No acetyl-CoA keeps *Plasmodium* at bay

Victoria Jeffers[1,*] and Matthew A. Child[2,*]
[1]Department of Molecular, Cellular and Biomedical Life Sciences, University of New Hampshire, Durham NH 03824, USA
[2]Department of Life Sciences, Imperial College London, South Kensington Campus, London SW7 2AZ, UK
*Correspondence: victoria.jeffers@unh.edu (V.J.), m.child@imperial.ac.uk (M.A.C.)
https://doi.org/10.1016/j.chembiol.2022.02.003

Acetyl-coenzyme A is an important metabolite and regulates diverse cellular processes, including metabolism and epigenetics. In this issue of *Cell Chemical Biology*, Summers et al. (2022) describe an essential parasite enzyme, acetyl-coenzyme A synthetase, as a target of two antimalarial small molecules active against liver and blood stages of the parasite.

Despite a concerted effort, malaria remains a global health challenge, with the burden of disease unfairly borne by the developing world. The etiologic agent of malaria, *Plasmodium* spp., is a member of the wider apicomplexan phylum that includes other pathogenic parasites such as *Toxoplasma gondii* and *Cryptosporidium* spp., which are responsible for toxoplasmosis and cryptosporidiosis, respectively. With the ever-present threat and seemingly unavoidable occurrence of antimalarial drug resistance, there is a great need for new therapeutic molecules and targets. Supporting these efforts, the Medicines for Malaria Venture's (MMV) malaria box and subsequent pathogen box provided researchers with curated libraries of commercially available, drug-like small molecules to galvanize drug discovery and research for malaria and other neglected infectious diseases (Spangenberg et al., 2013; Veale, 2019). All of the MMV malaria-targeted compounds are active against the blood stages of *Plasmodium falciparum*, but the molecular targets of most remain unknown.

Acetyl-CoA is a key molecule in cellular carbon metabolism and participates in many critical metabolic pathways in different subcellular compartments. Reflecting its inability to passively diffuse across biological membranes, enzymes responsible for acetyl-CoA biosynthesis are typically distributed throughout different subcellular compartments. In *P. falciparum*, the apicoplast-resident pyruvate dehydrogenase complex generates acetyl-CoA to support fatty acid synthesis within the organelle but appears to be dispensable during the parasite asexual stages; it does not contribute significantly to the nucleo-cytoplasmic pools of acetyl-CoA (Cobbold et al., 2013). An alternative route of acetyl-CoA biosynthesis occurs in the mitochondrion via the branched-chain α-keto acid dehydrogenase complex, which feeds acetyl-CoA into the TCA cycle for production of ATP. As acetyl-CoA is not easily transported across membranes, cellular processes in the cytoplasm or nucleus that require acetyl-CoA cannot rely solely on mitochondrial pools and must use additional sources. The related apicomplexan parasite *Toxoplasma gondii* expresses two enzymes, ATP citrate lyase (TgACL) and acetyl-CoA synthetase (TgACS), and both contribute to acetyl-CoA synthesis in the nucleo-cytosolic compartment. The functional redundancy of TgACL and TgACS was recently confirmed through a study of their synthetic lethality (Kloehn et al., 2020), supporting the critical nature of acetyl-CoA for apicomplexan parasite survival. In *T. gondii,* synthesis of acetyl-CoA would be a challenging process to therapeutically target, as inhibition of both TgACL and TgACS would likely be required to reduce the subcellular pool of acetyl-CoA. Promisingly, *P. falciparum* lacks a homolog of ACL, suggesting that a single enzyme (PfAcAS) is responsible for acetyl-CoA production in the nucleocytosolic subcellular compartment—a potential drug target.

In addition to its contribution to metabolism, acetyl-CoA is an essential substrate for epigenetic modification of chromatin structure (Figure 1). Nucleosomes are composed of an octamer of four different histone types (histones H2A, H2B, H3, and H4) around which the DNA is wrapped. The histones are acetylated by lysine acetyltransferases that catalyze the transfer of acetyl group from acetyl-CoA to the epsilon amino group of a lysine residue. Histones are abundant in positively charged lysine and arginine residues, and this net positive charge facilitates a tight interaction with negatively charged DNA. Acetylation of a lysine residue neutralizes this positive charge, loosening the interaction between DNA and the nucleosome, making the DNA sequence more accessible to DNA binding proteins and the transcriptional machinery, and promoting activation of transcription. This process is balanced by the action of the histone deacetylases, which remove acetylation marks from the histones to suppress transcription. Regulation of gene expression is thus tightly linked to the availability of nuclear pools of acetyl-CoA, allowing cells to rapidly reprogram gene expression in response to changes in nutrient availability or metabolic requirements (Trefely et al., 2019).

