

Loving the poison: the methylcitrate cycle and bacterial pathogenesis

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Abstract

Propionate is an abundant catabolite in nature and represents a rich potential source of carbon for the organisms that can utilize it. However, propionate and propionate-derived catabolites are also toxic to cells, so propionate catabolism can alternatively be viewed as a detoxification mechanism. In this review, we summarize recent progress made in understanding how prokaryotes catabolize propionic acid, how these pathways are regulated and how they might be exploited to develop novel antibacterial interventions.

INTRODUCTION

Propionate is one of the most abundant short-chain fatty acids (SCFAs) and is a common by-product of bacterial fermentation. Consequently, propionate is produced in large quantities (50–100 mM) in the gastrointestinal tract of mammals [1, 2]. Plants, insects and micro-organisms utilize several alternative pathways for metabolizing propionate as a carbon source. For example, propionyl-CoA is the by-product of β -oxidation of odd-chain fatty acids, of branched-chain fatty acid catabolism, of branched-chain amino acid degradation and cholesterol catabolism. Remarkably, though, propionate (or one of its derived catabolites) is lethally toxic to cells if it is allowed to accumulate. Therefore, there is a trade-off between the beneficial effects of propionate catabolism (its skeleton is a useful carbon source) and propionate toxicity. In this review, we address recent advances in our understanding of how cells catabolize propionic acid, what regulates this and why propionate and its catabolites are toxic. We also discuss how propionate catabolism plays an important role in virulence, and how this can be targeted for the development of new antimicrobial interventions.

To begin with, we will discuss how cells metabolize propionate. Three different mechanisms have been proposed: (i) the 2-methylcitrate cycle, (ii) the methylmalonyl pathway and (iii) the incorporation of propionyl moieties into cell wall lipids. In mammalian cells, the methylmalonyl-CoA pathway is the principal means by which propionyl-CoA toxicity is alleviated. In contrast, and in most

bacteria, mycobacteria, yeast and filamentous fungi, the 2-MC cycle is the main pathway used for the detoxification and catabolism of propionate [3], and we will focus mainly on this pathway for the remainder of this review.

THE METHYLCITRATE CYCLE

The methylcitrate cycle was originally thought to be present only in fungal species such as *Candida lipolytica* and *Aspergillus nidulans* [4]. However, in the late 1990s, the methylcitrate cycle was reported in *Salmonella enterica* and *Escherichia coli* [5, 6]. As shown in Fig. 1, propionate and oxaloacetate undergo an aldol condensation reaction to form 2-methylcitrate (2-MC), followed by an isomerization to yield 2-methylisocitrate, and finally, a cleavage reaction yielding succinate and pyruvate. This sequence of reactions is catalyzed by three core enzymes; methylcitrate synthase (MCS, encoded by *prpC*), methylcitrate dehydratase (MCD, encoded by *prpD*) and 2-methylisocitrate lyase (MCL, encoded by *prpB*) [7]. These reactions in the MCC are directly analogous to those catalyzed by citrate synthase, aconitase and isocitrate lyase in the glyoxylate cycle (Fig. 2). However, several variations upon this theme exist, and some organisms encode additional MCC-related enzymes such as PrpE and PrpF (Fig. 3). *PrpE* encodes propionyl-CoA synthase. In *S. enterica*, acetyl-CoA synthase can substitute for PrpE, so *prpE* only becomes essential for growth on propionate in an *acs* mutant background [8]. In contrast, PrpF plays an altogether more interesting role. Whereas PrpD functions to

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Abbreviations: CoA, coenzyme A; ICL, isocitrate lyase; 2-MC, 2-methylcitrate; 2MCA, 2-methyl-*cis*-aconitate; 4MCA, 4-methyl-*cis*-aconitate; MCC, methylcitrate cycle; MCD, methylcitrate dehydratase; MCL, methylcitrate lyase; MCS, methylcitrate synthase; MS, malate synthase; SCFAs, short chain fatty acids; TCA, tricarboxylic acid.

