Achilles' heel and a therapeutic target for the development of new classes of antibiotics? In this review, we draw lessons from oncology and attempt to address some of the open questions related to feasibility of targeting bacterial central metabolism as a strategy for developing new antibacterial drugs.

In recent years, the dramatic rise in microbial drug resistance has led organizations such as the Infectious Diseases Society of America and the World Health Organization to warn of a "post-antibiotic era" when pathogenic microbes will be recalcitrant to the current antibacterial regimens (Alanis, 2005; Boucher et al., 2009; Raviglione, 2006). Indeed, some totally drug-resistant pathogenic bacteria have already eroded earlier advances in treatment and control (Rowland, 2012).

Existing antimicrobial drugs have been identified from phenotypic screens on the basis of their ability to kill bacteria or inhibit their growth. They act by inhibiting a limited spectrum of cellular processes essential during logarithmic growth, namely biosynthesis of proteins, RNA, DNA, cell wall, and folic acid. Although there is continued success in finding inhibitors against such targets (Bax et al., 2010; Makarov et al., 2009; Phillips et al., 2011), the frequency is low and dwindling remarkably compared with the "golden era" of antibiotic drug discovery (Fischbach and Walsh, 2009; Payne et al., 2007). Furthermore, pathogenic bacteria have developed sophisticated intrinsic drug resistance mechanisms that are hard wired in the microbial metagenome as a natural phenomenon and are difficult to fight (Bhullar et al., 2012; D'Costa et al., 2011, 2006). Given the current gap between our ability to develop novel antibiotics and the pressing need for drugs active against drug-resistant and drug-tolerant populations (a nonheritable form of "resistance" or tolerance to antibiotic stress; a subpopulation of microbes insensitive to antibiotics concentrations that are otherwise cidal to the majority of the clonal population), there is motivation to consider alternative essential cellular processes as a target space for the development of antibacterial classes.

Bacterial pathogens respond to specific nutritional cues within the host microenvironment. Microbial pathogenicity is dictated by virulence factors that are required to establish infection at a given site and by metabolic pathways required to support growth (Brown et al., 2008). The host can in fact be considered as "a growth medium" that is exploited by microbial pathogens to multiply and cause diseases (Brown et al., 2008; Garber, 1960). Restricting access to essential nutrients is an integral part of innate immunity, a concept that was originally proposed by Louis Pasteur. To establish infection, bacterial pathogens reprogram their metabolic network, balancing biosynthetic processes with sufficient ATP biogenesis to support both growth and survival (Eisenreich et al., 2010; Liu et al., 2012; Schnappinger et al., 2003; Stavru et al., 2011). These altered metabolic pathways are absolutely essential for survival in vivo and represent an appealing space to expand the repertoire of antimicrobial drug targets. Historically, central metabolism has been considered unattractive for developing antimicrobials because of the possible lack of selectivity since most metabolic enzymes are conserved from bacteria to human. Nevertheless, it is important to remember that most clinically used antibiotics also target conserved cellular processes.

Success in targeting bacterial central metabolism will emerge from understanding of precisely how cells regulate the flux of nutrients into pathways that are essential for biosynthesis and energy metabolism under relevant growth conditions (Figure 1). Such an understanding necessitates the use of methods to untangle metabolic flux and pathway regulation that are not often used in mainstream drug discovery (Munger et al., 2008; Rabinowitz et al., 2011). Herein we review recent findings that support the therapeutic value of targeting bacterial central metabolism, examine the associated challenges, and outline a framework for targeting this niche. We focus our attention on intermediary carbon metabolism, drawing lessons from oncology and other