Target identification and validation campaigns are foundational to drug discovery, providing direct evidence of phenotypically relevant targets and target engagement in cells. These studies pave the way for more focused target-based screens and rational medicinal chemistry efforts to improve a molecule on its journey toward becoming an approved drug. In this issue of *Cell Chemical Biology*, Summers et al. (2022) present an outstanding example of a successful small-molecule target identification and validation study. Selecting for drug-resistant parasites that emerged following *in vitro* evolution, whole-genome sequencing of resistant clones identified acetyl-CoA synthetase (PfAcAS) as the likely target of the malaria box inhibitors MMV019721 and MMV084978 (Figure 1). This result suggested that inhibition of acetyl-CoA biosynthesis could be responsible for

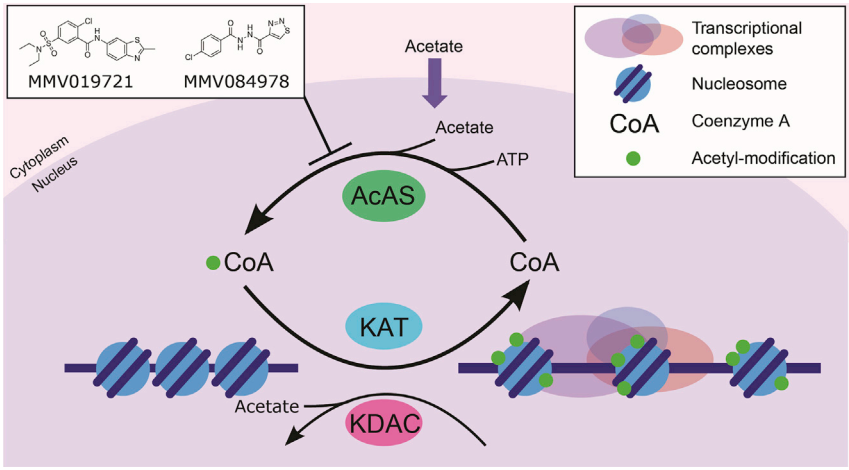

Figure 1. Inhibition of PfAcAS by MMV019721 and MMV084978 reduces pools of nuclear acetyl-CoA for histone acetylation

The action of lysine acetyltransferases (KAT) adds an acetyl group from acetyl-CoA to lysine residues on the histones, opening up chromatin structure and allowing transcriptional regulatory complexes to access the DNA. Pools of nuclear acetyl-CoA are generated by the enzymatic activity of PfAcAS using ATP and acetate that is either imported into the nucleus or released from acetylated histones by lysine deacetylases. Inhibition of PfAcAS by the small-molecule inhibitors MMV019721 and MMV084978 prevents cycling of acetate into acetyl-CoA and stalls histone acetylation. Consequently, gene expression is globally suppressed, leading to parasite death (Summers et al., 2022).

the growth-inhibitory phenotype associated with MMV019721 and MMV084978.

Metabolomic profiling determined that levels of acetyl-CoA were reduced in drug-treated parasites, indicating a mechanism of action whereby MMV019721 and MMV084978 inhibit acetyl-CoA biosynthesis (Summers et al., 2022). Satisfying the remaining tenets of a small-molecule target identification and validation campaign, Summers et al. (2022) provide genetic and biochemical evidence supporting PfAcAS as the phenotypically relevant target of MMV019721 and MMV084978. The introduction of the resistance-associated amino acid substitutions into the PfAcAS locus in otherwise wild-type parasites was sufficient to protect parasites from the effect of the drugs. Partial knockdown of PfAcAS levels was shown to shift the dose-response curves, resulting in hypersensitivity of these parasites to drug-induced growth inhibition. This knockdown approach defines a clear molecular link between the quantity of the protein target (PfAcAS) and the quantity of drug required to inhibit the target and elicit the phenotypic response (Summers et al., 2022). Homology modeling of PfAcAS using the crystal structure of acetyl-CoA synthetase from *Cryptococcus neoformans* (PDB: 5U29) indicated that the resistance-associated mutations clustered around the predicted active site of the enzyme. These data suggest a molecular mechanism of action whereby MMV019721 and MMV084978 directly block acetyl-CoA biosynthesis through competitive binding in the active site of PfAcAS. In vitro activity assays with purified recombinant PfAcAS demonstrated that MMV019721 and MMV084978 directly inhibit the breakdown of pyrophosphate—the first biochemical step in acetyl-CoA biosynthesis (Summers et al., 2022). Furthermore, these assays confirmed that the resistance mutations rescued PfAcAS activity in the presence of the drug. Analysis of enzyme rate constants in the presence of the drug confirmed discrete modes of inhibition for each molecule; MMV019721 was found to compete with CoA for binding to the active site of PfAcAS, while MMV084978 likely functions by preventing acetate from binding. Promisingly, limited activity was observed against the human ortholog HsAcAS for both MMV019721 and MMV084978. IC50 values for HsAcAS with MMV019721 and MMV084978 were >1000× and >50× those calculated for PfAcAS, respectively, indicating a good therapeutic window for selective inhibition of the parasite enzyme and not the host—a key requirement as these molecules continue on their journey from bench to clinic.