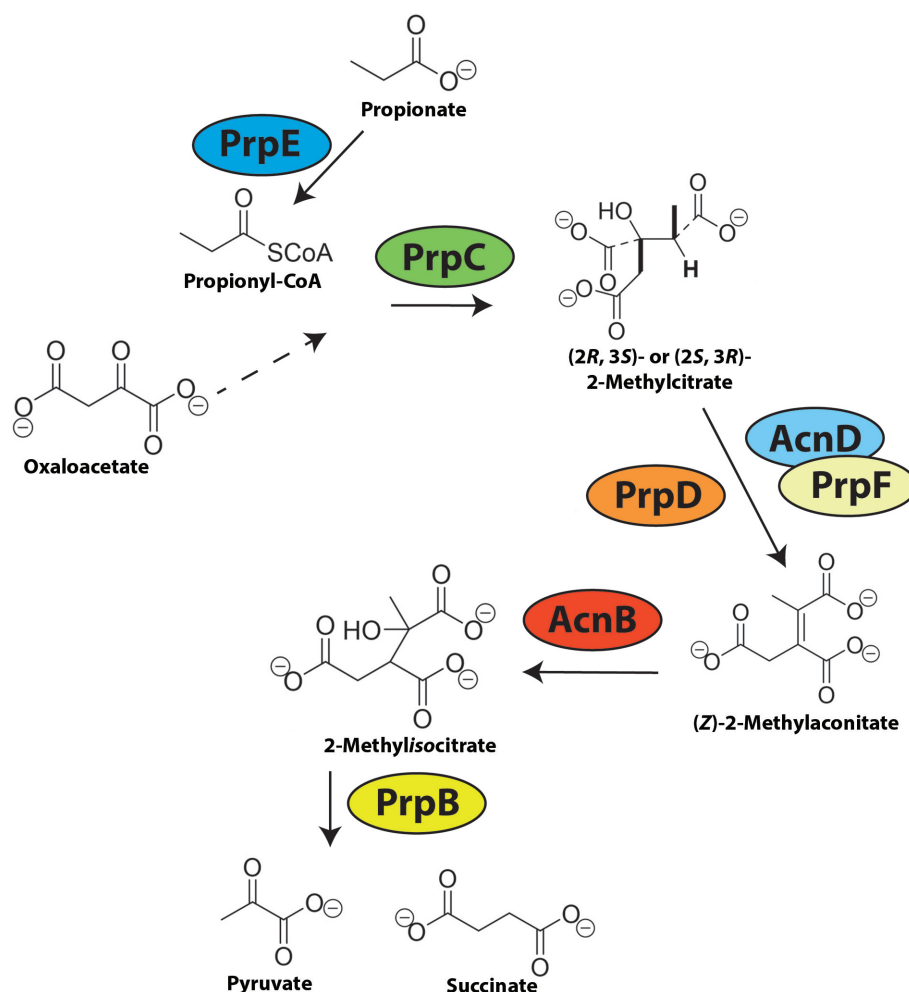


Fig. 1. In the majority of MCC arrangements, propionate is first activated to propionyl-CoA by propionyl-CoA synthase (PrpE). The propionyl-CoA is then condensed with oxaloacetate by methylcitrate synthase (PrpC), forming 2-methylcitrate. 2-MC is subsequently dehydrated to yield methyloaconitate. This step is accomplished by either methylcitrate dehydratase/hydratase (PrpD) or by the combined activity of methylcitrate dehydratase (AcnD) and methyloaconitate *cis-trans* isomerase (PrpF). Following dehydration, methyloaconitate is rehydrated by aconitase (AcnB) to yield methylisocitrate. This is then cleaved by methylisocitrate lyase (PrpB) or by isocitrate lyase (AceA in *M. tuberculosis*) to yield pyruvate and succinate.

dehydrate 2-methylcitrate (2MC) directly to 2-methyl-*cis*-aconitate (2MCA) (Fig. 1), an alternative route is to convert 2-methylcitrate into 4MCA (4-methyl-*cis*-aconitate, in a reaction catalyzed by AcnD) first and then isomerize the 4MCA into 2MCA (in a reaction catalyzed by PrpF) [9]. Quite why these different strategies for converting 2MC into 2MCA are employed is not clear, although one possibility is that since AcnD is an oxygen-sensitive Fe-S protein, PrpD functions when oxygen levels are high [10]. Some organisms, such as *Neisseria meningitidis*, have also been shown to encode additional genes outside of this ‘extended set’, presumably providing even greater flexibility in propionate metabolism [11]. It is noteworthy that none of these gene clusters encode any apparent propionate uptake mechanism, so a passive diffusion mechanism has been suggested. However, in at least one case

(*Corynebacterium glutamicum*) a proton-driven monocarboxylic acid transporter (MctC) has been shown to have high affinity for both acetate and propionate [12].