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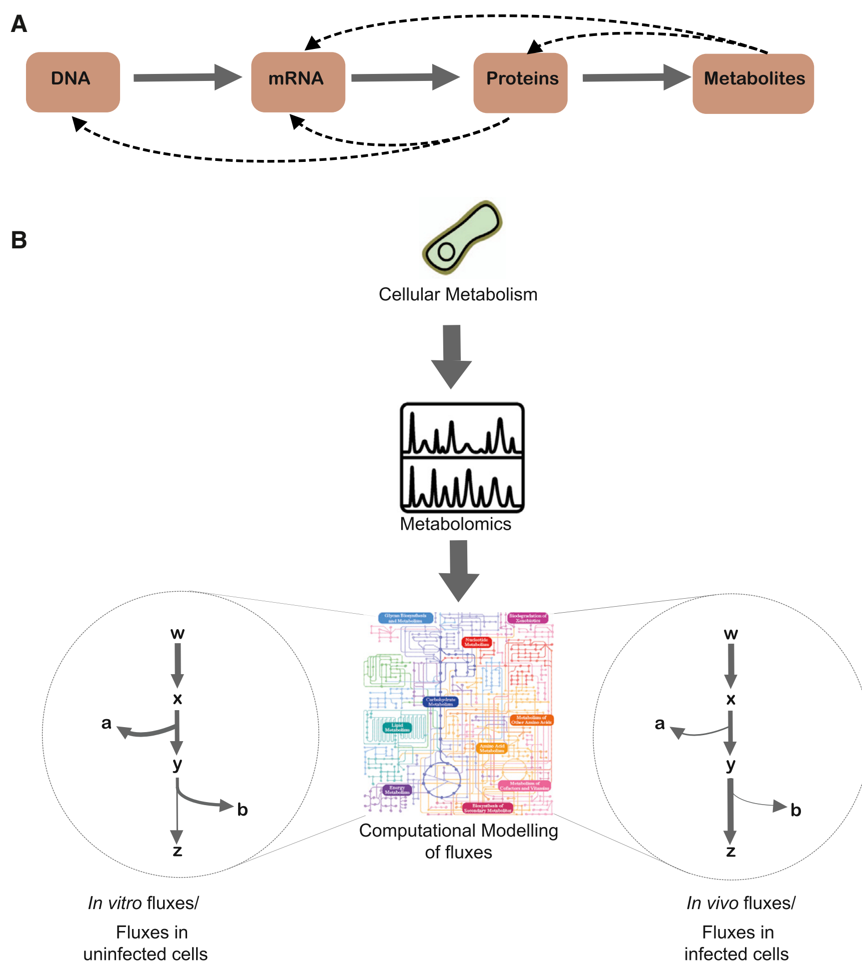

Figure 1. Interactions between Molecular Components Give Rise to Metabolic Network Responses

(A) Complexity arises through multiple overlapping regulatory mechanisms and metabolic feedback (black dotted arrows) in the components that mediate the primary flow of genetic information (gray arrows). Gene expression acts only on enzyme abundance, posttranslational modification may modulate abundance and kinetic parameters. Allosteric regulation exclusively modulates the kinetic parameters through noncovalent binding of effector molecules to noncatalytic sites.
(B) The actual in vivo reaction rate (i.e., metabolic flux in the cell) also depends on the reactant concentrations. Accordingly, metabolic fluxes are the net sums of underlying enzymatic reaction rates, which are an integral output of four physiologic elements interacting at the level of enzyme kinetics (protein abundance, kinetic parameters, metabolite concentrations, and metabolite thermodynamics). Bacterial infection alters the metabolic flux of host cells through certain parts of the metabolic network. In addition, the metabolic flux of the bacteria is different in vivo compared to the axenic cultures.

metabolic diseases where the renaissance of central metabolism has delivered preclinical and clinical drug candidates (Tennant et al., 2010; Vander Heiden, 2011).

Targeting Metabolism: Lessons from Oncology and Viral Diseases

The therapeutic potential of actively shifting the metabolic flux from one pathway to another has recently been shown in the modulation of the oncogenic mammalian pyruvate kinase (PYK) isoform 2 (M2PYK), a hallmark for cancer metabolism (Vander Heiden et al., 2009; Ward and Thompson, 2012). Oncogenic cells have preeminent rates of glucose uptake and yet the rates of mitochondrial oxidative phosphorylation remain unchanged, a phenomenon known as aerobic glycolysis or the Warburg effect.

Among the four PYK isoforms found in humans (Yamada and Noguchi, 1999), M2PYK plays a key role in cancer metabolism (Christofk et al., 2008). In an elegant approach to target the regulation of M2PYK activity, a switch in PYK expression to M1PYK isoform, an isoform expressed in healthy cells, lead to the reversal of the Warburg effect and reduction in tumor proliferation in nude mouse exenografts (Anastasiou et al., 2011; Christofk et al., 2008). To further validate the therapeutic potential of this approach, Anastasiou et al. (2012) analyzed the effect of constitutively modulating M2PYK to a highly active tetrameric state on tumor cell glycolysis. Treatment of tumor cells with a M2PYK activator depleted the glucose-derived carbon intermediates and suppressed the flux of carbon into oncogenic biosynthetic pathways, (Anastasiou et al., 2011; Christofk et al., 2008), leading to reduced tumorigenic proliferation both in vitro and tumor mouse models. Therefore, the bottleneck at the PYK level of an otherwise hyperactive metabolic pathway enables the flux redirection of glucose-derived carbons into anabolic pathways supporting macromolecular synthesis of cell proliferation building blocks (Anastasiou et al., 2011; Mazurek, 2011) (Figure 2). This result is an elegant example of knowledge-based modulation of disease metabolism by manipulating the metabolic flux as a therapeutic intervention that could be adapted to the development of novel antibacterial agents.