Global reduction of acetyl-CoA levels has critical metabolic consequences, but it also drastically reduces acetylation levels of histones and other gene-regulatory factors, hampering the parasite's ability to adapt by reprogramming transcription. The localization of PfAcAS to the parasite nucleus and observed reduction in global histone acetylation levels during inhibition of PfAcAS implicates it as a major source of acetyl-CoA for histone acetylation (Summers et al., 2022). A recent report that PfAcAS interacts with a chromatin remodeling complex and associates with the promoters of active genes (Bryant et al., 2020) also points to an integral role of PfAcAS in generating acetyl-CoA for the acetylation of histones. Furthermore, histones are not the only proteins that are acetylated in *P. falciparum*. Many other proteins involved in regulation of gene expression, metabolism, and protein synthesis are also modified by this post-translational modification (Cobbold et al., 2016; Miao et al., 2013). The distribution of acetylated proteins throughout multiple parasite cellular compartments implies that the reduction of acetyl-CoA during inhibition of PfAcAS likely affects the regulation of many different processes in addition to histone acetylation. Supporting this hypothesis, a recent study of the effect of pathogen box compounds on *P. falciparum* invasion and egress classified MMV019721 as a general growth inhibitor with a strong effect on invasion (Dans et al., 2020). Thus, the chemical inhibition of PfAcAS demonstrated by Summers et al. (2022) is a promising strategy for drug development, as the impact on the parasite is likely multi-factorial. As further studies proceed, it will be important to determine if PfAcAS is associated with other epigenetic regulatory complexes that direct its activity to specific gene loci under particular metabolic states to fine tune an appropriate transcriptional response.

REFERENCES

Bryant, J.M., Baumgarten, S., Dingli, F., Loew, D., Sinha, A., Claës, A., Preiser, P.R., Dedon, P.C., and Scherf, A. (2020). Exploring the virulence gene interactome with CRISPR/dCas9 in the human malaria parasite. Mol. Syst. Biol. *16*, e9569. https://doi.org/10.15252/msb.20209569.

Cobbold, S.A., Vaughan, A.M., Lewis, I.A., Painter, H.J., Camargo, N., Perlman, D.H., Fishbaugher,

M., Healer, J., Cowman, A.F., Kappe, S.H., and Llinás, M. (2013). Kinetic flux profiling elucidates two independent acetyl-CoA biosynthetic pathways in Plasmodium falciparum. J. Biol. Chem. *288*, 36338–36350. https://doi.org/10.1074/jbc.M113.503557.

Cobbold, S.A., Santos, J.M., Ochoa, A., Perlman, D.H., and Llinás, M. (2016). Proteome-wide analysis reveals widespread lysine acetylation of major protein complexes in the malaria parasite. Sci. Rep. *6*, 19722. https://doi.org/10.1038/srep19722.

Dans, M.G., Weiss, G.E., Wilson, D.W., Sleebs, B.E., Crabb, B.S., de Koning-Ward, T.F., and Gilson, P.R. (2020). Screening the Medicines for Malaria Venture Pathogen Box for invasion and egress inhibitors of the blood stage of Plasmodium falciparum reveals several inhibitory compounds. Int. J. Parasitol. *50*, 235–252. https://doi.org/10.1016/j.ijpara.2020.01.002.

Kloehn, J., Oppenheim, R.D., Siddiqui, G., De Bock, P.J., Kumar Dogga, S., Coute, Y., Hakimi, M.A., Creek, D.J., and Soldati-Favre, D. (2020). Multi-omics analysis delineates the distinct functions of sub-cellular acetyl-CoA pools in Toxoplasma gondii. BMC Biol. *18*, 67. https://doi.org/10.1186/s12915-020-00791-7.

Miao, J., Lawrence, M., Jeffers, V., Zhao, F., Parker, D., Ge, Y., Sullivan, W.J., Jr., and Cui, L. (2013). Extensive lysine acetylation occurs in evolutionarily conserved metabolic pathways and parasite-specific functions during Plasmodium falciparum intraerythrocytic development. Mol. Microbiol. *89*, 660–675. https://doi.org/10.1111/mmi.12303.

Spangenberg, T., Burrows, J.N., Kowalczyk, P., McDonald, S., Wells, T.N., and Willis, P. (2013). The open access malaria box: a drug discovery catalyst for neglected diseases. PLoS ONE *8*, e62906. https://doi.org/10.1371/journal.pone.0062906.

Summers, R.L., Pasaje, C.F.A., Pisco, J.P., Striepen, J., Luth, M.R., Kumpornsin, K., Carpenter, E.F., Munro, J.T., Lin, D., Plater, A., et al. (2022). Chemogenomics identifies acetyl-coenzyme A synthetase as a target for malaria treatment and prevention. Cell Chem. Biol. *29*, 191–201. https://doi.org/10.1016/j.chembiol.2021.07.010.

Trefely, S., Doan, M.T., and Snyder, N.W. (2019). Crosstalk between cellular metabolism and histone acetylation. In Post-translational Modifications That Modulate Enzyme Activity, pp. 1–21. https://doi.org/10.1016/bs.mie.2019.07.013.

Veale, C.G.L. (2019). Unpacking the Pathogen Box-An Open Source Tool for Fighting Neglected Tropical Disease. ChemMedChem *14*, 386–453. https://doi.org/10.1002/cmdc.201800755.