REGULATION OF METHYLCITRATE CYCLE GENE EXPRESSION – PRPR

PrpR is a transcriptional activator of the *prp* genes in some *Gamma*proteobacteria (*Enterobacteriales* and *Xanthomonadales*) and *Beta*proteobacteria (*Burkholderiales*). In most *Enterobacteriales* the propionate metabolism genes are organized in the divergon *prpR prpBCDE*, although in the majority of *Xanthomonadales* and *Beta*proteobacteria the propionate divergon has a slightly different arrangement – *prpR prpBC-acnD-prpF*. In some *Beta*proteobacteria, additional transcription factors regulate propionate metabolism [13].

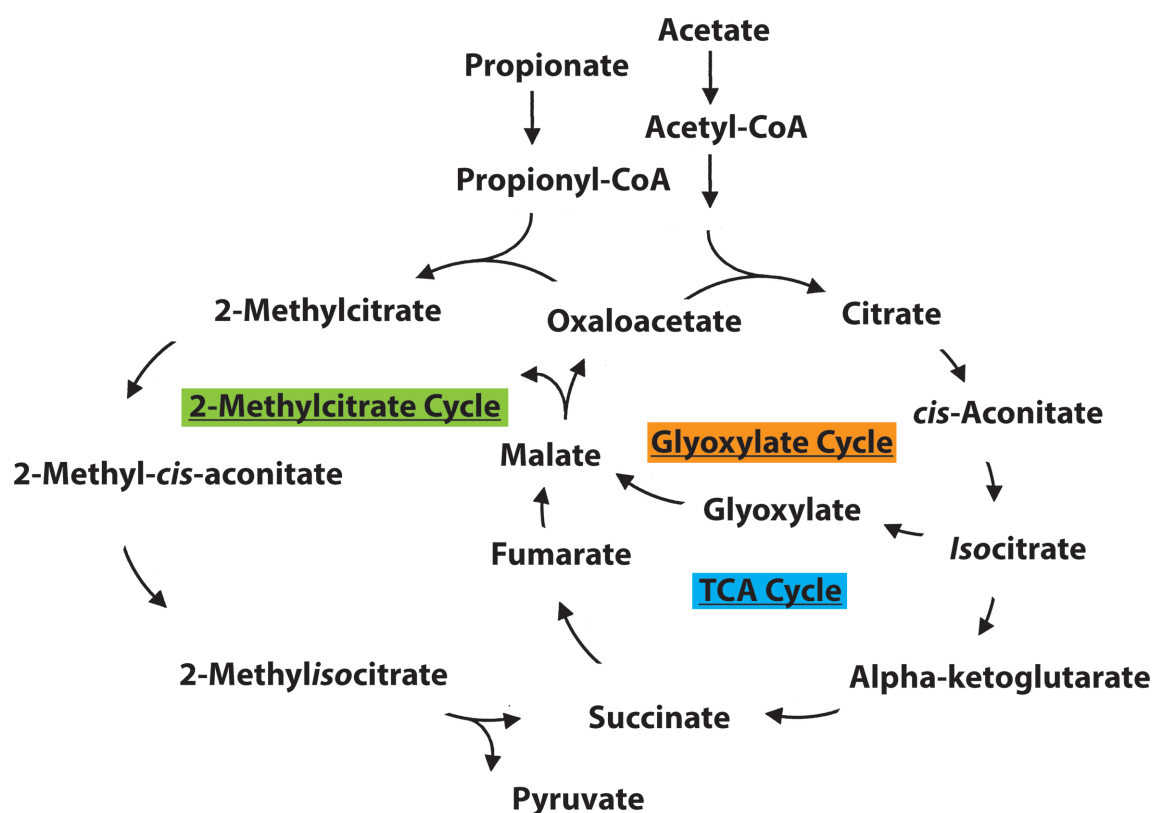


Fig. 2. The overlapping reactions encompassing the citric acid (TCA), glyoxylate and methylcitrate cycles in bacteria. Odd-chain fatty acids are degraded via the β -oxidation cycle to yield acetyl-CoA and propionyl-CoA units. These are then further oxidized by the citric acid cycle or the methylcitrate cycle, respectively. Anaplerosis during growth on even-chain fatty acid substrates is achieved by metabolism of acetyl-CoA via the glyoxylate cycle, which consists of the enzymes isocitrate lyase (ICL) and malate synthase (MS).