In infectious diseases, an insightful understanding of metabolic flux induced by enveloped viruses recently revealed numerous metabolic processes important for viral replication in vivo as targets for antiviral therapy (Munger et al., 2006, 2008; Vastag et al., 2011) (Figure 3). Infection by these viruses induces nucleotide biosynthesis, central carbon and lipid metabolism closely resembling the hallmarks of cancer metabolism such as anaerobic glycolysis (the Warburg effect) and elevated flux from glucose metabolism into both nucleotide biosynthesis and fatty acid metabolism. Using fatty acid biosynthesis inhibitors, Munger et al. (2008) chemically validated the suppression of viral replication during human cytomegalovirus (HCMV) infection, demonstrating the significance of increased flux through the tricarboxylic acid cycle and its efflux into fatty acids biosynthesis during infection (Munger et al., 2008). By quantifying the integrated network response to infection, an antiviral

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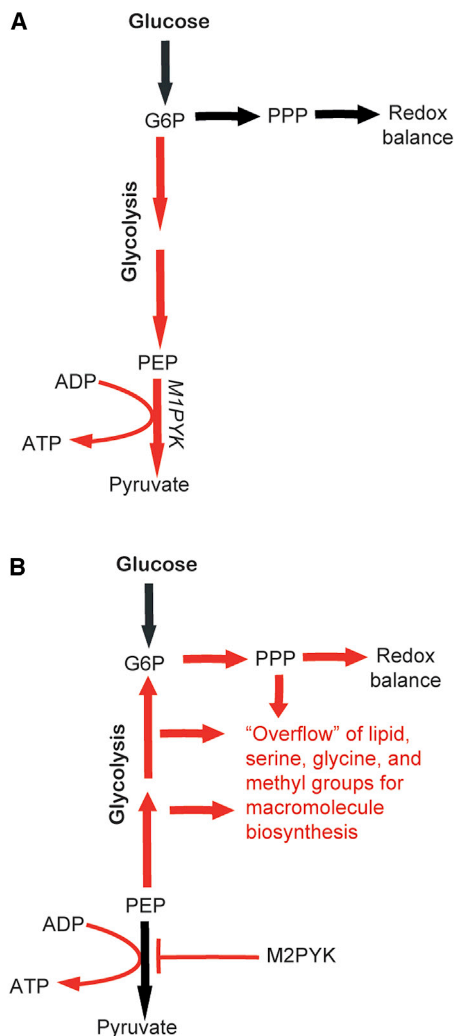

Figure 2. M2PYK in Cancer Metabolism: A Metabolic Bottleneck on Glycolysis and Its Effects on the Pentose Phosphate Pathway

(A) Pyruvate kinase isoform M2 (M2PYK) is present in very few types of proliferating normal cells but is present at high levels in cancer cells. M2PYK catalyzes the rate-limiting step of glycolysis, controlling the conversion of phosphoenolpyruvate (PEP) to pyruvate and thus ATP generation.

(B) Although counterintuitive, M2PYK antagonizes the Warburg effect by inhibiting glycolysis and the generation of ATP in tumors. M2PYK provides an advantage to cancer cells because, by slowing glycolysis, this isozyme allows carbohydrate metabolites to enter other subsidiary pathways, including the hexosamine pathway, uridine diphosphate-glucose synthesis, glycerol synthesis, and the pentose phosphate pathway (PPP), which are required for macromolecule biosynthesis and the maintenance of redox balance that is needed to support the rapid cell division. ATP, adenosine triphosphate; G6P, glucose-6-phosphate; PEP, phosphoenolpyruvate. Red lines with bars denote the constrained flux by M2PYK. Red pathways indicate the flux of carbons and their "overflow" into biosynthetic pathways supporting cancer proliferation by M2PYK.

drug target, which is important for the development of viral lipoproteins and envelopes, could be rationally identified. Such a remodeling of central carbon metabolism in both viruses and oncogenic cells is reminiscent of carbon storage in nongrowing but metabolically active (NGMA) bacteria (Rittershaus et al., 2013), a subpopulation known to be refractory to current antibacterials.

Approved Antimicrobials Targeting Central Metabolism

The development of selective antibacterials targeting central metabolism has historical precedent. A classical archetype is the development of drugs inhibiting the folate pathway (Bushby and Hitchings, 1968; Lombardo et al., 2004; Shah et al., 2004; van Miert, 1994). Folate is an essential cofactor required for many one-carbon transfer reactions and is a critical precursor for the biosynthesis of purines, pyrimidines, and amino acids. Dihydrofolate reductase (DHFR) plays a central role in maintaining cellular pools of tetrahydrofolate, a precursor for the synthesis of glycine, methionine, thymidine triphosphate, and purines. Inhibition of DHFR activity depletes the cell of essential metabolites for protein, RNA, and DNA biosynthesis, resulting in bacterial stasis (Bushby and Hitchings, 1968; Kwon et al., 2010; van Miert, 1994). Although both humans and bacteria encode a functional DHFR, trimethoprim (TMP) selectively inhibits many microbial DHFRs because of the bacterial specific cooperative binding properties of DHFR, DFHR-cofactor nicotinamide adenine dinucleotide phosphate, and TMP (Baccanari and Kuyper, 1993; Kwon et al., 2010). The high safety and efficacy of TMP reside in its selective inhibition of bacterial DHFRs, with an affinity of TMP several orders of magnitude higher for microbial DHFR compared with the human counterpart (Baccanari and Kuyper, 1993; Birdsall et al., 1983; Hitchings and Burchall, 2006).

Another clinical drug target in the folic acid biosynthetic pathway is dihydropteroate synthase (DHPS), the target of the first synthetic antimicrobial agent Prontosil (Domagk, 1935). DHPS catalyzes the condensation of ρ-aminobenzoic acid (ρABA) with 7,8-dihydropterin-pyrophosphate to form 7,8-dihydropteroate, a key convergent step in the pathway. Sulfanilmide drugs are structural analogs and competitive inhibitors of ρABA at the DHPS active site. Like TMP, the inhibition of DHPS critically depletes cellular folate levels inducing thymineless death (Then and Angehrn, 1973). Today, DHFR-DHPS inhibitor combinations continue to be used as a first-line therapy in the prophylaxis and treatment of HIV-associated secondary *pneumocystis carinii* pneumonia infections. Recent studies have revealed that ρ-aminosalicylic acid (PAS), a second-line anti-TB drug that has been clinically used for more than 60 years, inhibits the folate pathway by a unique mode of action. Instead of acting as a DHPS inhibitor as suggested from its chemical structure, PAS is in fact recognized as a substrate mimic of ρABA and is bioactivated by DHPS into the antimetabolite hydroxyl-dihydrofolate that subsequently poisons folate-metabolic dependent pathways (Chakraborty et al., 2013; Zheng et al., 2013).

The ability to develop bacterial selective inhibitors on enzymes involved in central metabolism has further been recently exemplified by the development of bedaquiline for the treatment of tuberculosis. Bedaquiline is an antitubercular drug targeting the F_0F_1 ATP synthase (Andries et al., 2005; Koul et al., 2007). In spite of the evolutionary conservation between mycobacteria and human, the mycobacterial ATP synthase is at least 20,000 times more sensitive to bedaquiline than human mitochondrial ATP synthase (Haagsma et al., 2009). Taken together, these landmark antimicrobials stimulate interest in exploring pathways whose sequence and architecture is similar to their human homologs.