PrpR is widely conserved across many species, although it was first described in *Salmonella enterica* serovar Typhimurium [14]. *S. enterica* PrpR is a sigma-54 family transcriptional regulator that requires 2-methylcitrate as a co-activator [15]. This accumulates during growth on propionate, and following binding of 2-methylcitrate to PrpR, transcription of the MCC gene cluster is rapidly induced, thereby decreasing the toxicity of propionate-derived metabolites [16]. However, a detailed analysis of the *C. glutamicum* transcriptional regulatory network revealed no obvious homologue of PrpR from *E. coli* or *S. enterica* [17]. Instead, the *C. glutamicum* *prpR* gene was found to encode a regulatory protein belonging to the HTH_XRE family, and a $\Delta prpR$ mutant of *C. glutamicum* was unable to induce expression of the *prpDBC2* genes in the presence of propionate [18]. Intriguingly, *C. glutamicum* PrpR was noted to have moderate similarities to the regulator of acetate metabolism, RamB [19]. However, whereas RamB can act as both a repressor and an activator [20], PrpR acts exclusively as an activator. Nevertheless, the similarity in the amino acid sequence between RamB and PrpR hinted at a common ancestry, with each regulator apparently evolving separately to eventually control degradation of the structurally closely related but different carboxylic acids, acetate or propionate [18].

Additional insight into the genetic regulatory control of propionate catabolism came from studies on *Mycobacterium tuberculosis* (Mtb). In this organism, PrpR was shown to act as a transcriptional activator of the *prpDC* and *icl1* genes (*icl1* encodes isocitrate lyase – one of the enzymes involved in the glyoxylate shunt). Strikingly, *ramB* was shown to be negatively regulated by PrpR. This suggested that PrpR plays a key role in coordinating expression of the enzymes involved in the glyoxylate shunt and methylcitrate pathways [21]. PrpR was also shown to regulate Mtb *dnaA* expression, indicating that it may also be involved in controlling the initiation of chromosomal DNA replication. Consequently, PrpR appears to be an important element of the complex regulatory system(s) required for the persistence of Mtb within macrophages, controlling both the catabolism of host-derived fatty acids and the initiation of chromosomal replication [22].

OTHER REGULATORS OF PRP GENE EXPRESSION

Although PrpR is the principal regulator of *prp* operon expression in enterobacteria, several other proteins are also involved. These include σ^{54} , integration host factor (IHF)