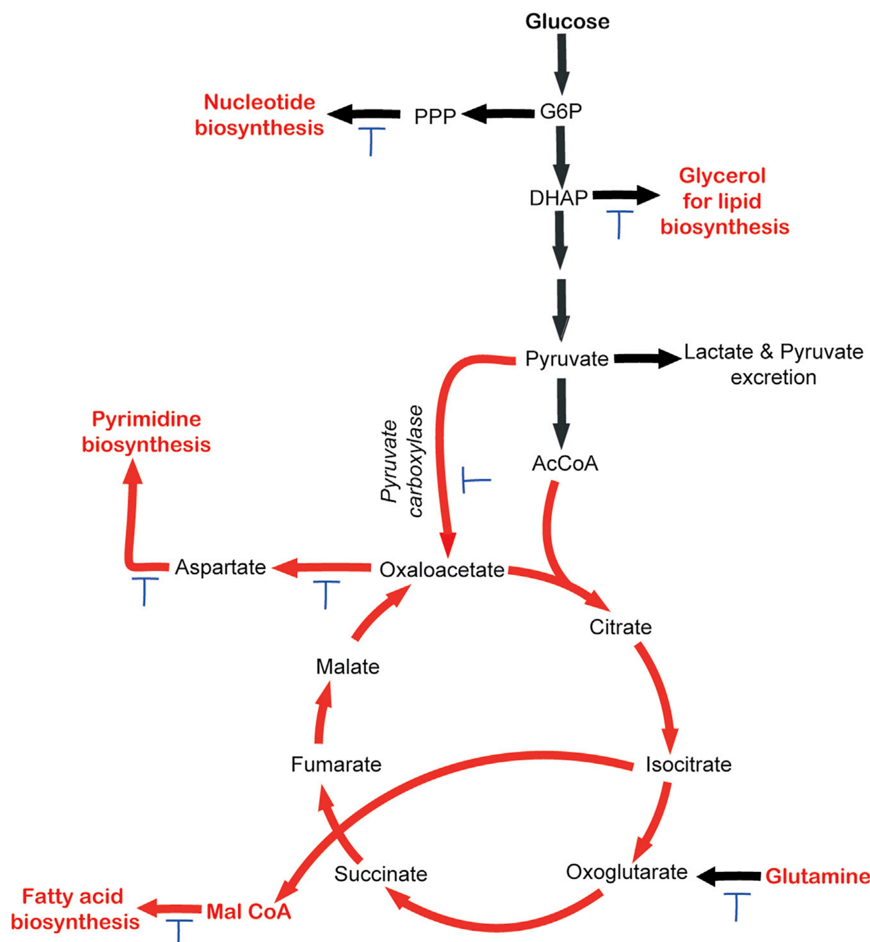

Figure 3. Hijacking Host Cell Metabolism: Central Carbon Metabolism during a Viral Infection
Viruses (hepatitis C virus, herpes simplex virus [HSV] human influenza virus, HCMV) hijack host cell metabolism during infection by rerouting central carbon metabolism toward macromolecule biosynthesis pathways—fatty acid biosynthesis and nucleotide biosynthesis—to support the biosynthesis of viral subunits. HSV infection specifically increases flux from glycolysis to de novo pyrimidine biosynthesis via glucose anaplerotic pathway pyruvate carboxylase, and aspartate transaminases. In line with these flux routes, small interfering RNA knockdowns of the glucose anaplerotic pathway way pyruvate carboxylase and aspartate transaminase-2 impaired HSV replication, demonstrating potential antiviral targets emerging from central metabolism. Red pathways show major flux changes following viral infection. Areas of potential inhibition are depicted in blue lines with bars on top. AcCoA, acetyl coenzyme A; DHAP, dihydroxyacetone phosphate; G6P, glucose-6-phosphate; Mal-CoA, Malonly-CoA.

Defining Essentiality for Metabolic Drug Targets

Historically, ideal drug targets have been genes whose function is important for growth or survival under a chosen condition, so-called essentiality (the demonstration that the abrogation of function of a specific gene/gene product results in the loss of bacterial viability [or phenotypic alteration] that is sufficient to affect bacterial survival either in vitro or in vivo), and are amenable to chemical inhibition, a property commonly referred to as *druggability* (the likelihood of being able to modulate the function of a target with a druglike small molecule, usually estimated by classifying it with known gene families that have previously been successfully targeted with drugs). The increase in the wealth of information on functional genes required for growth and survival under different disease relevant conditions (Becker et al., 2006; Sassetti and Rubin, 2002) led to the concept of a minimal genome. Such a functional requirement for growth has been used to prioritize potential drug targets (Hasan et al., 2006; Payne et al., 2007). Genetic essentiality to validate a drug target falls short of evaluating the vulnerability (a measure of the minimum inhibition threshold limiting bacterial growth and mimicking the gene null phenotype; such an indicator defines whether a proposed drug target requires partial or complete inhibition and can be used to rationally prioritize drug targets for whole-cell and pathway based phenotypic screens) of the target to partial inhibition, which mimics the chemical inactivation by a drug (Wei et al., 2011). Such a validation is even more significant for central metabolic enzymes where the intracellular substrate concentrations of the metabolites are higher or close to the K_M of the respective catalyzing enzyme (Bennett et al., 2009).

The integration of chemistry and biology expertise has redefined the criteria for target validation in drug discovery. The "essentiality"-driven target validation failed to separate the attributes of a well-validated target, as evaluated pharmacologically by distinguishing it from the biological properties of a vulnerable pathway, whose impairment abrogates growth under disease relevant conditions. Deciphering this property is critical and carries discrete, yet overlapping, information of both biology and chemistry. Chemical validation of proposed essential targets is imperative because, unlike gene inactivation that completely abolishes activity, it enables the quantification of the minimal inhibition threshold required to mimic the gene null phenotype (in the cell, the drug will seldom exhibit 100% target occupancy). Conditional expression of essential genes enables the titration of the gene product to quantify such a threshold (Gandotra et al., 2007; Woong Park et al., 2011; Zhang et al., 2000). In a compendium, such chemically validated essential targets can be ranked to prioritize drug targets for early stage drug discovery (Yin et al., 2004), thus ensuring the maximum resource allocation to the most vulnerable drug targets. The utility of hypersensitized strains has been repeatedly documented as a platform to generate new leads from phenotypic screens (DeVito et al., 2002; Phillips et al., 2011; Wang et al., 2006), even for pathways that have been recalcitrant to inhibition to date (Abrahams et al., 2012; Barry et al., 2009; Mak et al., 2012). The use of a pathway-sensitive screen in hypoxic conditions to identify novel ATP depleting antimycobacterials that are active on both active and NGMA mycobacteria demonstrates the power of these tools

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in phenotypic screening (Mak et al., 2012). Finally, given that essentiality of metabolic targets is often conditional and/or contextual, it is important to highlight that most methodologies used to identify antibacterial drug candidates do not reproduce any relevant conditions relevant for infection in vivo. This is a serious bottleneck that is starting to be appreciated (Fahnoe et al., 2012; Pethe et al., 2013; Stanley et al., 2014; Zlitni et al., 2013) and taken into consideration at the discovery and assay development stage.