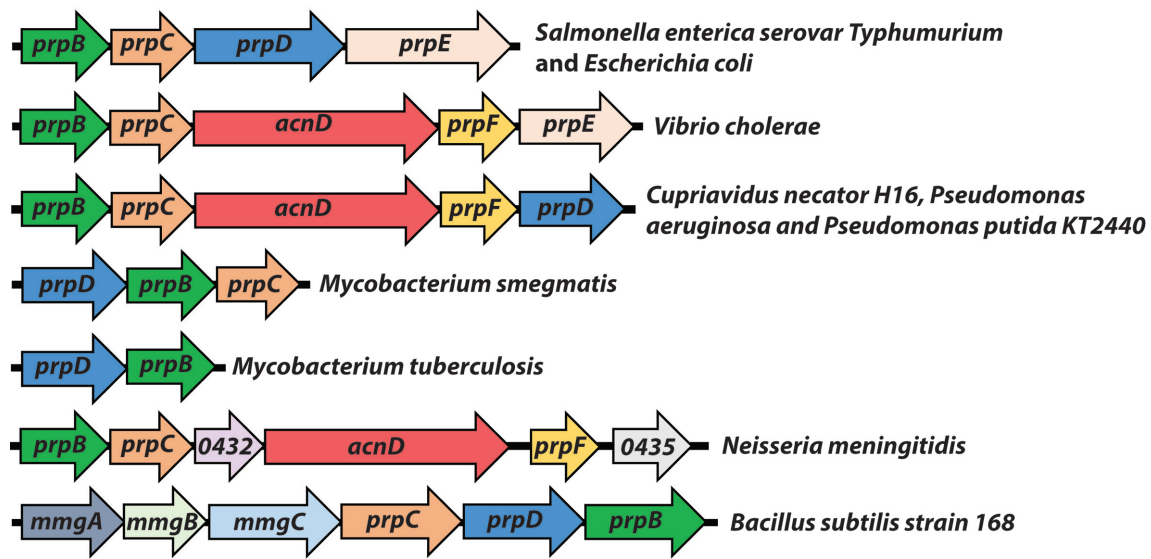


Fig. 3. At least seven structural variations of the *prp* operons have been described thus far for bacteria. The combined activity of AcnD and PrpF replaces the activity of PrpD in some organisms. Interestingly, though, and in spite of the apparent redundancy, some *prp* operons encode both AcnD/PrpF and PrpD. Further variations include the *N. meningitidis* *prp* operon, which contains two additional uncharacterized genes, NMB0432 (a putative membrane protein) and NMB0435 (a putative acetate kinase). Mutants deficient in NMB0432 or NMB0435 are unable to utilize propionic acid. The *P. aeruginosa* *prp* operon appears to lack an obvious *prpE* homologue; presumably this function is encoded by an unlinked gene external to the operon. Similarly, *B. subtilis* strain 168 also does not appear to encode a PrpE homologue. Instead, this function is encoded within the mother cell metabolic gene (*mmg*) operon, which is involved in fatty acid degradation. Crucially, the *M. tuberculosis* genome does not encode the main terminal enzyme of the MCC, PrpB (methylisocitrate lyase). This is because in *Mtb* the glyoxylate shunt enzyme, isocitrate lyase, is capable of both isocitrate lyase and methylisocitrate lyase activity, so no additional enzyme is required.

and the cyclic AMP receptor protein (CRP) [23]. *E. coli* cells with defects in CRP or adenylate cyclase activity (*crp* or *cya* mutants, respectively) exhibit reduced transcriptional activation of *prpR* and *prpBCDE*, and the effect on *prpBCDE* is independent of *prpR*. This suggests that propionate metabolism is subject to catabolite repression, and that this is likely mediated through CRP-dependent control of both *prpR* and the *prpBCDE* operon [24].

Expression of the *prp* genes is also known to be regulated by small RNA molecules. *Neisseria* metabolic switch regulators [NmsRs] act as switches controlling the transition between cataplerotic and anaplerotic metabolism in *N. meningitidis*. The expression of several TCA cycle-associated genes (including *prpF*, *prpB*, *acnB* and *prpC*) were down-regulated by the direct action of NmsRs. Expression of the NmsRs themselves was under the control of the stringent response through the action of the (p)ppGpp synthase, RelA [25], suggesting that the use of propionate may become crucial in this organism under conditions of nutrient limitation [26].

The activity of many small RNAs in bacterial pathogens depends on the RNA chaperone Hfq. Among the direct targets of NmsR_A, three proteins (GltA, PrpB and PrpC) were also found to be overexpressed in an Hfq deletion strain of *N. meningitidis* [11]. This suggested that NmsRs may act in concert with Hfq. Hfq-dependent repression of *prpB*

expression was subsequently shown to be mediated by two *trans*-acting sRNAs, RcoF1 and RcoF2 [27]. Northern blot analysis revealed that both the single *prpB* and the bicistronic *prpB-prpC* mRNAs were up-regulated upon the deletion of *hfq* or a double deletion of *rcoF1* and *rcoF2* [28], thereby confirming the genetic link between RcoF1/2, Hfq and *prp* gene expression. By regulating *prpB*, RcoF1 and RcoF2 appear to be the first *trans*-acting sRNAs with a direct host-associated function in *N. meningitidis* [27].

In *S. enterica*, the *prpBCDE* genes undergo antagonistic regulation by σ^S at the RNA and protein levels. Expression of the *prpBCDE* operon was stimulated by σ^S at the RNA level, yet at the same time, also down-regulated at the protein level. This negative effect of σ^S on the abundance of the Prp proteins is consistent with the fact that $\Delta rpoS$ mutants grow better (compared with the wild-type) on propionate as a sole carbon source. In *E. coli* K12, the *prp* genes are also positively controlled by σ^S at the RNA level [29]. However, and since *rpoS* disruption did not improve *E. coli* K12 growth on propionate, *prp* regulation appears to be different in *E. coli* and *S. enterica*. This may be related to the finding that in contrast to the situation in *S. enterica*, the *prp* operon of *E. coli* is interrupted by repetitive elements [30]. Dual antagonistic control of *prp* expression at both the RNA and protein levels may be a mechanism that allows *S. enterica* σ^S to

maintain finely balanced Prp expression, with greater responsiveness to changing environmental conditions [30].

In *E. coli*, RNase R expression is fourfold higher under anoxic compared with oxic conditions. Intriguingly, RNase R has been shown to hydrolyze *prpBCDE* transcripts, potentially to ensure that propionate degradation only occurs under oxic and not anoxic conditions. Thus, the *prp*-operon of *E. coli* K12 is also regulated at least partly at a post-transcriptional level to prevent the anaerobic metabolism of propionate [31]. This may be a method to prevent futile expenditure of metabolic energy, 2-MC toxicity and/or metabolic roadblocks during anoxic conditions.

Recent studies have demonstrated that propionate catabolism is also regulated post-translationally. For example, propionate metabolism in *Mycobacterium smegmatis* is regulated by lysine acetylation [32] and a cAMP-dependent protein lysine acetyltransferase in *Mycobacterium bovis* BCG (KATmt) has been shown to be necessary for growth on propionate as a sole carbon source [33].

ALLEVIATING PROPIONATE TOXICITY

Although many aerobes (and also some anaerobes) can use propionate as a sole carbon source, the addition of propionate into growth media stunts the growth of most micro-organisms, even in the presence of other carbon sources [34, 35]. Due to this property, SCFAs such as propionate are widely used as preservatives in the food industry. However, and in spite of its widespread use, we still do not have a clear mechanistic insight as to how propionate prevents microbial growth.

The importance of the 2-MC cycle in propionate detoxification has been demonstrated in *E. coli*, *M. smegmatis*, *M. tuberculosis*, *C. glutamicum*, *S. enterica* and several other bacteria. However, the mechanisms of propionate toxicity appear to be extremely diverse. Early studies argued that growth inhibition might be caused by generic, non-specific mechanisms such as the dissipation of the proton motive force, decreased intracellular pH or accumulation of the propionate anion in the cytoplasm [1]. More recent work has challenged these notions. For example, in *Rhodobacter sphaeroides* propionyl-CoA inhibits pyruvate dehydrogenase, and in *S. enterica* 2-MC inhibits the gluconeogenic enzyme, fructose 1,6 biphosphatase (FBPase) [7]. 2-MC has also been shown to be toxic in *Aspergillus nidulans* [36]. Another potentially toxic effect was described for *E. coli*, where it was found that homocysteine accumulates after propionate addition [37]. Consistent with this hypothesis, enhanced expression of the potential homocysteine-consuming enzyme, MetE, was noted in *C. glutamicum* [38]. In addition, propionyl-CoA has been described as toxic in *E. coli* [39], *R. sphaeroides* [40] and *Aspergillus nidulans* [41].

B. SUBTILIS

Recent work has examined growth on propionate or the scavenging of branched fatty acids and branched-chain amino acids as separate drivers for the evolution of a methylcitrate cycle. In *B. subtilis*, lack of an obvious

propionyl-CoA synthase and a general inability to grow on propionate suggest that the function of the methylcitric acid cycle in this organism is to cope specifically with propionyl-CoA, and not propionate, making this pathway potentially distinct from the propionate-metabolizing pathways of other species [42]. What, then, is the source of the propionyl-CoA in these circumstances? Instead of a PrpE-like propionyl-CoA synthase homologue, the *B. subtilis* mother cell metabolic gene (*mmg*) operon comprises six ORFs, *mmgABCDEF*, the first three of which were shown to encode enzymes involved in fatty acid β -oxidation. The final round of the β -oxidation cycle of methyl-branched fatty acids or the carbon skeleton derived from isoleucine catabolism would yield one acetyl-CoA and one propionyl-CoA. The acetyl-CoA would enter the citric acid cycle via the citrate synthase activity of MmgD. However, MmgD also has 2-methylcitrate synthase activity, and can likewise condense propionyl-CoA with oxaloacetate to begin the 2-MC cycle. The remaining steps are encoded by *mmgEF* and *citB* (aconitase) [42]. Further atypical methylcitrate cycles may be present in organisms that do not grow on propionate as a sole carbon source. A key question is whether metabolic defence against toxic levels of propionate acts as an additional driver to maintain this operon.