Current Platform and Approaches to Identify Inhibitors of Metabolic Targets

The germ theory by Luis Pasteur described the body as a culture vessel (Pasteur, 1878). More than a culture vessel, the human body is a chemostat where essential nutrients important for microbial growth are continually replenished to enable establishment, maintenance of infection, and development of disease (Brown et al., 2008; Garber, 1960). Accordingly, the impediment of any step between establishment and maintenance of *Mycobacterium tuberculosis* (*M. tuberculosis*) infection can result in loss of virulence (Dick et al., 2010; Gouzy et al., 2013; Muñoz-Elías and McKinney, 2006; Woong Park et al., 2011) or resistance of the host to infection (Walburger et al., 2004; Zhang et al., 2013). As a result, the interest in further understanding the microenvironment where microbes reside during infection has opened up potential avenues for chemotherapy (Marrero et al., 2010; Munger et al., 2006, 2008; Muñoz-Elías and McKinney, 2005; Puckett et al., 2014).

A converging finding from many of these studies is the poor predictive value of in vitro culture conditions used to study microbial physiology and to evaluate drug candidates (Brinster et al., 2009; Pethe et al., 2010; Brown et al., 2008; Garber, 1960). Accordingly, the need to develop predictive assay conditions has seen the emergence of new alternative protocols to identify central metabolic inhibitors (Fahnoe et al., 2012; Pethe et al., 2013; Stanley et al., 2014; Zlitni et al., 2013). A central feature in all these phenotypic assays is the use of minimal media, devoid of the replete nutrients in the standard broth. Accessibility to most nutrients per se is more difficult from the host than in nutrient rich media; thus, if there is need to use a "default culture medium" for phenotypic screening, it should be defined and minimal at best in order to chemically probe the totality of the biosynthetic pathways. For instance, the presence of vitamins or amino acids in *M. tuberculosis* whole cell-based screens should be avoided since they usually cannot be acquired from the host (Beste et al., 2013, Dick et al., 2010; Gouzy et al., 2013, 2014, Tullius et al., 2003). Nevertheless, the precise composition of the culture broth medium remains to be established for most pathogenic bacteria. Likewise, the amount of metals such as iron, copper, or zinc must be adapted to reflect physiological concentration in target organs or cellular compartment (Corbin et al., 2008; Samanovic et al., 2012; Schaible and Kaufmann, 2004). The inventive identification of inhibitors targeting glycine and biotin biosynthetic pathways in *E. coli* growing on minimal media using metabolomics illustrates the utility of nutrient limitation in early stage drug discovery (Zlitni et al., 2013). The platform presented in this study is promising to identify additional small-molecule inhibitors of essential metabolism pathways and should be easily adaptable to most bacteria. In addition, inhibitors targeting the glyoxylate shunt (Fahnoe et al., 2012), ATP metabolism (Mak et al., 2012), and electron transport chain (Pethe et al., 2013), together with some whose mechanism of action is yet to be understood but are active on NGMA cells (Stanley et al., 2014), demonstrate the power of screening for inhibitors using in vitro conditions mimicking at least some key aspect of the in vivo niche the bacilli reside in.

In an elegant counter immunity screen, Zhang et al. (2013) recently demonstrated that the mycobacterial tryptophan biosynthetic pathway could be targeted by small-molecule inhibitors to promote the clearance of *M. tuberculosis* in synergy with the immune system in infected animals (Zhang et al., 2013). These findings together with others (Gouzy et al., 2013; Lee et al., 2013; McKinney et al., 2000) provide a proof of principle that the metabolic pathways required for intracellular survival in an immune-competent host can be exploited as potential targets for therapy. This kind of concept could only emerge in a physiologically relevant system that can reproduce essential aspects of the host immunity. Similarly, the remarkable efficacy of bedaquiline in treating mycobacterial infections has recently been attributed to its enhanced bactericidal activity in nonfermentable carbon sources (Koul et al., 2014). This is noteworthy because mycobacterial populations residing in human macrophages and in vivo primarily utilize lipids as a source of carbon (Lee et al., 2013; Timm et al., 2003). Fittingly, bedaquiline has enhanced bactericidal activity in macrophages compared to its extracellular activity (Dhillon et al., 2010). The relevance of screening in infected macrophages (Brodin et al., 2010), a relevant physiological environment for *M. tuberculosis* and other pathogens, has recently led to the identification of the energy metabolism inhibitor Q203 (Pethe et al., 2013). Interestingly, Q203 is 10 times more potent against bacteria replicating inside macrophages compared with classical culture broth medium. Analogous to bedaquiline, Q203 selectively inhibits the respiratory cytochrome *bc1* complex in mycobacteria in spite of architectural conservation of the *bc1* complex in many species, including humans (Pethe et al., 2013).