ENTEROBACTERIA

Although the reasons for retaining the methylcitrate cycle are clearly diverse, genomics has also revealed the repeated loss of this cycle in some species. *Shigella* sp. and the enteroinvasive *E. coli* arose from distinct *Escherichia* ancestors, acquiring the capacity to invade epithelial cells. The genome sequences of these species have undergone substantial reduction, with multiple metabolic pathways becoming lost. One such victim of genome reduction is the methylcitrate pathway, which appears to have been specifically deleted from many of these enteroinvasive pathogens, although it is found in all other *E. coli* genomes [43, 44]. Additionally, four genes (*prpDBCE*) found in *Campylobacter coli* but not in *C. jejuni* are involved in the methylcitrate cycle, which could account for the enhanced ability of *C. coli* strains to grow on odd-chain fatty acids compared with *C. jejuni* [45, 46]. However, the precise driver(s) for the loss of this pathway in certain pathogens remains to be elucidated.

Exploring the molecular basis for propionate toxicity has led to unexpected insights into microbial metabolism. In *S. enterica*, deletion of the methylcitrate synthase, PrpC, results in severe growth inhibition when propionate is added to the growth media. This is because, in the absence of PrpC, the production of 2-MC by citrate synthase (GltA) [1], which has a relatively relaxed substrate specificity, inhibits fructose-1,6-bisphosphatase (FBPase), a key enzyme in gluconeogenesis. The lack of growth due to inhibition of FBPase by 2-MC^{GltA} could be overcome by increasing the expression of FBPase or by adding micromolar amounts of glucose (the ultimate product of the pathway in which FBPase is involved) to the medium – thus providing a definitive target of 2-MC inhibition [47]. Similarly, in

M. tuberculosis, the sole annotated FB Pase (encoded by *glpX*), is also non-competitively inhibited by 2-MC [48].

The relaxed substrate specificity of certain 2-MC cycle enzymes suggests that the methylcitrate cycle is potentially capable of overcoming bottlenecks in cellular metabolism. For example, due to its promiscuous catalytic activity, PrpC has been shown to complement a deletion in *glpA* (encoding citrate synthase) in both *S. enterica* [1] and *E. coli* [49]. Such observations indicate that the methylcitrate cycle can fulfil atypical functionalities when required [49]. The metabolic redundancy of PrpC may also explain why the citrate synthase of *Pseudomonas aeruginosa* (GltA) is not generally essential for growth [50].

MYCOBACTERIA

Mycobacterial sp. have an unusual way of dealing with excess propionyl-CoA. The mycobacterial cell wall has a high lipid content and contains several important lipid groups, including poly- and di-acyltrehaloses (PAT/DAT), sulfolipids (SL-1) and phthiocerol dimycocerosates (PDIM) [51]. These lipids are produced and actively secreted during infection to subvert the host immune system, eventually leading to *M. tuberculosis* persistence. These bioactive lipids can be esterified with up to five multiple methyl-branched long-chain fatty acids, which provides an effective ‘sink’ for excess propionyl-CoA. Like PDIM, the storage lipid triacylglycerol (TAG) can also serve as a sink to alleviate the accumulation of potentially toxic propionyl-CoA [51–53].

The mycobacteria also employ an intriguing feedback mechanism to regulate free propionyl-CoA levels in the cell. Propionylation of acyl-CoA synthase in *M. bovis* BCG leads to diminished synthesis of propionyl-CoA from propionate [33]. Indeed, a considerable number of propionylated proteins have been identified in this species, possibly defining another ‘sink’ for excess propionyl-CoA. Moreover, because the 2-methylisocitrate lyase activity of isocitrate lyase (ICL, a key component of glyoxylate shunt) is essential for the methylcitrate cycle in mycobacterial sp., propionylation of ICL may have a dual impact, i.e. to simultaneously modulate ICL activity in response to the propionyl-CoA concentration and sequester excess of propionyl-CoA to relieve the toxicity of this compound [54].