Unless a metabolic target is validated clinically, we advocate for the use of a whole cell-based screen to identify new antibacterial targeting central metabolism. Indeed, target-based drug discovery is overly reductionist in concept for two primary reasons. The drug-organism relationship is reduced to drug-target interplay without integrating the complexity of systems pharmacology. In addition, bacterial metabolic networks are very robust; this enables them to maintain stable function in the face of environmental perturbations. One of the advantages of probing central metabolism in whole cells in a predictive culture broth medium is a complete recapitulation of the metabolic fluxes and compensatory or bypass system the bacteria possesses to multiply or survive. The in vivo efficacy of bioactive inhibitors from these screening conditions should be evaluated prior to intensive lead optimization to clearly validate the physiological relevance of the targeted pathway in establishing infection or persistence.

Modulation of Bacterial Metabolic State to Kill Phenotypic Drug-Tolerant Bacteria

In addition to bacterial drug resistance, drug tolerance and "dormancy" are two major causes of treatment failure for most

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Table 1. Metabolic Pathways that Impair Bacterial Growth and Drug Tolerance

Metabolic Pathway	Impact	References
Fatty acid anaplerosis and gluconeogenesis	impairs the establishment and maintenance of infection	Blumenthal et al., 2010; Marrero et al., 2010; Muñoz-Elías and McKinney, 2005
Vitamin biosynthesis	impairs the establishment and maintenance of infection	Dick et al., 2010; Duckworth et al., 2011; Woong Park et al., 2011
Respiration (ATP biosynthesis and proton motive force)	impairs bacterial growth and promotes killing of NGMA cells	Gengenbacher et al., 2010; Mak et al., 2012; Pethe et al., 2013; Rao et al., 2008; Andries et al., 2005
Nicotinamide adenine dinucleotide biosynthesis	eliminates NGMA cells and impairs the establishment of infection	Kim et al., 2013
Amino acid biosynthesis	impairs the establishment of infection	Beste et al., 2013; Gouzy et al., 2013, 2014; Tullius et al., 2003
Proteolysis deregulation	proteome degradation through Clp proteases leading to eradication of NGMA cells	Conlon et al., 2013; Schmitt et al., 2011
Metabolite supplementation	stimulate the eradication of NGMA cells by aminoglycoside	Allison et al., 2011
Indole metabolism	stationary phase signaling molecules induces drug tolerance	Vega et al., 2012

serious bacterial infections. Given that most antibiotics are inept at killing NGMA cells (Balaban et al., 2004; Rao et al., 2008) and the innate dynamic tolerance to antibiotic stress in microbes (Wakamoto et al., 2013), new approaches are needed to eliminate these subpopulations. Actively shifting the characteristic metabolic flux of drug-tolerant NGMA microbes toward an actively growing state offers a fresh prospect to control such populations. Recent studies have demonstrated that shifting the bacterial metabolism from a quiescent to a replicating state increases sensitivity to antibiotics. Allison et al. (2011) demonstrated that upon addition of the metabolites mannitol or fructose, bacterial persisters became hypersensitive to aminoglycosides (Allison et al., 2011). Mannitol and fructose stimulated bacterial central metabolism by inducing the proton motive force in NGMA cells, thereby enabling aminoglycoside uptake and consequent killing.

The application of such an approach for drug discovery was recently shown when a compound library was screened against persistent *E. coli* in combination with ampicillin (Kim et al., 2011). A polycyclic small molecule C10 accelerated the reversion of persister cells to normal cells and potentiated ampicillin killing (Kim et al., 2011). Although no persister specific metabolic pathway was identified for C10 potentiation, the notion of metabolic modulation to enhance the efficacy of current antibiotics provides key insights into the Achilles' heel of persistent cells. Possible future metabolic approaches for eliminating persister cells include collapsing the proton motive force (Allison et al., 2011; Farha et al., 2013; Rao et al., 2008), dissipating intracellular nutrient storage in mycobacteria (Baek et al., 2011), or inducing reactive oxygen species (ROS) production (or inhibiting ROS-protective genes) in order to increase the potency of bactericidal antibiotics (Brynildsen et al., 2013; Grant et al., 2012) (Table 1). The application of selective manipulation of cellular ROS regulatory systems in aberrant cells has recently gained momentum as a therapeutic approach in oncology (Cabello et al., 2007; Pelicano et al., 2004; Schumacker, 2006; Trachootham et al., 2009), an initiative that will probably benefit the antibacterial drug discovery field.

Challenges and Opportunities in Targeting Central Metabolism in Bacteria

The opportunity to develop drugs targeting central metabolic pathways against bacteria is broad for all diseases where metabolism is important for pathogenesis such as malaria (Storm et al., 2014) and intrinsic pathologies such as chronic neuropathic pain (Patti et al., 2012). Success in targeting central metabolic pathways for bacterial pathogens will emerge from understanding the pathophysiology in metabolism using the power of metabolomics to yield novel drug targets as demonstrated in virology and oncology. The key challenge in targeting central metabolism is the paucity in understanding the convoluted nature of metabolic networks together with how bacteria adapt and regulate these pathways to meet their bioenergetic demands (Figure 1A) (Heinemann and Sauer, 2010). Understanding how central metabolism is regulated in vivo is essential in the development of stratified treatments against both drug-resistant and persistent bacteria. It is also critical to understand pathway redundancies or bypass mechanisms within a complex metabolic pathway to envision whether it might be necessary to block multiple points within such a network. Such an understanding has been key in the development of sulfonamides as anti-infective and anticancer agents.

A recent report on bacterial metabolome analysis demonstrated that the concentrations of metabolites in central metabolism are higher or close to the K_M of their consuming enzymes (Bennett et al., 2009). This has strong implications in flux regulation. Metabolite concentrations greater than 50% of the enzyme K_M indicate that these metabolic steps can be efficiently governed by enzyme inhibition. However, such enzymes can also be easily activated and have most of their active sites occupied, thus compelling greater potency and longer drug residence times for molecules targeting these pathways (Copeland et al., 2006). Alternatively, the development of antimetabolites

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recognized as a substrate by specific metabolic enzymes can circumvent this problem and thus presents an alternate approach to discover antibacterials (Chakraborty et al., 2013; Zheng et al., 2013). Inhibitors targeting central metabolism are being developed within the pharmaceutical industry for many different applications such as cancer, diabetes, and other metabolic disorders (Cabello et al., 2007; Hawser et al., 2006; Pelicano et al., 2004; Schumacker, 2006; Trachootham et al., 2009; Vander Heiden, 2011). In targeting bacterial central metabolism, it is plausible to ''piggyback'' off the extensive platforms from oncology and other metabolic diseases such as obesity and hyperlipidemia. For instance, access to preexisting metabolism curated libraries may allow for the extensive screening of a vast number of compounds without the cost of time and synthesis, meaning that medicinal chemistry can be devoted on the optimization of lead compounds.

The paucity of tractable leads resulting from both phenotypic and target based screens for antibacterials has partly been attributed to the limited chemical diversity in the libraries used for screening (Payne et al., 2007). This is because the libraries utilized were built on medicinal chemistry efforts largely focusing on human therapeutic targets (Payne et al., 2007). Therefore, by pursuing bacterial targets with high sequence or structural homology to eukaryotic proteins, the full potential of our current chemical collection could be exploited for antibacterial drug discovery as demonstrated by Miller et al. (2009). Early evidence suggests that the existing collection of eukaryotic inhibitors (for cancer and other metabolic disorders) present in pharmaceutical libraries can be mined for their activity against structurally related bacterial targets (Johnson et al., 2009; Magnet et al., 2010; Miller et al., 2009; Schreiber et al., 2009), with a focus on selectivity once a promising lead molecule has been identified. Such an approach to generate novel antibacterial agents targeting new biosynthetic processes warrants investigation.

Targeting metabolic enzymes also offers unique challenges within the drug discovery process because of the low druggability of many central metabolic enzymes (Hopkins and Groom, 2002, 2003). Their active sites are usually shallow and often accommodate hydrophilic substrates thus; the opportunity to build a good inhibitor is poor due to the lack of hydrophobic patches. Nevertheless, the paradigm is changing, and interest in these targets is growing in oncology and other metabolic disorders (Teicher et al., 2012; Vander Heiden, 2011). Alternative assay formats, innovative screening paradigms, and chemical strategies remain to be developed. Many of these tools are already in place in industry from more established fields on cellular metabolism such as oncology or other discovery programs targeting metabolic disorders. The transfer of this expertise to anti-infective biology will accelerate the drug development of new antibacterials targeting central metabolism.

In summary, targeting central metabolism in bacterial pathogens represents an attractive proposition in the future development of novel antibacterial drugs. While some of these enzymes are unique to bacteria, many of the enzymes are sufficiently similar to allow exploitation of chemical libraries designed for mammalian metabolic enzymes, and yet different enough to achieve selectivity, which can be established as a new chemotype against bacterial infections.

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