An *M. tuberculosis* isocitrate lyase mutant, $\Delta icl1$, has a synthetic lethal phenotype and fails to grow in rich media containing cholesterol. In *M. tuberculosis*, ICL also doubles as a 2-methylisocitrate lyase. It was therefore hypothesized that this growth inhibition might be linked to the accumulation of one or more toxic cholesterol-derived intermediates produced by the methylcitrate cycle, which accumulate in the bacteria when *icl1* is non-functional. Recent work has shown that $\Delta icl1$ ‘bypass’ mutants, which either alleviate methylcitrate toxicity or reduce the catabolic flux of propionyl-CoA, are able to rescue the growth defect by alleviating the accumulation of toxic metabolites. This led to the identification of a previously uncharacterized protein, Rv3723/LucA, which functions to integrate cholesterol and

fatty acid uptake in *M. tuberculosis* [55]. Similar experiments in other key pathogens will likely uncover more uncharacterized proteins dedicated to propionate metabolism and/or alleviating toxicity.

PROPIONATE METABOLISM AS AN ANTIMICROBIAL TARGET

Given the abundance of propionate in many infection scenarios, the 2-MC cycle has been identified as an attractive target for the development of new anti-bacterial agents. Blocking this pathway in several notable pathogens results in extreme sensitivity to propionate and reduced virulence. For example, several studies have indicated that the primary carbon source for *M. tuberculosis* is fatty acids/cholesterol when residing within host macrophages [56–58]. In support of this, PrpC was found to be required for intracellular growth in macrophages, indicating that propionyl-CoA metabolism is critical for maintaining the intracellular lifestyle [34, 58, 59]. Indeed, deletion of *prpC* impaired the ability of *M. tuberculosis* to grow in media containing cholesterol as a primary carbon source, and recent work has established that an *M. tuberculosis* *prpCD* double mutant also exhibits a growth defect in murine bone marrow-derived macrophages [60]. These observations are consistent with the notion that *M. tuberculosis* obtains a significant amount of its carbon requirements from cholesterol [61]. Building on these observations, an unbiased chemical screen was recently used to identify compounds that inhibit propionate catabolism and thereby diminish *M. tuberculosis* survival within macrophages. One such compound, V-13-009920, inhibited PrpC and prevented *M. tuberculosis* growth in cholesterol-containing media [60]. These results highlight the potential of targeting this pathway in prominent human pathogens as an antimicrobial strategy.

In *S. enterica* serovar Typhimurium, propionate exposure has been shown to decrease the expression of the *Salmonella* pathogenicity island 1 (SPI-1). This effect is dependent upon the transcriptional regulator HilD, which is post-translationally modified by propionyl-CoA [62]. Furthermore, pre-incubation of *S. enteritidis* with propionate and butyrate resulted in decreased epithelial cell invasion [63]. Moving to a whole-animal infection model (pigs), supplementation of the diet with a mixture of organic acids including propionate resulted in decreased *Salmonella* recovery from the mesenteric lymph nodes [64]. Thus, simple dietary supplementation with SCFAs, especially propionate, may be a promising intervention strategy for decreasing *Salmonella* infection in farm animals [65]. Interestingly, it has also been suggested that the production of propionate by intestinal microflora may be a protective mechanism to control the host response to commensals. This way, the host would avoid local inflammation and tissue damage [66].

The methylcitrate cycle gene cluster also plays an important role in *N. meningitidis* infection and transmission. In this organism, the *prp* genes are located on a large genomic island, absent from closely related non-pathogenic *Neisseria* species such as *N. lactamica*. This may be important because

the ability to utilize propionic acids may confer an advantage in colonizing the adult nasopharynx, which is rich in propionic acid-generating bacteria [25, 67]. From an evolutionary perspective, this plays an important role in the transmission and disease epidemiology of *N. meningitidis* by providing a selection pressure for the retention of a horizontally acquired propionate utilization pathway [25]. Recent work has shown that another respiratory pathogen, *Pseudomonas aeruginosa*, also utilizes the methylcitrate cycle to catabolize and detoxify propionate in the airway environment. Here, the propionate is derived from the breakdown of tracheobronchial mucin by cohabiting fermentative anaerobes [68]. However, the precise contribution of the *P. aeruginosa* methylcitrate cycle to propionate detoxification and virulence has not been explored further.

In summary, the last few years have yielded major advances in our understanding of how different species of bacteria detoxify propionate – an abundant SCFA in many infection scenarios. Moreover, it is also becoming clear that a better understanding of these pathways, and of the ecology of infection sites, can potentially lead to new antimicrobial interventions. Along the way, we are also learning much about the evolution, acquisition, or loss of metabolic pathways; a voyage of discovery that is being catalyzed by unprecedented access to genome sequence information.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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